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3D Printing of Dairy-based Ingredients and Investigation into Irish Consumer Acceptance of 3D Food Printing Applications

Thesis presented by

Megan M. Ross, B.Sc.

for the degree of

Doctor of Philosophy

in

Food Science and Technology

May 2022

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Declaration

I hereby declare that the work submitted is entirely my own and has not been submitted to any other university or higher education institute, or for any other academic award in this university.

Signature:

Megan Ross Date 5th May 2022

Megan Ross

This thesis is dedicated to my family who have supported me throughout this fulfilling journey

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Many thanks to the technical staff in the school of Food and Nutritional Sciences (Avril McCord, Eddie Beatty (now retired), Theresa Dennehy, Dr. Therese Uniacke-Lowe and Johann Scollard) who have guided me and shared their invaluable knowledge over the course of this degree. Thanks also to Chief Technical Officer Dave Waldron for always being so willing to help and give advice when I needed it, it is really great to have someone like you on our team.

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I would also like to thank my family for loving and supporting me throughout these four years. Thanks to my mum, Anne, for listening to my non-stop chatter about everything 3D, food or printing even when you mightn't have been as excited as I was; and thanks to my Dad, Edward, for showing so much interest in the engineering side of my project that he considered buying a 3D printer for himself just to be able to support and learn alongside me.

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Abstract

Three-dimensional (3D) food printing is a type of additive manufacture in which foods with certain rheological characteristics are mechanically layered to create 3D structures from a digitally model. 3D food printing can potentially offer consumers a range of benefits, such as personalised nutrition, customisable textures and unique structures and shapes. This multidisciplinary thesis outlines research completed in three key areas: engineering, food science and consumer science, reflecting the importance of evaluating 3D food printing in this holistic manner. The primary objectives of this work was three-fold: (1) to develop a practical 3D food printer design suitable for printing dairy-based ingredients; (2) to identify and develop suitable dairy-based recipes and investigate factors affecting their printability; and (3) to explore determinants of Irish consumer willingness to try 3D food printing applications.

The rationale behind two selected 3D food printer designs (Cartesian and Delta), as well as a brief comparison between the functionality of both designs, are reported. The effects of printing parameters (i.e., nozzle diameter, distance to print bed, print speed etc.) on print quality are also discussed. Certain physicochemical factors such as pH and structural protein content were found to significantly affect the printability and texture of a basic processed cheese recipe. Samples with a higher pH (pH 5.8) tended to print less accurate grids and were significantly softer and less gummy, chewy and resilient than those with lower pH (≤ pH 5.6). Printed processed cheese recipes formulated with fresh curd (high structural protein content) resulted in significantly harder prints, yet printed grids inaccurately due to the material dragging during printing. Viscosity profiles were created for each processed cheese recipe using rheological methods and correlated with absolute printing precision values to identify a range of suitable recipes for accurate printing. The effect of various factors on the printability and functionality of Micellar Casein Concentrate (MCC) suspensions was also investigated. Increasing calcium chloride concentration (5 mM) of suspensions lead to significantly harder printed samples, which had lower dissolution and solubility rates than control or printed samples with lower calcium chloride concentration (1 mM). MCC suspensions printed in porous lattice structures were found to dissolve at a quicker rate than those printed in a hemisphere structure due to

a higher surface area to volume ratio. An example of possible 3D-printed product concepts demonstrating the potential of MCC as a printing material is presented.

In order to achieve a balanced and comprehensive understanding of 3D food printing technology from a number of perspectives, consumer acceptance research was incorporated into this study to compliment the food science and engineering narrative. Using qualitative methods, a series of themes were identified as forming Irish consumers' perceptions of 3D food printing applications. Consumers' affinity for naturalness and a strong association for unprocessed, homemade meals were considered barriers to acceptance of 3D-printed foods. As an extension of this study, data from quantitative research further revealed perceived personal relevance as a significant determinant affecting the dependent variable (i.e., willingness to try 3D food printing applications in the food service sector). Trust in science was found to diminish the negative effects of novel food technology neophobia on willingness to try. Potential solutions for negating factors affecting consumer acceptance are also discussed, which may be of benefit to those looking to market 3D food printing applications in the Irish marketplace.

The findings from these studies present an opportunity for food sector stakeholders to utilise this knowledge as part of their foundation to build upon and create novel 3D food printer designs and printable formulations which are suitable for acceptance in the consumer market.

List of publications and conference contributions

The following list of publications arise from work undertaken during my PhD studies, and most are included in this thesis for examination purposes. At the time of writing the material presented in Chapter 4 and Chapter 6 were submitted to peer-reviewed journals and are currently in review. It should be noted that the chapters in edited books listed (see titles marked with an *) are included in the appendix in the published format.

Peer-reviewed papers

Ross, M.M., Crowley, S.V., Crotty, S, Oliveria, J, Morrison, A.P., and Kelly, A.L. (2021) Parameters affecting the printability of 3D-printed processed cheese, *Innovative Food Science and Emerging Technologies*, **72**, 102730.

Ross, M.M., Collins, A.M., McCarthy, M.B., and Kelly, A.L. (2022) Overcoming barriers to consumer acceptance of 3D-printed foods in the food service sector, *Food Quality and Preference*, **100**, 104615.

Ross, M.M., Crowley, S.V., and Kelly, A.L. Application of micellar casein concentrate in 3D-printed food structures, *Innovative Food Science and Emerging Technologies*, (In review).

Chapters in edited books

***Ross**, **M.M.,** Kelly, A.L., and Crowley, S.V. (2019) Potential applications of dairy products, ingredients and formulations in 3D printing, in, *Fundamentals of 3D food printing and applications*, Godoi, F.C. Bhandari, B.R., Prakash, S., and Zhang, M. (eds.), Massachusetts, United States: Academic Press, pp. 175-206.

***Ross, M.M.,** Burke, R.M., and Kelly, A.L. (2021) 'Culinary Applications of 3D Food Printing' in Burke, R.M., Kelly, A.L., Lavelle, C., and vo Kientza, H.T. (ed.) *Handbook of Molecular Gastronomy*: *Scientific Foundations, Educational Practices, and Culinary Applications*, Florida, United States: CRC Press.

Poster presentations

Ross, M.M., Kelly, A.L., Crowley, S.V., McCarthy, M.B., and Morrison, A.P. (2019) Dairy and public perceptions: the building blocks of 3D food printing applications, 48th Annual Food Science and Technology Conference hosted by University of Limerick and IFSTI, Limerick, Ireland, December 16th, 2019.

Oral presentations

Ross, M.M., and Kelly., A.L. (2018) 3D Food Printing Applications for Dairy Systems, 2018 Global Food Science Student Competition, Jiangnan University, Wuxi, China, November 14-18th, 2018. [Received bronze award and 2000 YMB/~€250 prize money for oral presentation category]

Ross, M.M., and Kelly, A.L. (2018) 3D Food Printing Applications for Dairy Systems, 47th Annual Food Science and Technology Conference 2018, University College Cork, Cork, Ireland, December 6-8th, 2018.

Ross, M.M., Crowley, S.V., McCarthy, M.B., Morrison, A.P., and Kelly, A.L. (2019) Dairy Structures and Consumer Acceptance: The Building Blocks of 3D Food Printing Applications, Internal Food and Nutritional Sciences conference, December 13th, 2019.

Ross, M.M., Crowley, S.V., McCarthy, M.B., Morrison, A.P., and Kelly, A.L. (2021) 3D Printing of Cheese: Evaluation as Process Technology and Consumer Product, IDF International Cheese Science and Technology Symposium, [Virtually], June 7-11th, 2021.

Ross, M.M., Crowley, S.V., Morrison, A.P., and Kelly, A.L. (2021) 3D Printing Applications for Cheese and Milk Protein Ingredients, Dairy Science and Technology Symposium, [Virtually], June 21st – 24th, 2021.

Ross, M.M., Crowley, S.V., McCarthy, M.B., Morrison, A.P., and Kelly, A.L. (2021) 3D Printing Applications for Cheese: Process Evaluation and Public Perceptions, IDF International Cheese Science and Technology Symposium, [Virtually], July 11th – 14th, 2021.

Kelly, A.L., and **Ross, M.M.** (2021) 3D Printing and Dairy Product Innovation, INNOVA, [Virtually], September 30th, 2021.

Introduction and research objectives

The overarching theme surrounding this thesis is the development of a deeper understanding of 3D food printing using a multifaceted approach. This body of work draws on three disciplines, food science, engineering and consumer science, to generate comprehensive insight and contribute to the knowledge currently available on 3D food printing. During the initial stages of research, a thorough review of the literature available was completed (**Chapter 1**) and a version of this written manuscript was published as a book chapter in 'Fundamentals of 3D Food Printing and Applications' (See Appendix). The overarching aim in reviewing the literature across the three multi-disciplinary research areas of interest was two-fold; (1) Generate a solid foundation of knowledge of aspects involved in 3D food printing, 3D food printer technology and consumer acceptance of novel food technologies, and (2) Locate and identify key areas of interest and gaps in knowledge in the existing literature and use this information to guide the focus of the topics covered in each experimental chapter.

In terms of the engineering viewpoint of the project (**Chapter 2**), two traditional plastic printing 3D printers were purchased with the intention of modifying them both to print food using two different mechanisms, a syringe extrusion method and a peristaltic pump method. A number of brief experiments were conducted with the aim of developing an understanding of the fundamentals of printing parameters such as nozzle diameter and material viscosity, and their effect on printability of different food materials. This preliminary knowledge helped to develop the foundations for a deeper insight into what factors effect printability of more complex dairy-based formulations such as processed cheese (**Chapter 3**) and purified protein fractions (e.g., Micellar Casein Concentrate, MCC) (**Chapter 4**). A range of analytical techniques and methods, such as microscopy and rheological characterisation, were used in order to describe the degree of printability of each of the formulations that were 3D-printed.

In order to obtain a comprehensive understanding of the area of 3D food printing, consumer science research was incorporated into this project to compliment the food science and engineering portions of this body of work. The overarching objective of this consumer science research was to gain insight into consumer behaviour and attitudes towards 3D food printing, and how this might drive the overall design and messaging of 3D-printed food applications in order to build consumer trust. Ethical approval was sought and received to conduct focus groups with the objective of

gaining insight into Irish consumers' initial impressions of 3D food printing applications (**Chapter 5**). The primary themes generated from the qualitative data were then used to design and create an online survey which was distributed to Irish consumers to gather quantitative data. This data was then used with the primary aim of determining which factors significantly contribute to Irish consumer willingness to try 3D food printing applications (**Chapter 6**). Both quantitative and qualitative studies were completed in parallel by Finnish research partners, VTT (Technical Research Centre of Finland), as part of a collaboration who carried out the same study in both Finland and Belgium in order to compare consumer perceptions across different European countries. Findings reported in this thesis may be beneficial to both food manufacturers and food technology development specialists when formulating products suitable for 3D printing, but that are also presented in such a way that is acceptable to consumers.

CHAPTER 1

Literature review

Megan M. Ross

Declaration

This chapter was written by author MMR and reviewed by their co-authors, APM, SVC, and ALK. A version of this chapter has been published as: Ross, M.M., Kelly, A.L., and Crowley, S.V. (2019) Potential applications of dairy products, ingredients and formulations in 3D printing, in, *Fundamentals of 3D food printing and applications*, Godoi, F.C. Bhandari, B.R., Prakash, S., and Zhang, M. (eds.), Massachusetts, United States: Academic Press, pp. 175-206.

Abstract

Three-dimensional (3D) printing has become a technology of considerable interest in the food sector. This review provides a detailed description of the potential of 3D food printing in terms of the research output currently available, with a specific focus on those involving dairy-based ingredients. The constituents found in milk, such as protein, fat and lactose, each have unique structural characteristics that lend themselves to the possibility of printing, which are discussed in detail. A selection of 3D printing technologies which have been used to print foods are also described, as well as the analytical tools which are used to characterise printed food structures. As this novel technology enables consumers to become more proactive in the food manufacturing process, becoming both consumer and producer, factors affecting consumer acceptance of 3D food printing are important to consider. Challenges currently facing 3D food printing are also discussed.

1. 1 Introduction

3D printing is being investigated for a broad range of applications, including printing of replacement parts for automobiles (Schniederjans, 2017), printing of tissues and organs in regenerative medicine (Murphy and Atala, 2014) and, quite recently, the printing of food as solid structures with unique visual and textural attributes (Sol *et al*., 2015; Sun *et al*., 2018). As a novel technology, 3D printing has gathered some momentum in recent years, but has yet to enter most home kitchens and food businesses. Up until recently the 3D printing of certain foods, such as chocolate and other confections, has been the primary subject of both academic research (Hao *et al*., 2010; Lanaro *et al*., 2017; Mantihal *et al*., 2017; Yang *et al*., 2018b) and commercial applications (byFlow, 2021; Choc Edge, 2022). However, the range of foods which have been investigated for their printing properties has expanded to include fruits and vegetables (Derossi *et al*., 2018; Pant *et al*., 2021; Severini *et al*., 2018b), pasta (Van der Linden, 2015), meat (Blutlinger *et al*., 2021; Bulut and Candoğan, 2022; Dick *et al*., 2019; Yang *et al*., 2022), cheese (Le Tohic *et al*., 2018; Ross *et al*., 2021) and more novel foods such as plant-based meat and fish alternatives (Aleph Farms, 2021; Ko *et al*., 2021; Revo Foods, 2022) and sustainable insect protein snacks (Severini *et al*., 2018a). Since 2020, there has been an increase in the number of scientific studies focusing on the printing of dairy-based foods, where before that little was known about optimising the printing of dairy formulations, and no current-generation 3D printer was specifically engineered to print these materials.

The dairy category spans a broad range of products and ingredients with diverse compositions, functionalities and structures, making dairy a promising source of raw materials for the 3D printing of food. The printability of dairy products (e.g., cheese, yoghurt, butter) and ingredients (e.g., protein isolates, edible lactose, milk minerals) is not well characterised, despite the considerable opportunities they present for the preparation of foods that are both nutritious and flavoursome. There is significant scope to exploit the strong structure-forming properties of dairy systems during 3D printing through, for example, manipulation of gelling mechanisms, melting behaviour and ingredient interactions. To realise the potential of dairy in 3D printing, different model formulations will need to be tested and optimised for printability, with successfully printed objects subjected to multiscale characterisation to determine their post-printing integrity and functionality.

The evolution of 3D dairy will be interwoven with the broader narrative of food printing. Lupton (2017) studied online news outlets for their coverage of developments in 3D printing of food between 2013 and 2016 and organised results into the five major promissory themes of Futuristic (e.g., 'Kitchen of the future'), Creative (e.g., 'Customised foods'), Healthy (e.g., 'Foods for people with dysphagia and the elderly'), Efficient (e.g., 'Food for plane travellers') and Sustainable (e.g., 'Use of alternative edible substances in food products'). Indeed, the opportunities afforded by 3D printing of dairy can be said to fit within these thematic areas. The inherent 'promise' of 3D food printing should be tempered by the many practical challenges the technology presents to food researchers, which are not limited to issues of retail price and user-friendliness. In terms of personalised nutrition, many formulated products used currently in 'healthy' nutrition (e.g., elderly, dysphagia) applications, for example, contain milk protein ingredients as their primary protein source, yet little is known about their printability.

As well as the processing-related challenges related to the effective and safe printing of such foods, there are questions associated with appropriate methodologies for analysing printed structures, which may have levels of geometrical complexity that are unprecedented for food. More broadly, much of the health-orientated imaginings of food printing are characteristic of a pervading discourse in food and nutritional science that has been strongly criticised as 'nutritionism' (Scrinis, 2008); for example, some imagine a dietary approach that merges the 'quantified self' movement with 3D food printing, in which self-tracking of biomarkers dictates the nutritional profile of the printed meal (Khot *et al*., 2017). Furthermore, 3D printing as 'additive manufacture' is frequently referred to as being 'waste-free' and therefore sustainable, but this characterisation is not so straightforward. If dairy-derived ingredients are to be used in printing applications, then their entire life cycles, encompassing associated by-products, emissions and consumer food waste behaviour, will dictate the degree to which the printed object is indeed environmentally friendly. These and other issues need to be considered in parallel with technological developments in food printing. Nonetheless, efforts to realise the potential of 3D printing in the food industry continue apace. Many individuals are more concerned with diet, desiring greater control over what they eat without sacrificing modern conveniences (Petty, 2017). Three-dimensional printed snack vending machines are being developed at present, with the object of delivering healthy printable snacks on the go (Gao *et al*., 2015). In this way,

3D printing is envisaged as facilitating the accurate ‘metering’ of ingredients/nutrients in printed meals.

In this chapter, an overview of printing technologies of potential relevance for dairy printing will be provided. The structural transformations that occur in dairy systems will then be discussed, with the aim of gaining insights into future possibilities in printable dairy. The literature on the topic of 3D printing of dairy systems will then be reviewed. Some analytical tools to analyse dairy systems before, during and after 3D printing will then be proposed. To conclude this chapter, an overview of the challenges and limitations associated with 3D food printing as well as future perspectives will be explored.

1.2 Overview of relevant 3D printing technologies

Over the past decade, the range of 3D printing technologies has expanded rapidly, particularly for the printing of nonedible materials, such as plastics and metal powders; however, food printing technologies are also being developed and are beginning to emerge in the consumer world. The equipment and materials available today are far more sophisticated than when 3D printing technology was first developed (Hull, 1986), when the first patent was issued for a stereolithography apparatus that could print plastics. A decade ago, 3D printers were an expensive hobby costing about the same price as a family car; in more recent times, a basic printer incurs roughly the same cost as a low-end laptop (Miller, 2016). This section will contain a brief overview of how a 3D-printed product is created from computer code and the printing technologies of potential relevance to dairy applications, as well as the advantages and disadvantages of these technologies.

The majority of printing techniques require modelling software such as computer-aided design (CAD) or 3D scanning equipment to create a digital file of the 3D design (Goyanes *et al*., 2016). This file will contain information pertaining to the dimensions of the object to be printed. As seen in Fig. 1.1, the most common file format for the 3D model is Standard Tessellation Language (STL), which can be transformed into ‘G-code’ using a slicing programme such as Slic3r or CuraEngine, both of which are commonly used open-source software applications in 3D printing. This slicing software works by taking the object in the STL file and ‘slicing’ it into individual layers, which then act as printing instructions in the form of G-code, guiding where the printer must print the material in order to form the shape (Hamilton *et al*., 2018).

Fig. 1.1. Example of slicing software used by Choc Creator. A step-by-step guide is shown from basic sketch of an idea to final 3D-printed product (Choc Edge, 2022).

A common technology that has been used when printing desserts and confectionary is fused deposition modelling (FDM) (Fig. 1.2). This type of technology operates by extruding heated food material from a nozzle or syringe that is guided within the Cartesian coordinate space (x-, y-, and z-axis) during printing, with the rate of deposition being controlled by computer-regulated motors. This allows defined layer-by-layer geometries to be constructed using the most efficient pathway possible, with minimal excess material being deposited in the process. As each layer is extruded, the food material cools and hardens, providing a physical support for the subsequent layers (Wegrzyn *et al*., 2012).

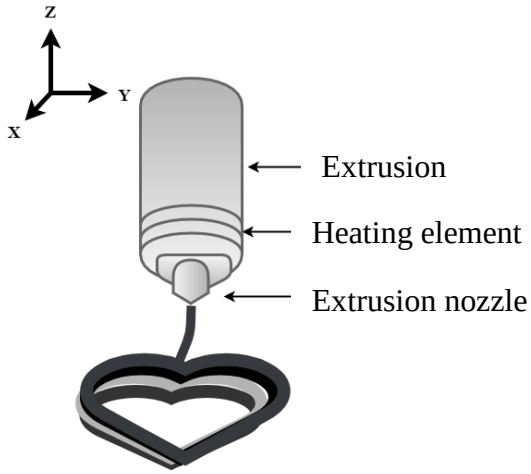

Fig. 1.2. Fused deposition modelling (FDM) - Food material in liquid/semiliquid state is extruded through a narrow orifice and deposited onto a pre-programmed design, layer by layer.

This form of printing often produces a visible layer-by-layer definition that may not be suitable for applications that, for example, require a smooth finish, such as detailed chocolate-based designs. Printing using FDM technology also has the advantage of being able to directly dose structure-building agents (e.g., hydrocolloids, transglutaminase) during printing by incorporating a side inlet embedded in the nozzle

(Schutyser *et al*., 2018). Examples of FDM applications that are available on the market (at the time of writing) include Choc Creator, Foodini, Focus printer and Procusini (Sun *et al*., 2018); these machines are claimed to be able to print confectionary-/chocolate-based structures and fruit-/vegetable-/cereal-/meat-based structures.

Another form of FDM printing technology is co-extrusion printing. This technology was patented by Guillemette and Peters (2015), who mentioned the potential for using co-extrusion printing in food applications. In the food industry, extrusion technologies are commonly used to make pasta, dried snack foods, breakfast cereals and confectionary (Ding *et al*., 2006); however, extrusion technology can be combined with 3D printing to provide customisable layering, enrobing or filling capabilities in various food stuffs. The technology enables users to combine materials in different ways through different nozzle configurations. For example, a food material, such as semi-molten chocolate, could be extruded through a nozzle, while a different food material, such as strawberry jam filling, flows through a narrower nozzle embedded within the main nozzle. In another configuration, different ingredients or formulations could be extruded from adjacent nozzles, creating side-by-side layers in a visibly stratified product. Guillemette and Peters (2015) have claimed that their co-extrusion technology has the potential to incorporate localised preservatives, alternating flavours or colours in layered food products as a useful method to build functional, sensorial and visual complexity.

This type of technology might also be useful in the pharmaceutical industry, where purified lactose, a dairy-based ingredient, is used as a bulking agent in pills and tablets. Three-dimensional printing pharmaceutical-grade lactose and medical drugs into tablets is a cost-effective production method for on-demand manufacturing, as well as individualised dosage (Sadia *et al*., 2016). Bi-layer tablets are used as a controlled-release formulation to provide a way of successful drug delivery system into the body (Divya *et al*., 2011). Khaled *et al*. (2014) demonstrated the manufacture of relatively complex pharmaceutical formulations printed into bilayer tablets using an inexpensive desktop 3D printer. It was found that the 3D-printed formulation could match the controlled-release performance of a commercial manufactured bi-layer tablet using conventional tablet compression methods. The authors proposed that there was clear potential for 3D printing to allow entirely new formulation types, for instance, new geometries and complex multilayer or multi-reservoir tablets and others.

Calcium phosphate is a mineral naturally present in milk and also in human bone. Xilloc (2017), a Dutch medical engineering company, developed a bone implant consisting of pure pharmaceutical-grade calcium phosphate that can be used in non-load-bearing areas such as the skull. Calcium phosphate was found to be printable using bio-ceramic printing technology, a type of FDM where calcium phosphate is made into a wetted mixture which can then be 3D-printed. Calcium phosphate forms durable but porous structures when printed. This may be an interesting area to explore for food researchers, where edible-grade milk minerals (i.e., calcium phosphate, zine, magnesium, iron, etc.) may produce hard, rigid structures using 3D printing technology.

When printing with dry, un-wetted powders, however, a different form of technology called powder bed printing (PBP) is used to fuse or melt the particles together to create a solid structure. Selective hot air sintering and melting technology is a type of PBP and operates using hot air, which in turn fuses powdered food substances into a layered structure (The-CandyFab-Project, 2008). Initially, the powdered food material is spread out evenly and thinly using a levelling roller and subsequently undergoes melting when exposed to hot air. The roller then proceeds to level freshly introduced powder over the melted surface, fusing the layers together. This process is repeated to build the desired structure layer by layer in this fashion (Holland *et al*., 2018). This method can also use lasers in lieu of hot air to melt and fuse particles together (Fig. 1.3). This technology is called selective laser melting, also known as selective laser sintering (SLS). Diaz *et al*. (2014) patented this technology as a method of producing edible structures. The authors claim that in conjunction with a non-melting powder component, a ‘binder’ can be used to provide structural support. The binder component will melt at the temperature of the laser (140-180°C) and connect the non-melting component particles. Sources of binders that may be used in the SLS process include polysaccharides, fats and waxes, and also proteins like those found in milk (i.e., casein).

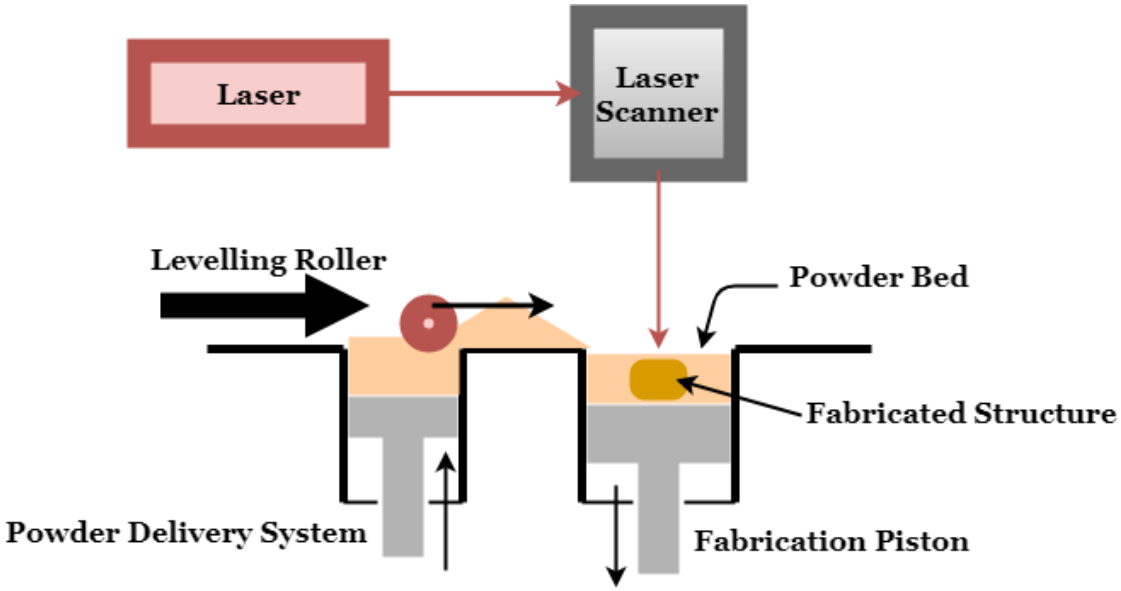

Fig. 1.3. Principle of a laser sintering: a laser melts the fine layer of powdered material to cause it to fuse together into a solid structure. The levelling roller scrapes each layer to provide an even surface for the subsequent lasering.

Liquid binding (LB) is another variation of PBP technology, which was originally patented by Bredt and Anderson (1999) as 3D printing. This 3D-printing method, when applied to food, involves edible objects being produced by jetting a liquid food or 'binder' onto a print bed layered with edible powder. This powder can be composed of a water-soluble protein and/or a hydrocolloid to fabricate solid structures, as described by Godoi *et al*. (2016). The binder joins adjacent powder particles together due to dissolution-fusion or cross-linking of surface particles, thereby creating a 3D matrix. The technology can also be adapted to print flavoured edible binders which will allow the creation of interesting flavoured powdered structures, which can be rehydrated with a liquid if desired (Wegrzyn *et al*., 2012). There is limited evidence of this technology being applied in dairy formulations.

1.3 Structure-forming mechanisms involving milk components

Milk is a complex biological fluid consisting of multiple macronutrients potentially suited to 3D printing due to their structure-forming capabilities. The solids in bovine milk are comprised of lactose, fat and protein, in order of decreasing abundance, in addition to minerals, vitamins and other minor compounds (Fox, 2008). The physio-chemistry of major milk components will be described in this section to establish the principle mechanisms that could be exploited in the printing of solid dairy structures.

1.3.1 Milk proteins

Two protein families are contained within the milk system, namely casein and whey protein. These proteins are present in bovine milk at a ratio of 80:20 casein: whey protein. Casein and whey proteins have different structures and functionalities, with each family further subdividing into different proteins with unique properties (O'Mahony and Fox, 2013). Whey products are widely used as ingredients in food formulations due to their excellent functional and nutritional properties. Native whey proteins are globular in structure and, following heating (>70°C), will denature and aggregate. In order to gel, whey proteins must be present at sufficient levels of protein (~ 6%) and will gel on heating or cooling. However, many factors affect the gelation of whey proteins, such as pH, heating rate, temperature, time and solutes (Lucey, 2008).

It is generally agreed that whey protein gels can be classified as being either fine-stranded or particulate, or a mixture of both (Foegeding *et al*., 1998). These types of gels differ in functional properties. Fine-stranded gels are translucent and retain large quantities of water, while particulate gels are opaque and readily expel water. This influences mouthfeel *in vivo* where, for example, fine-stranded whey protein emulsion gels have smooth surfaces, express minimal fluid during chewing, with a low degree of cohesiveness and adhesiveness. In comparison, particulate gels have rougher surfaces, release higher levels of moisture during chewing and have a high degree of cohesiveness and adhesiveness (Gwartney *et al*., 2004). The type of gel formed can be controlled through certain factors and therefore manipulated to create a gel that will be 'printable', without being too thick or too liquid, in order to form a structure. For example, filament gels will form equally well at pH 2.0 and 7.0 in conditions of low ionic strength, whereas particulate gels will form at a higher ionic strength at or close to pH 7.0 (Foegeding, 2006). Changing the pH or salt concentration of the dairy formulation could thus enable the user to control the texture of the desired printed food. Table 1.1 shows these physicochemical differences between fine-stranded and filament gels in more detail.

Table 1.1. Table with physicochemical property differences between fine-stranded and filament whey protein gels (Note: pI = isoelectric point).

Filamentous gel	Particulate gel
▪ Transparent	▪ Opaque
▪ Higher elasticity (less syneresis)	▪ Lower elasticity (more syneresis)
▪ Slow forming – also break down slower in mouth (good control of flavour release)	▪ Random/unordered formation – faster forming – break down quickly in mouth
▪ Formed at low ionic strength	▪ Formed at high ionic strength
▪ Can be used to fortify juices/water	▪ Can be used to fortify milks
▪ Formed at pH away from pI	▪ Formed at pH close to pI
▪ Whey proteins strongly charged	▪ Whey proteins not strongly charged
▪ Gels made up of strands of protein	▪ Gel made up of protein particles
▪ Strands are 10-20 nm thickness	▪ Particles are micron sized (can be used as fat replacers
▪ Brittle at low pH (rubbery at neutral pH)	▪ Brittle
▪ Good water-holding capacity	▪ Poor water-holding capacity

Whey proteins, compared to caseins, are considered more ordered in their structure. Whey proteins form gels due to a combination of noncovalent (e.g., hydrophobic) and covalent (e.g., disulphide linkages) bonds between denatured proteins (Anema and Li, 2003). In yoghurt, for example, whey protein gelation is important, as milk must be heated to allow for denaturation of the protein, which, in turn facilitates interaction with casein and therefore incorporation of whey proteins into the casein-based gel matrix (Anema, 2008). Whey proteins can undergo extensive aggregation during heating and form strong gels on cooling (Damodaran and Parkin, 2017). This cold-set gelling mechanism is amenable to FDM, as the solution could be heated prior to printing to maintain a flowable consistency and promote denaturation and subsequently undergo cooling on the printer bed to initiate gelation. However, at temperatures of 70°C and above, an increased viscosity could lead to fouling issues

on extruding through a narrow orifice, where the coagulated particles clog the printing nozzle (Chandan, 1997).

Caseins micelles are large, self-associated assemblies of individual caseins linked together with calcium phosphate, and are porous and roughly spherical colloidal particles (Chandan, 1997). The casein micelle has a dynamic structure and is continuously exchanging components (i.e., ions) with the aqueous phase in which it is suspended to maintain chemical equilibria. Micelles also respond to changes in the surrounding environment, such as temperature, pH and pressure, accordingly. There are four subtypes of casein proteins, called α_{S1}-casein, α_{S2}-casein, β-casein and κ-casein. The κ-casein is located at the surface of the casein micelle and stabilises the structure sterically and electrostatically. This particular protein is easily accessed by chymosin, or other proteinases with comparable specificity, which cleaves a bond (Phe_{105}-Met_{106}) specific to κ-casein. This, in turn, initiates the gelation of milk by allowing coagulation of the caseins in the presence of calcium, which is the basis of cheese-making (Huppertz, 2013).

Acid-induced gelation is also a method of coagulating casein and is commonly used in the manufacture of soft cheeses and yoghurt. Unlike rennet-induced gelation, bacterial cultures are added to liquid milk which ferment lactose into lactic acid, thereby lowering the natural pH of milk from pH 6.7 to pH 4.6, the isoelectric point of casein, where electrostatic repulsion is minimised (Lucey and Singh, 2003). Acidification may also be achieved through the addition of mineral acids (e.g., hydrochloric acid) in combination with warming, which is used to produce the ingredient acid casein.

Caseins are relatively heat-stable as a consequence of their open structure and do not denature or unfold on heating (Holt *et al*., 2013). When combined with hydrocolloids (thickening and gelling agents derived from carbohydrates, animals and seaweeds, such as carrageenan), caseins can generate network structures which stabilise multiphase dispersions (Puvanenthiran *et al*., 2003). Verbeken *et al*. (2006) combined κ-carrageenan, milk proteins and modified starch to form gelled, sterilised dairy desserts. The study found that modified starch imparts a large influence on the complex modulus of the gel due to the exclusion effect of the swollen starch granules, which act as non-interacting fillers, which in turn concentrates the carrageenan-milk protein solution in the continuous aqueous phase, allowing a strong gel to form. Such

approaches could potentially be applied to printed dairy products, depending on their desired attributes and functionality.

1.3.2 Milk fat

Milk is an oil-in-water emulsion, consisting of fat globules protected by a thin layer called the milk fat globule membrane (MFGM) suspended in the aqueous phase, which stabilises the emulsion through lowering of the interfacial tension surrounding the fat globule. In the manufacture of butter, the fat fraction of milk (i.e., cream: 40 % milk fat) is separated from skim milk and pasteurised. The cream is then churned at cold temperatures (approximately 14°C) using an augur or similar disruptive force to disrupt the MFGM, leading to an inversion to a water-in-oil emulsion. Buttermilk is then separated from the butter grains, and the butter is subjected to a second churning stage in order to further remove excess buttermilk. At this stage, moisture levels can be adjusted and salt or cultures added if desired. Ghee is clear butter fat which originated from India, and is typically made by heating butter and evaporating the remaining moisture, resulting in a buttery-flavoured product with an extended shelf-life (Kratochvil and Vedlich, 1974).

Triglycerides (TG) are the primary fat component present in liquid milk, along with carotenoids and vitamins. TG content can be controlled though the diet of the animal, by feeding sources of unsaturated or saturated fatty acids. Milk fat has an extremely diverse fatty acid profile and therefore a varied TG composition, which contributes to a wide range of melting temperatures, spanning from -40°C to 40°C (Rønholt *et al*., 2014). Butter has been used as decoration and ornamentation on hotel and restaurant tables for decades, as the unique texture of butter allows it to be easily moulded, shaped and sculpted. Schnell (2013) discussed the work of Pamela Simpson and Caroline Brooks, who were renowned for their famous butter sculptures, including a life-size bust of Teddy Roosevelt, the 26th US president.

There are many factors that can affect the structure of fat that may be relevant during printing of dairy products. This includes whether the butter has been subjected to thermal treatment, which can influence crystal polymorphism (changes in crystal fat structure). In addition to this, the number and sizes of fat crystals created, as well as crystal-crystal interactions within the product, are critical determinants of texture (Rønholt *et al*., 2013). The process of fat crystal formation and growth are vital elements in developing the fat crystal network within a dairy fat product such as butter. Consequently, properties such as spreadability, mouthfeel, appearance and product

functionality are affected by the fat crystal network. It is generally accepted that the presence of fat globules produces a softer product in comparison with milk fat-based products without any fat globules present (Mulder and Walstra, 1974).

Three-dimensional butter printing has never been studied to the authors' knowledge; however, the rheology of butter has been well characterised and may give some indication of how the butter might print when subjected to a certain stress, e.g., on being pushed through a narrow orifice or nozzle during printing. When a small stress is applied to butter, the crystal network may distort, resulting in reversible breakage of some crystal bonds. In contrast, when a large stress is applied, this will rearrange the network or cause fracturing, resulting in irreversible breaking of the bonds (Rønholt *et al*., 2014). Although studies have yet to be performed on the printability of butter and other dairy fat-based products, butter is known to be easily sculpted, holds its shape well at refrigeration and ambient temperatures, and has been well studied for its melting-solidification behaviour.

Oleogels are an interesting development in emulsion science, which have been proposed as an alternative replacement to trans and saturated fats (Dassanayake *et al*., 2011), and often rely on the structuring function of dairy proteins. Oleogels can be defined as an organic liquid (i.e., liquid fat) entrapped within a thermoreversible 3D protein gel network (Stortz *et al*., 2012). According to Marangoni and Garti (2011), the major approach in producing oleogels is to incorporate specific molecules (i.e., polymers such as proteins) into the oil components to alter the physical properties of the oil.

This leads to a decrease in fluidity, giving rheological properties that are similar to those of fats. Oleogels have been effectively used by Stortz *et al*. (2012) to replace hardstock fats (i.e., solid phase in margarine) in creams and cookies, and potentially other dairy-based foodstuffs such as cheese and ice-cream. Oleogels could be a potentially printable substance to enhance nutritional profiles of food through encapsulation through the addition of plant sterols, lycopene or coenzyme Q10 to the protein-oil matrix (Zetzl and Marangoni, 2012; Zhang *et al*., 2015). This could be achieved using 3D printing technology, where the nutrient can be added at the last step as the material leaves the nozzle and cools on the print bed.

1.3.3 Lactose

Lactose, the sugar in milk, is a disaccharide of glucose and galactose joined by a glycosidic linkage. Lactose is present in whey and can be recovered in purified form

through a crystallisation process for food or pharmaceutical use (Paterson, 2017). Lactose is used in a variety of different food applications, such as ice cream, to increase viscosity or improve texture without imparting an excess level of sweetness. Lactose has flavour-, aroma- and colour-absorbing properties, which can be beneficial in a product that requires the release of fragrant aromas or intense flavours over time (e.g., confectionary). In the baking industry, the role of lactose in the Maillard reaction or browning process is taken advantage of in creating a caramelised flavour and improved appearance of baked products. Lactose is primarily used in the production of humanised infant formula, where its main role is to correct the balance between carbohydrate and protein of cow's milk to make it more comparable to human breastmilk. Lactose is relatively slow to digest and has bifidogenic activity, meaning the infant has prolonged energy and maintains a healthy intestinal flora in the gut. Lactose can also be added to other powders in order to increase the free-flowing properties of powdered foods (Holsinger, 1997).

In terms of 3D printing, during an SLS process, powders must be free-flowing in order to create an even surface for the laser to penetrate a thinly layered powder. In this type of 3D printing, the powder bed process, sugars such as lactose provide the scaffolding or framework of the printed structure. Factors affecting the flowability include the compressibility and density of the powders themselves (Berretta *et al*., 2014; Godoi *et al*., 2016).

In comparison with other sugars, lactose has a relatively low level of solubility (Holsinger, 1997). This becomes important when lactose is added to a formulation and printed using FDM technology, as the solution must remain homogenous and flow readily without clogging the nozzle. However, solubility of lactose when dissolved can be controlled through muta-rotation or interconversion between the two forms of lactose, α and β, each form having distinctly different solubilities. Of the two, α-lactose has a lower solubility in water at temperatures < 93.5°C; therefore, when an excess quantity of a-lactose is added to water, the initial solubility will be limited. However, some α-lactose will be converted to β-lactose, resulting in an increase in solubility and enabling more α-lactose to be dissolved. This process will continue until the final equilibrium between α-lactose and β-lactose is achieved. The rate of muta-rotation is affected by pH, where pH values < 2.0 and > 7.0 result in very rapid muta-rotation. Temperatures above 70°C also allow muta-rotation to occur in minutes rather than hours at room temperature (Huppertz and Gazi, 2016). Lille *et al*. (2018) studied the 3D printing properties of skimmed milk powder (SMP) which contains 53% lactose,

and semi-skimmed milk powder (SSMP) which was hydrolysed to create a lactose-free powder. Comparing the printing quality between these two food materials may be useful in determining how lactose may contribute to structure. Using 60% SSMP paste solution, the authors were able to print a square grid (25 mm × 25 mm) with excellent printing precision and shape retention (i.e., no warping or spreading of the layers). In contrast, 50% and 60% SMP prints sticky and dry, crumbly prints, respectively. However, it is not understood completely whether lactose is the contributing factor to a lower-quality print, and further research must be considered in this area.

1.4 Studies of printable dairy structures

Despite recent progress with 3D food printing technology, only a limited range of food products have been successfully printed. Dairy materials that have been demonstrated in 3D printing applications will be discussed in this section, including MPC (Milk Protein Concentrate), WPI (Whey Protein Isolate), Lactose, SSMP, SMP, sodium caseinate (NaCas), micellar casein concentrate (MCC) and processed cheese.

MPC is a dairy ingredient typically produced using membrane filtration technology (i.e., ultrafiltration and/or diafiltration), where the retentate of the filtration process is concentrated by evaporation, followed by a spray-drying step. MPC is composed of casein and whey proteins and usually contains a certain amount of lactose and minerals (O'Kennedy, 2009). This ingredient has been demonstrated to produce printable structures at high protein concentrations when combined with NaCas (Liu *et al*., 2019). NaCas is manufactured by adding sodium hydroxide to acid casein (e.g., casein prepared from milk by acid precipitation) curds or powder to create a solution of pH 6.6, under which conditions the caseins become more soluble and functional (Carr and Munro, 2004). Samples (NaCas dispersions (20 % w/v) with added MPC to make a composite gel of a range of protein concentrations) with 400 and 450 g/L of total protein were determined to be optimal in terms of printing performance. The addition of MPC to NaCas was shown to allow water to be distributed equally in the sample and form gel-like structures. However, samples with < 400 g/L total protein lacked the storage modulus necessary to maintain the structural integrity of the printed object, and were, therefore, deemed unsuitable for printing. Consequently, the total concentration of the printing material has been shown to be an important consideration during preliminary trials in creating suitable formulations designed for printing (Liu *et al*., 2019).

MPC has also been used as a printing ingredient when combined with another dairy-based ingredient, WPI (Liu *et al*., 2018b). Produced using microfiltration, followed by concentration and spray-drying, WPI is a high protein ingredient (> 90% total protein) with no to low levels of lactose (< 1%) or fat (Mehra *et al*., 2021; Meng *et al*., 2021). With the aim of producing a high-protein mixture with optimum printing performance, Liu *et al*. (2018b) combined MPC and WPI in a ratio of 5:2 along with glycerol and xanthan gum as a plasticiser and structuring agent respectively. Pure MPC pastes were shown to be dry and fragile, whereas the addition of WPI had a softening effect on the paste. Interestingly, at a powder addition ratio of 4:3 (MPC: WPI), the paste showed a doubling of the relaxation time of that at a ratio of 5:2, which would suggest that a critical concentration value for WPI exists before the structure of the paste becomes compromised and collapses. WPI (at 12%, w/w, addition) was also shown to have softening effect when added to gelatine-supplemented Greek yoghurt where 3D-printed samples exhibited reduced resilience. The addition of WPI proved beneficial in improving the flowability of Greek yoghurt through the printing nozzle, where the protein isolate prevented complete cross-linking within the gel network, creating a smoother, yet weaker gel suitable for the printing process (Riantiningtyas *et al*., 2021). Though MPC was shown to improve hydration in the study mentioned previously involving NaCas (Liu *et al*., 2019), it appears that WPI has a greater ability to increase the amount of free water within the sample than MPC. Findings such as this are important to acknowledge when selecting ingredients and their quantities to formulate printable foods.

SSMP is a dairy ingredient in which the fat/cream in whole milk (3.5% fat) is removed by centrifugation and then reincorporated at approximately half the level of the original milk; the reduced-fat milk is then evaporated and spray-dried to generate a powder. SMP is a similar product except that the fat is not reincorporated after it has been removed (Lewis and Deeth, 2009). SSMP has been demonstrated to be compatible with 3D printing after reconstitution to a high solids content (60% w/v). Lille *et al*. (2018) conducted 3D printing experiments with protein- and fibre-rich foods. Print quality (e.g., precision, shape) was assessed visually from photographs using a scale, ranging from 1 = poor quality to 5 = good quality. The authors used lactose-free SSMP, in which the lactose, a disaccharide, was enzymatically hydrolyse into its component monosaccharides, glucose and galactose. SSMP at 60% solids scored 5 in print quality, SMP (60%) scored 1 and starch (15%) scored 3. The superior printability of SSMP was suggested to be due to its higher fat and lower carbohydrate

content, allowing a more free-flowing print. Although SMP or starch were unsuitable for printing when either were used alone, Lille *et al*. (2018) observed that a mixture of starch (10%) and SMP (15%) was more printable (print quality value = 4).

Derossi *et al*. (2018) used SMP as an ingredient during a study on the 3D printing of fruit-based snacks for children. Fresh bananas, dried mushrooms, canned white beans, lemon juice, pectin and ascorbic acid were used in combination with the SMP to create a cube-shaped snack containing 5 % -10 % of the total daily energy requirement for a child. X-ray microtomography analysis of the snacks showed that the most structurally uniform sample was printed at a flow level of 130%, which indicates the volume of material extruded during printing (this setting is normally selected in the slicing software of the printer), and a print speed of 70 mm/s compared to those at a lower flow rate (100 % and 70 %) and print speed (50 and 30 mm/s). A flow of 130 % means a 30 % increase in speed of the mechanism extruding the paste (e.g., motor driving extrusion pump) and therefore a 30 % increase in millimetres of paste extruded from the nozzle; printing speed refers to the rate at which the physical nozzle of the printer moves. At a lower flow rate (i.e., 70% and 100%), the cube-shaped snack appeared irregular, depositing broken lines during printing which caused large cavities in the structure. Joshi *et al*. (2021) observed that adjusting the ratio of HDMP (Heat Desiccated Milk Powder – milk which has been vacuum-dried using a pan-drier resulting in a high fat dairy product) and SSMP added to a set quantity of cornflour and water allowed the authors to create mixtures with varying rheological properties. Formulations with a higher ratio of SSMP tended to have more viscous mixtures with a higher yield stress than formulations with a high ratio of added HDMP. This flow behaviour was reflected in the printing accuracy of the respective formulas, where SSMP dominant samples resulted in thinner, more accurate lines, whereas formulas with high quantities of HDMP tended to spread during printing due to a lack of structural integrity. Joshi *et al*. (2021) attributed this finding to the high fat content in HDMP acting as a lubricant, leading to formulations lacking in shear-thinning behaviour, which is a key attribute of printable food materials.

Many milk protein ingredients exhibit thermoreversible sol-gel transitions and could be used at lower concentrations than the previous SMP/SSMP examples by exploiting phase transitions induced by temperature changes. NaCas suspensions at high concentrations display such thermoreversible properties and have been studied in 3D printing applications (Schutyser *et al*., 2018). NaCas displays a thermoreversible transition from a solution to a gel at or above a critical concentration (140 g/L) with

added salt (1.53 M NaCl), existing as a liquid at >50°C and a solid gel at 5°C (Carr and Munro, 2004). Schutyser *et al*. (2018) 3D-printed hot NaCas-based emulsion systems that solidified on a cold printing stage. The authors used a nozzle with a side inlet to incorporate coloured vegetable oil droplets into the protein matrix during printing. Using the dispenser with a side inlet created a unique structural design with a controlled spatial distribution of oil droplets. According to the authors, this technique may afford more freedom and creativity in the customisation of healthy foods, where vitamins and minerals can be encapsulated and incorporated into a protein matrix.

MCC is similar to NaCas in that it forms cold-set thermoreversible gels. MCC is manufactured using microfiltration of milk in order to concentrate the casein protein (without precipitation) and remove whey protein, lactose and soluble minerals (Schuck *et al*., 1994). Nutritionally, MCC is much higher in minerals (especially calcium and phosphorous) than NaCas, and, functionally, it generates more opaque gels. MCC will form a thermoreversible gel at low temperatures, and on subsequent 'melting', should not display any alteration in functionality compared to the initial liquid (U.S. Dairy Export Council, 2015). Amelia and Barbano (2003) confirmed the thermoreversible behaviour of MCC by using 18% MCC held in a suspension. A gel was formed when the solution is cooled to 4°C, but reverted back to a liquid when heated from 4 to >22°C. Lu *et al*. (2015) also studied the thermoreversible properties of MCC, and showed that MCC formed a cold-set gel at ≤38°C at 23% protein concentration.

Daffner *et al*. (2021b) studied the effect of a series of factors on the rate of aggregation of cold-acidified casein (MCC)-whey protein (WPI) suspensions, which the authors suggest to be directly correlated with printability. They determined that samples with an aggregation rate above the chosen threshold of 250 Pa/10 K displayed adequate shape retention and gel-firmness and were therefore, deemed suitable for printing. Daffner *et al*. (2021b) concluded that samples with higher overall protein concentration (10 % MCC and 2.5 % WPI) and lower cold-acidification pH values (pH 4.8) contributed to faster aggregation rates. In an additional study, Daffner *et al*. (2021a) explored the effect of adding dairy-fat in the form of cream (up to 5 % addition) to a casein (8 % MCC)-whey protein (2 % WPI) suspension. Protein-fat formulations formed solid-like structures suitable for printing when heated initially at higher pH (pH 6.9/7.1), followed by cold-acidification to a lower pH (pH 4.8/5.0). The authors contributed this phenomenon to a decrease in the amount of κ-casein on the surface of the casein micelle, which in turn is attached to the milk fat globule,

therefore, resulting in lower steric forces and allowing for a higher incidence of aggregation (Daffner *et al*., 2021a).

Cheese has also been reported to be suited to 3D printing applications, with mozzarella or burrata cheese being printed on pizzas (Garfield, 2016), for example, and processed cheese or mascarpone being printed into various shapes (Le Tohic *et al*., 2018). Certain cheeses may be more appropriate for 3D printing than others, which may depend on factors such as their composition (e.g., fat, protein, lactose) and functional properties (e.g., meltability, flowability). However, at the time of writing, published research on the 3D printing of different varieties of cheeses is solely limited to processed cheese (Le Tohic *et al*., 2018; Lipton *et al*., 2010).

Researchers at University College Cork, Ireland, first identified 3D printing potential in processed cheese in 2015 when trialling a variety of commercially manufactured cheeses that may be suitable for the printing process (Le Tohic *et al*., 2018). Processed cheese is manufactured using methods that in some way mimic the 3D printing technique, where ingredients are mixed at a high temperature under vacuum and then moulded into a cylindrical shape. The extrusion mechanism was modified from a traditional 3D printer designed to print plastic such as polylactic acid. Processed cheese was shown in preliminary tests to be suitably flowable to be distributed *via* a narrow nozzle, but also sufficiently viscous to set into a layered structure during printing. The effect of speed of printing on the structure and textural properties of the cheese was studied, whereby samples were printed at flow rates of 4 or 12 mL/min, respectively. These were compared against an untreated processed cheese and melted (but not extruded) cheese sample to differentiate between the changes in properties. There was not a comparable difference between the two extruded cheeses in texture and softness; however, there was a more significant difference when the structures of the cheese samples were observed under a confocal laser scanning microscope. The untreated cheese showed a well-characterised and uniform distribution of round fat droplets within the continuous protein phase, whereas the melted cheese displays fat droplets which are visibly larger than those of the untreated cheese, presumably due to the fat coalescing on melting. In contrast, the more slowly printed cheese presents a discontinuous protein phase and random fat regions varying in size and shape. Cheese printed at 12 ml/min resulted in smaller and more uniform lipid fat droplets than that of cheese printed at 4 ml/min. The higher shear rate being utilised during faster printing was suggested to have contributed to greater fat globule disruption, resulting in this particular type of structure formation. Consequently, the disruption of

fat globules confirms the rheological and textural data, where the interruption of the protein and fat phase produced a softer, more easily meltable cheese. Another comparable difference found was that the printed cheese was notably darker in colour with a lightness value, L*, of 80.8 for high-speed printed cheese than the untreated cheese sample with an L* value of 83.3. This is also due to the modification to the fat globule size and morphology and non-uniform protein network formation, where the cheese printed at a faster rate was slightly darker as a consequence of the presence of larger fat globules than cheese printed at a slower rate (Le Tohic *et al.*, 2018).

As evident from the growing number of research studies involving printable dairy-based formulations published since 2017, there is increased interest in the development of 3D-printed dairy ingredient snack concepts with a range of compositions and applications. However, there is still an abundance of opportunity for growth in this area of research, especially with regards to understanding the effect of various factors (such as pH, structural protein content, calcium supplementation or chelation etc.) on printability. These factors, and more, will be expanded on in detail in Chapter 5 and Chapter 6 involving processed cheese formulations and highly concentrated micellar casein suspensions, respectively.

1.5 Analytical tools for 3D-printed dairy applications

To print effectively, an understanding of the impact of heating/cooling and shearing forces on the macro- and microstructure within a food product is vital. Food formulations can be constructed using this information to add and subtract or manipulate the ingredients to suit the application. Using analytical techniques to examine the foodstuff is essential to gathering this information such as gel strength, textural properties (e.g., hardness, stickiness), colour, porosity, melting characteristics and viscosity. Analysis of 3D-printed food material may be divided into pre-printing (e.g., melting, gelation and powder properties) and post-printing (e.g., texture profile analysis [TPA], colour, microscopy, X-rays) testing. Some tools used to measure these parameters will be discussed in this section, beginning with rheology.

Understanding the rheological and mechanical properties of various dairy products is important in the design of flow processes in 3D printing (Foegeding *et al.*, 2011). Rheometers are used to characterize the flow and deformation behaviour of a food material. They can apply oscillatory as well as rotational motion to the spindles, which can quantitatively measure parameters such as shear rate, percent deformation and shear stress. The gelation point of a dairy ingredient-based gel can be measured using

small-amplitude oscillatory rheometry, for example. The solution is subjected to sinusoidal deformation or waves using a specific head geometry (e.g., cone and plate, parallel plate) to suit the viscosity of the solution. The gel point is defined as the point at which the storage modulus (G') becomes higher than the loss modulus (G"), indicating that the fluid has transitioned from fluid-like flow behaviour to solid elastic behaviour (Phillips *et al*., 1994).

Rheometers can also apply large step changes in stress and strain to determine viscoelastic properties as well as flow properties (Metzger, 2006). This can become useful in comparing flow properties of various dairy-based food ingredients and, therefore, determining optimum flow conditions suitable for printing that food type. Rheometers also tend to offer a wide range of sampling accessories, such as temperature control units to study materials under a wide range of conditions, which can simulate thermal processing or printing conditions over time (ATA Scientific Instruments, 2010). This may be applicable to printing in examining how food will flow through a nozzle, whether it will increase in viscosity over time with constant temperature and shear rate or whether the structure will be disrupted and become more liquid as more shear is applied.

In contrast, a rotational viscometer has a far more limited range of applications, where the equipment only measures viscosity (η) of various liquid/ semisolid foods by rotating the spindle in one direction. The rotational or shear rate can be increased or decreased depending on the initial viscosity of the food. For example, in a chocolate printer used in a study by Mantihal *et al*. (2017), the chocolate was melted in a syringe whilst being mixed using a rotating auger within the reservoir. The temperature of the food can be altered or maintained through the use of a heating and cooling jacket surrounding the sample container. This allows the sample to be subject to temperature sweeps, mimicking the printing process, where the food may be heated in the syringe or reservoir, and cooled on a pre-chilled printer stage (ATA Scientific Instruments, 2010).

Melting properties are important attributes to measure, especially in the context of dairy-based foods such as cheese. For example, a Discovery Hybrid Rheometer (TA Instruments, United States) was used in a processed cheese 3D printing study by Le Tohic *et al*. (2018) in order to assess the flow behaviour and melting properties of cheese samples in the linear viscoelastic region (LVR). Using a dynamic temperature sweep test and a stainless steel parallel plate attachment, the LVR was determined

using a dynamic sweep test using the method described by Prow and Metzger, (2005). It was found that the ratio of G' to G", called the loss tangent, or tan (δ), represents the temperature at which the food material yields to a solid-to-liquid transition (Foegeding *et al*., 2011). Another method of determining progression in changes in texture is through the use of Low Frequency-Nuclear Magnetic Resonance (LF-NMR). Some studies have used this technology to measure water distribution in dairy-based gels intended for printing (Liu *et al*., 2018b; Liu *et al*., 2019). LF-NMR, a rapid, non-destructive method of analysis, works on the principle of magnetic fields where the resonant frequencies of the molecules within the sample are measured and converted into transverse relaxation time (T_2). Formulations with low T_2 values show little to no movement of moisture within the sample, implying a more solid structure; conversely, higher T_2 values indicate a more fluid-like structure with a high degree of free water (Liu *et al*., 2022; Yang *et al*., 2018a). This is useful information in terms of printing to determine the most suitable viscosity for a dairy product, both for extrusion without any issues of clogging, but also for determining the point where the product gets too fluid-like or 'runny' and is uncontrollable during the printing process (Lanaro *et al*., 2017).

Computational Fluid Dynamics (CFD) is a relatively new technique that has been used to quantify and measure the printability of food materials including gels made from rice, pseudo-cereals, beans and seeds (Guo *et al*., 2020). To the authors' knowledge, this technique has not been used in evaluating dairy-based printing applications, but an assumption could be made that this method would be suitable for any flowable semi-solid or gel-like food material. Using CFD simulations, Guo *et al*. (2020) were able to determine the distribution of shear rate, viscosity and pressure of a variety of plant-based gels during a simulated extrusion process. The authors of this study concluded that this method of analysis produced results bearing a more realistic resemblance of the flow behaviour than those which are obtained *via* more traditional rheological methods (e.g., flow and oscillation testing).

In Powder Bed Printing (PBP) applications, powder properties such as particle size, flowability and wettability are important to consider. Holland *et al*. (2018) suggests that a mixture of small and larger particles is the most suitable for use in 3D printing. Smaller particles will fill the gaps created between larger particles, therefore reducing unwanted porosity in the finished product. Particle size can be measured using a laser diffraction particle size analyser, which measures the angular variation of light intensity of a laser beam as it passes through a dispersed particulate sample. The sizes

of the particles are inversely related to the measurement of the angles of light scattered (Levoguer, 2013).

Flowability also plays an important role in PBP, especially when the powder must be spread evenly, allowing the build-up of thin layers. Low flowability (i.e., restricted movement of the powder) will lead to insufficient recoating of the previous layer, therefore creating weak and porous structures (Berretta *et al*., 2014). Yang and Evans (2007) showed that high particle sphericity improves powder flowability. The authors also concluded that coarse powders flow more easily than fine powders, and factors such as high temperature and humidity affected the flow negatively. Flowability of powders can be analysed using shear cell testers such as the Brookfield Powder Flow Tester, where a sample is sheared and compressed simultaneously using whilst measuring the torque resistance of the powder (Salehi *et al*., 2017).

Wettability of particles is also an important physicochemical property, especially in LB printing technology. The volume of binder dispensed onto the powder bed and the amount of binder absorbed by the particles dictates the quality of resolution and structural properties of the print. Low wettability of fine particles results in powder bed instability, and high wettability combined with low flowability may cause the particles to clump together, negatively affecting the quality of the print (Shirazi *et al*., 2015). The Washburn method is an analytical technique that can be used to determine wettability of a powder, where a column with powder lowered into contact with a liquid and the uptake of liquid are monitored as a function of time. Displacement or mass uptake is plotted against time to obtain the wettability (Thakker *et al*., 2013).

Accuracy and consistency of the print are important selling points of 3D printing. It is essential to be able to evaluate the performance and precision of the print in order to avoid any errors or discrepancies between samples. Lanaro *et al*. (2017) described a line test in which the calculated amount of extruded chocolate could be compared to the actual amount of extruded chocolate. Using a calculated flow equation integrating syringe plunger displacement, nozzle diameter and syringe internal diameter values, the amount of extrudate was made to equal the inner diameter of the needle. To conduct the line test, the printer was programmed to extrude chocolate in a back-and-forth motion, where the diameter of the line could be measured. To do this, image analysis software was used and, as the food was extruded, the predicted diameter of chocolate extruded could be compared to the actual value, which is a useful calibration and optimisation technique in 3D printing.

Texture analysis is the collective term for the destructive compression tests: TPA and uniaxial compression testing. TPA is an instrumental method of measuring critical sensory attributes (Steffe, 1996). TPA uses two uniaxial compressions and results in a plot from which parameters such as degree of firmness, cohesiveness, resilience, springiness and adhesiveness can be calculated (Chen and Opara, 2013). Uniaxial compression, however, works by only compressing the food sample once in one single direction. This method yields values for Young's modulus, fracture stress/strain, work of fracture and hardness (Hamann *et al*., 2006). Uniaxial compression can also be used to test structural stability and softness of a food substance post-printing, where a flat-headed probe attachment is used to compress the food to a certain point and then released. The force needed to compress the sample to a certain height is measured, where a larger force equals a harder, more solid food, and a lesser force equals softer, more elastic food. For example, Le Tohic *et al*. (2018) used this method to compare hardness between printed substances versus non-printed processed cheese cylinders. It was found that the printed processed cheese required less force than non-printed cheese, leading to the conclusion that printed cheese was softer than the control.

This technology can also be used to determine optimal geometries to achieve certain sensory characteristics in printed chocolate (Mantihal *et al*., 2017). Snap quality is an important parameter in chocolate manufacture, with a stronger snap often being more desired, as it can suggest a higher-quality chocolate. In the above study, three different structural supports were tested for a printed hexagonal prism-shaped chocolate: a crossed biaxial support, a parallel support and no support present, as seen below in Fig. 1.4. It was found that the chocolate design with cross-support required a higher force to break the chocolate structure, indicating a better snap quality than the other designs.

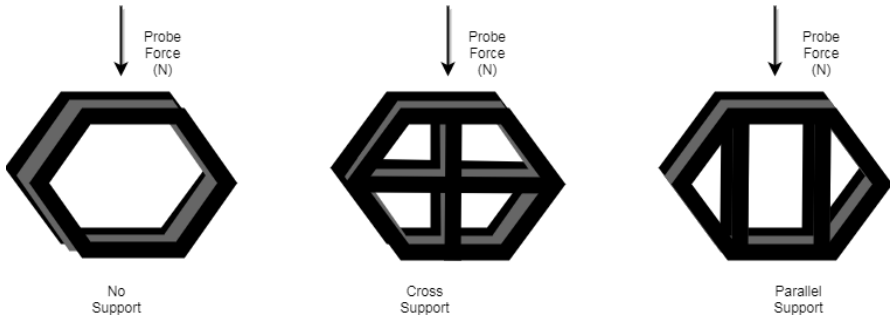

Fig. 1.4. Three different structural designs of printed chocolates analysed for snap quality using uniaxial compression (adapted from Mantihal *et al*., 2017).

Another important group of methods that can be helpful in analysing 3D-printed products is microscopy. Microscopy allows the observation of the types of aggregates, location of non-gelling components such as fat, structure porosity and homogeneity of the gel. Confocal scanning laser microscopy (CSLM) is a more recent development in microscopy technology, which is relatively non-invasive to the sample. The sample, in liquid, solid or powder format, is optically sectioned into x-, y- and z-axes, which allows the viewer to observe each layer individually (Sozer, 2016). Foegeding (2007) used CSLM to view the effect of varying the protein content of gels on their fracture strength. The authors found that gels containing 6% protein had a lower fracture stress, higher fracture strain and a porous structure, and protein clusters rotate during deformation. In comparison, gels containing 12% protein had higher fracture stress and lower fracture strain, and their microstructure showed considerably less change in deformation. Le Tohic *et al*. (2018) also used CSLM, in combination with staining, to observe microstructural changes between printed processed cheese and untreated processed cheese. CSLM enables users to stain particular items of interest (i.e., proteins, phospholipids, fat globules) to differentiate between different components within the structure (Keogh and Auty, 1998). Another form of microscopy, Scanning Electron Microscopy (SEM), has also been used by others to compare differences in surface-level microstructure which can complement observations made at a visual level of printed structures. For example, Liu *et al*. (2018b) showed that a cross-section of printed MPC had a loose and porous network of protein particles, suggesting that this paste was not fully hydrated, whereas cross-sections of samples containing WPI were more homogenous in appearance and did not contain any visible powder particles, indicating a more hydrated sample with enhanced flow properties.

Porosity can influence flavour release, texture and degree of syneresis. This value can be measured using X-ray microtomography. X-ray microtomography is a powerful non-destructive technique which provides direct images of internal structures and is typically used to assess microstructural details (Babin *et al*., 2007). X-ray microtomography can be used to determine the air bubble/ pocket size, distribution, orientation and their position within the food matrix. Defects such as altered support structures and abnormal porosities can be quantified through the use of this technology (Guillermic *et al*., 2018). Such defects can be caused by undesirable air pockets created within the food matrix which can cause gaps during printing travel across the print bed. The use of X-ray microtomography could also be a potentially useful tool

when combined with textural analysis data and/or a trained tasting panel to identify any correlation between porosity and flavour intensity or texture.

Applying a vacuum to minimise air inclusion can remedy this issue (Liu *et al*., 2018a). Kuo and Lee (2014) suggested that a more porous structure enabled a maximum rate of sodium release during initial gel compression, and flavour released at a faster rate. However, porous gels can also lead to greater degrees of syneresis which is typically undesirable in certain products such as yoghurt, but desirable in cheese (Harwalkar and Kalab, 1986). X-ray microtomography has also been used in non-3D-printed food applications by Laverse *et al*. (2011a, b) e.g., cream cheese and yoghurt. In these cases, this technology was used to determine an accurate percentage of the volume of the fat present and also the spatial distribution of those fat globules. Similar to the processed cheese study by Le Tohic *et al*. (2018), this technology is useful in understanding how 3D printing might structurally alter morphology and distribution of fat globules and what implications this might have on texture.

Shape stability during post-printing processes are also important parameters to consider. The vast majority of 3D food printers are limited to single-step processes, and any cooking/baking steps require an additional separate step. Lipton *et al*. (2015) considered the possible implications of combining the printing and cooking process a single step on the food structure. In order to compare deformation and shape consistency, the authors measured the height, length and width of a 3D-printed cube-shaped dessert directly after printing and again after baking. It was found that an increase in butter quantity in the formulation led to a decrease in shape stability. The mixture became easily extrudable when butter quantities were increased by 150%; however, it was unable to retain its shape at room temperature.

Determining the colour change of dairy-based foods before and after printing can be meaningful. At times, this can be desirable if Maillard browning is required to achieve a caramel-like flavour; however, in many other applications, a change in colour may not be desirable. Colour measurements of food samples can be assessed using a Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) or similar colorimeter. The equipment must be calibrated using a referenced plate, and then Hunter L*, a* and b* values can be recorded in triplicate at the surface of the food samples. The L-value describes lightness and varies from 0 (black) to 100 (white). The magnitude of a* and b* both vary between -300 and 299, which describe green to magenta and blue to yellow, respectively. Relative differences in L* (ΔL), a* (Δa), and b* (Δb) enable

the user to determine overall colour difference, ΔE, using a non-3D-printed sample as a reference (Le Tohic *et al*., 2018).

1.6 Research needs, challenges and opportunities

In spite of the most recent developments, the majority of technologies for 3D food printing are still undergoing research and in the growth phase. This is due to the challenges that researchers face in terms of creating a technology that will incorporate traditional methods of cooking and baking whilst modifying these foods to suit the 3D printing process and still remain appetising. Consumer acceptance of new technologies is another factor that creates obstacles for the emergence of 3D printing. Other considerations in terms of research needs, challenges and opportunities include how 3D printing will affect food supply chains, whether the food is considered safe and whether 3D printing will help or hinder our current relationship with food.

1.6.1 Safety and acceptance

Food safety is one of the most important aspects of food manufacture. Due to 3D printing being a novel food technology, it will undoubtedly be met with a reasonable amount of scepticism by the general public. The name '3D printing' is another aspect that generates confusion amongst the general public. People may associate printing with ink and high level of processing if care is not taken to introduce the technology with due transparency and clarity regarding its operating principle (Brunner *et al*., 2018). Consumers may also view novel food technologies with suspicion (Popa and Popa, 2012); however, not all new food technologies and food trigger the same 'neophobia'. Both the technology itself and an individual's own experience and knowledge background play a determining role in the evaluation process. Repeated exposure to different types of novel food and visual representations of them can also contribute to making new processing techniques and new food more familiar to consumers, which may positively affect consumer attitudes (Brunner *et al*., 2018; Caulier *et al*., 2020).

The importance of 'naturalness' is also a key implication of how 3D printers will be accepted by consumers. Consumers may have conflicting interests in that they may view the potential convenience and creativity associated with 3D printing positively, but may also link the technology with undesirable, overly processed and unhealthy foods (Román *et al*., 2017). A qualitative study conducted online in Australia (n = 30) reported that individuals expressed concern about the safety of 3D-printed foods where it was believed that chemicals and additives were added to these foods in order

for the food to from 3D-structures. According to certain participants, 3D-printed foods were seen as meddling with nature and unnecessary (Lupton and Turner, 2018). These comments provide evidence that consumers require a multi-faceted approach in addressing their primary concerns regarding the novel technology. Providing transparent information about the printing process to reassure consumers of its safety has been shown to be ineffective in diminishing novel food technology neophobia (Brunner *et*., 2018); however, linking 3D food printing with already familiar and accepted food technologies, such as extrusion or moulding, may enhance consumer perceptions of the novel technology (Bruhn, 2007; Lupton, 2017).

Nutrition-focused 3D food printers are being developed (Natural Machines, 2016), although much coverage has been given to food printers focused on convenience food products (Garfield, 2016) and luxury food items (Choc Edge, 2022). Some 3D food printing companies, like Ohio-based Beehex, utilise the novel technology in producing snacks which can be personalised to an individual's flavour and texture preferences, but also, to their nutritional needs. For example, if a customer requires ingredients to support joint care and maintain their energy levels during the day, they may select a snack bar which is formulated with a high-protein base, contain healthy fat sources in the form of peanut butter and flax seeds, and incorporate glucosamine to support joint health (Beehex, 2022). If consumers were to associate 3D-printed foods with nutritional foods that enhance their specific health goals, this may improve consumers' perception of the technology (Caulier *et al*., 2020).

1.6.2 Research Challenges

In order for 3D printing technology to be feasible in industry and for everyday use, the individual food formulations must have suitable rheological characteristics for printing, such as a flowable consistency that will maintain a constant viscosity during printing (Dankar *et al.*, 2018). Other challenges associated with the practicalities of use include speed of printing, cost of equipment and materials and, in most cases, a separate cooking or baking step often being required as an additional step after the printing process (Sun *et al*., 2015).

At the time of writing, there are a limited number of studies carried out on the fundamental principles of how the printing process affects dairy food microstructures. Food matrix interactions at a microscopic level are potentially important in order to understand physical and rheological behaviour and textural and sensorial traits of foods (Aguilera, 2005). Further research into the effects of 3D printing on both

properties and texture is needed to allow greater diversification in more application areas. The lack of understanding of how structural change occurs during printing, which can influence printing accuracy and shape stability, is one of the biggest challenges in 3D food printing to date. For example, a study by Lipton *et al*. (2010) showed that, when a dessert product with reduced fat was baked after printing, this led to increased shape stability after baking, whereas an increase in egg yolk concentration improved width/length stability but lowered height stability following baking.

It was also reported by Lille *et al*. (2018) that a high yield stress was necessary to achieve acceptable shape stability post-printing, whilst a high solids content (samples with 60% SSMP) was shown to be most effective in retaining shape and structure in post-processing by oven drying. This also shows the added challenge of compatibility of 3D printing technology with traditional food processing methods such as baking (Lille *et al*., 2018). The ideal situation is to incorporate cooking (e.g., baking, steaming) while the product is being printed to eliminate two-step print-then-cook processes. To the authors' knowledge, there are no such printers available on the market at present. However, a study by Blutlinger *et al*. (2021) has demonstrated that lasers of differing wavelengths can be used to cook thin layers of chicken meat; however, it must be noted that these developments are in the preliminary stages and further research must be carried out to ensure the safety and feasibility of this technique.

Furthermore, there are additional technological challenges to tackle, as illustrated by the printing of batters, where the food material is strongly dependent on oscillations during its pumping process to maintain viscosity, which will consequently have an impact on the final quality of the printing (Millen, 2012). Three-dimensional printing dairy-based products could benefit from the addition of novel additives, such as bacterial nanocellulose (BNC), a product of fermented waste coconut water, to improve shape stability and structural integrity of the food matrix. BNC could potentially be suited to 3D printing due to its own natural 3D nanofibrillar arrangement of pure cellulosic fibres, which will enable a stronger and more enforced printed food structure (Gama *et al*., 2016). Other ingredient combinations have been used to create a multitude of texture varieties in order to recreate the texture that can be lost through the printing process. Cohen *et al*. (2009) printed various combinations of hydrocolloids to develop a certain 'mouth-feel matrix', measuring the strength and texture spectrum of a food material. This study showed that pure xanthan gum and gelatin added independently to formulations had more of an effect on the strength of

gel, rather than smoothness, with 0.5% gelatin resulting in a milk-like texture and 4% gelatin producing a mushroom-like texture. When two hydrocolloids were combined, the degree of textural smoothness was affected, where 1% gelatin and 4% xanthan gum brought about a risotto-like texture, and 1% gelatin and 8% xanthan gum created a tomato-like texture.

1.6.3 Industrial advantages and disadvantages

Other benefits predicted for 3D printing of food materials include flexibility, configurability and high-material-use efficiency (Hopkinson *et al*., 2006). The flexibility of 3D printing technology will enable the use of alternative dairy-based food ingredients in creating improved products with respect to nutritional content and health benefits. Broadly speaking, enterprises have been interested in the environmental benefits of 3D printing (SPI Laser LTD, 2017). This might apply more notably to the dairy industry, as it falls under increasing scrutiny and increased concern regarding its sustainability (Von Keyserlingk *et al*., 2013). Three-dimensional printing would potentially allow goods to be printed and sold close to their point of consumption. The novel technology presents an opportunity to 'reshape' the manufacturing supply chain to produce cheaper products through a reduction in the number of stages in the traditional supply chain (Berman, 2012). Additive manufacturing technology offers the opportunity to redesign products and with fewer components. The combined effect is the reduction in the need for storage, transportation and packaging (Gebler *et al*., 2014).

Several researchers have investigated the use of 3D printing in the spare parts supply chain, where printers would have the ability to print their own replacement parts and therefore reduce manufacturing downtime. According to Siddiqui *et al*. (2016), 3D printing is a viable option in printing parts for food processing operations, for example, feed spacers for membrane filtration technology, which is commonly used in to separate specific particles (e.g., fat, lactose, proteins, bacteria, salts) from milk depending on the pore sized used (Mercier-Bouchard *et al*., 2017). Feed channel spacers are placed on top of the filter membrane to facilitate feed flow and retentate recovery while promoting turbulence. Three-dimensional printing technology is considered a suitable tool in rapid, low-cost production of spacers with a thin and complex geometry design that may improve cleanability (Siddiqui *et al*., 2016). However, 3D food printing also has practical limitations. In the case of FDM printing, food materials must be converted to a paste, for which not all foods are suitable

candidates (Dankar *et al*., 2018). This can be due to potentially undesirable changes in texture or taste or negative interactions with other food contained within the paste (e.g., syneresis, browning, off-flavours). Pastes might also be limited by their shelf-life due to their high moisture content leading to a possible microbial risk. According to Lipton *et al*. (2015), regulatory agencies, mechanical engineers, chemists and other experts will have to collaborate to ensure that the right materials, processes and standards are in place.

Speed of printing is a common concern amongst industrial companies considering implementing the technology in their manufacturing lines. Printing speed has improved in recent years but has yet to reach speeds capable of efficient production standards. To put this into context, the Choc Creator chocolate 3D printer is capable of printing speeds of 20 mm/s (Choc Edge, 2022). Choc Edge (2022) states that, to create a design which is up to 3.75 cm in height and 4 cm in width/length, will take between 20 and 45 min, depending on intricacy of the print.

One other final concept that is moving 3D printing a step further in terms of creativity and innovation is the term '4D printing'. This term is relatively new and much less developed than 3D printing. Wang *et al*. (2017) have introduced this concept through their experiments with pasta, where the initially flat-shaped pasta will twist and curl up in a unique pattern when hydrated and/or heated. The authors took 3D-printed edible films made of common food materials such as protein, cellulose or starch in order to achieve this '4D' effect. How such an effect could be achieved using dairy-based 3D printing is an interesting question.

Overall, 3D printing is an exciting technology at present, presenting researchers with many opportunities and challenges. Three-dimensional printing has captured the imaginations and attention of researchers, businesses and citizens. Successful exploitation of the diverse functional and nutritional properties of dairy ingredients could be an important contributor to progress in the field of 3D food printing.

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THESIS HYPOTHESES

The experimental work in this thesis is multidisciplinary in nature and can be divided into three key elements: engineering, food science and consumer science. In terms of the overall aims of the work, the experimental studies could be condensed into the following hypotheses:

- A temperature-controlled syringe-based feed delivery system can be implemented on a modified traditional plastic 3D printer to 3D-print dairy-based foods;
- A peristaltic pump feed delivery system will be suitable (i.e., extrude material in a manner that will encourage stable and layered structures) for printing multiple feeds on a Delta-type printer;
- Physico-chemical factors such as pH and calcium supplementation can be used to modify and customise the printability (e.g., printing precision) and functionality (e.g., melting, dissolution, solubility etc.) of dairy-based food ingredients such as processed cheese and micellar casein concentrate;
- A comprehensive and structured online survey can be applied (based on preliminary exploratory qualitative analysis) in order to establish a model which captures > 40% of the variance in the data gathered to be considered a robust and comparable data set to other similar studies in this area (e.g., Brunner *et al*., 2018 – model explains 42 % of the variance in consumer attitudes towards 3D food printing).

The following experimental chapters aim to systematically explore these hypotheses and provide convincing resolutions to each proposed research problem. By providing answers to these questions, this goal of this thesis is to allow readers and interested parties to gain insight into the unique solutions 3D food printing has to offer. Individuals can use this thesis in part or as a whole to learn more about 3D food printing and how the three disciplines ultimately interact and complement each other, to give a comprehensive overview of 3D food printing applications, especially as applied to dairy systems.

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CHAPTER 2

3D food printer customisation:

Investigation into design rationale and process variables

Megan M. Ross

Declaration

This chapter was written by author MMR and reviewed by their co-authors, ALK, SVC, and AM. MMR co-designed the study, carried out the 3D-printing experiments, TPA and viscosity measurements and analysed the data. Alan P. Morrison, Michael O'Shea and MMR co-designed and implemented the modifications made for the 3D printers. A version of this chapter is being prepared for submission to *International Journal of Gastronomy and Food Science*.

Abstract

Three-dimensional (3D) food printers are becoming more available on the commercial market. At present, these 3D food printers generally operate using the mechanism of syringe-based extrusion. However, for the purpose of comparison between the functionality of two printer types (Cartesian and Delta) which have differing delivery systems, two traditional plastic printers were converted into 3D food printers and fitted with syringe-based and peristaltic pump-driven delivery systems respectively. The rationale for the design processes involved for both printers is discussed. A selection of printing parameters (e.g., nozzle diameter, print speed, printing temperature etc.) were chosen to investigate their effect on printability of two shear-thinning food materials (cream cheese and ketchup). Understanding the relationship between these process variables forms the basis for additional work on more complex food systems.

2.1 Introduction

Three-dimensional (3D) printing is the process of forming physical 3D objects from a digital model. The working principle of a 3D printer is analogous to that of a more conventional 2D printer where the plane of printing is extended layer by layer in the vertical dimension. Printing 3D objects relies on using alternative materials (e.g., plastic) *in lieu* of ink to form successive layers of material on a support bed. 3D printing is a relatively new technology, which began to be developed approximately forty years ago in the early 1980s. The first 3D printer was a primitive form of what is known as today as 'laser sintering' (Yanar *et al*., 2020). Chuck Hull, the father of the first commercialised 3D printer, described the technology in the patent 'Apparatus for production of three-dimensional objects by stereolithography' (Hull, 1986). 3D printing has since spread across many fields of industry, most notably in engineering, architecture and medical technology (Nadagouda *et al*., 2020; Su and Al'Aref, 2018).

In more recent times, 3D printing has progressively appeared in the realm of gastronomy and food manufacture. 3D food printers are capable of printing many food substances (e.g., meat and fruit and vegetable purées) however, there is no universal printer available to date which can 3D-print all types of foods. Though there are commercially-available 3D food printers on the market at present, other researchers have chosen to design and modify traditional 3D printers to be suitable to print food materials of their choosing. The majority of these designs are FDM-style printers, where the basic design utilises a syringe-based system which allows food materials to be mechanically extruded from the syringe nozzle. In the case of the 3D food printer design used by Lille *et al*. (2018), the extrusion mechanism is based on compressed air which allows the user to adjust the system pressure depending on the desired flow-rate and rheological characteristics of the printed foods. For example, high viscosity foods with relatively little flowability may require more pressure to achieve the same flow-rate as foods which flow naturally under gravity. Plunger-based syringe mechanisms, similar to those used by Chen *et al*. (2019) and Ross *et al*. (2021), operate using stepper motors to drive gears or a drive shaft which mechanically rotates a helical screw attached to a plunger. While plunger and compressed-air systems generally print in limited batch volumes, augur or screw-type systems have also been implemented by others to deliver a continuous feed of food material to the extrusion nozzle (Liu *et al*., 2018). As far as the authors' are aware, the use of peristaltic pumps to drive continuous extrusion in a 3D food printing application is a novel technique

which has not been implemented before in literature. Peristaltic pumps operate on the principle of positive displacement, which allows incremental movements of fluid along flexible piping (Klespitz and Kovács, 2014).

Temperature control is a feature which has been increasingly utilised in the printing of temperature-dependent food materials, for example, processed cheese (Ross *et al*., 2021) or thermoreversible dairy-based gels (Daffner *et al*., 2021). Daffner *et al*. (2021) modified a Creality Ender 3 3D printer which was retrofitted with a syringe with a double-walled cooling jacket. This temperature control mechanism operates in a similar way to that of Ross *et al*. (2021) to maintain temperature of the food material within the syringe barrel. A screw plunger was driven by an attached stepper motor which allowed the printer to extrude food materials from the syringe nozzle. The added cooling jacket allows the materials within the syringe to maintain a low temperature, which can be beneficial depending on the gelling mechanism of the intended printed food; in this case, concentrated milk proteins were acidified with citric acid at 2°C (i.e., cold-acidification). The cooling jacket was connected to a water bath which was set to the desired temperature, so it would be assumed that the syringe could also be heated to warmer temperatures, which might be beneficial for food materials requiring higher temperatures to flow.

Printability has become a term that is commonly used by others to describe the characteristics of food materials, however, there is no universally accepted definition for the term 'printability'. A basic definition of printability can be stipulated as being the capacity of the printing material to flow uninhibited during extrusion, yet form a self-supporting 3D structure once that stress is removed (Guo *et al*., 2019; Liu *et al*., 2018; Ouyang *et al*., 2016). A review by Wilms *et al*. (2021) listed the various rheological and/or processing parameters that other authors have used to characterise printability, such as critical shear rate (Kern *et al*., 2018), yield stress (Lille *et al*., 2018) and extrusion volume to print speed ratio (Lanaro *et al*., 2017). Another method commonly used by others is a comparison between model-object correspondence depending on differences in formulation or processing treatment. This is typically measured subjectively by visual comparison of images taken of each samples, however, others have utilised imaging software to measure and detect differences in dimensional measurements of the sample in comparison to those of the original model (Kim *et al*., 2018; Tan *et al*., 2020).

This chapter will be split into two sections (section A and B); in the first half, the modifications which were made to both a Cartesian and delta 3D printer are discussed in detail, followed by a comparison of a both printers and their respective advantages and disadvantages with respect to design and functionality. The second half then outlines a number of studies which examined the process variables involved in 3D printing, such as print speed, nozzle diameter, printing distance, and rheological properties of printing food materials (e.g., texture and viscosity).

Part A: 3D printer modifications

2.2 3D food printer customisation

In the following section the reasoning for the use of each 3D printer will be detailed, in addition to outlining the design modifications made to both along with their advantages and limitations.

2.2.1 Customisability

Before purchasing a 3D printer to carry out experimental work, there were a number of decisions to be considered. As mentioned previously, others have taken the route of modification and reconstruction of traditional plastic printers in order to print food (Daffner *et al*., 2021; Lille *et al*., 2018), however, commercial 3D food printers have also been used in food research (Zhu *et al*., 2019). Both options have advantages and disadvantages which must be evaluated, especially with considerations of the potential future use of the printer. A conventional 3D food printer would require little to no modifications to print food, whereas a traditional plastic 3D printer may require redesigning of the print head and heating system. However, with a pre-designed 3D food printer there is little scope for any post-manufacture alterations, which may limit the functionality of the printer. By purchasing a plastic-printing 3D printer, there was potential for a much broader range of modifications to be made to the printer, which was beneficial in terms of expanding the number of controllable variables during research. In addition to this, 3D food printers are usually much more expensive to purchase (e.g., at the time of writing the most commonly purchased 3D food printers can be purchased between €3,400 (Natural Machines, 2021) and €3,900 (byFlow, 2021) than the 3D plastic printer. To put this into context, the more expensive printer used in this study, the Rostock 301/Delta printer, cost approximately one tenth of the cost of a Foodini 3D food printer.

2.2.2 Types of FDM printers

Another key decision before purchase is the type of 3D printer. There are four main types of Fused Deposition Modelling (FDM) printers: Cartesian, delta, polar and SCARA or Selective Compliance Assembly Robot Arm (Fig. 2.1) (Manufactur3D, 2020). The most commonly used type of 3D printer is the Cartesian 3D printer, which uses the Cartesian coordinate system of x, y and z coordinates to orientate the print head and the print bed (Griffey, 2014). Driven by stepper motors, the print head has the capacity to move both left and right along the x-axis (i.e., longitudinal movement), but also upwards and downwards on the z-axis (i.e., ascent/descent movement), whereas the print bed (usually square in shape) can only move backwards and forwards on the y-axis (i.e., lateral movement). Combining these three movements along each plane allows the printer to move freely in every direction, utilizing the full area of the print bed (Fischer *et al*., 2020).

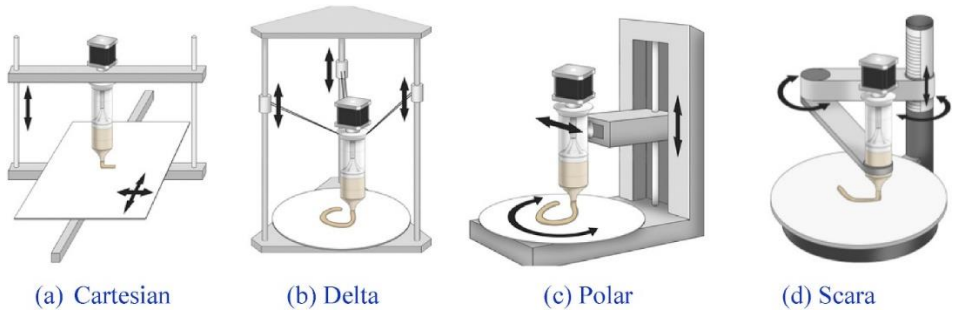

Fig. 2.1. Illustration of the various types of FDM printers available, including the primary difference between their movement paths (Obtained with permission from Sun *et al*., 2018).

The second most popular type of FDM printer, the delta, also uses the Cartesian coordinate system, but the primary difference is that the print bed (usually circular) remains stationary while the print head moves in all three planes of movement simultaneously. The print head is suspended by three arms which are connected to three upright struts which make up the x, y and z-axes (Griffey, 2014). The print head moves by changing the angle of the arms to hit a precise movement path which is calculated by the printer *via* a series of trigonometric functions (Manufactur3D, 2020). In general, Delta printers are taller than Cartesian printers due to need for longer struts to facilitate more complex movement of the print head. An increased frame height, and therefore z-axis length, naturally means that there is more build height available for 3D-printing taller models, which can be advantageous over the Cartesian printer (Manufactur3D, 2020). Delta printers are considered to be faster in printing than

conventional Cartesian printers; however, printing accuracy can often be sacrificed when printing speed is accelerated, which must be taken into account (Martel, 2017).

Polar 3D printers are not as commonly used as the Cartesian or the delta printers. The polar type of 3D printer uses the polar coordinate system, which operates in a similar vein to the Cartesian coordinate system except that, instead of using a square grid to describe coordinate points, the printer uses a circular grid (Fischer *et al*., 2020). Using the angle and radius length/separation distance of the arm and the print head rather than x, y and z-coordinates, the polar printer can move left to right and back and forth, but also rotate the arm which the print head is positioned in the z-direction. One advantage of using this type of printer is that larger objects can be printed in a smaller space (Manufactur3D, 2020). However, the main advantage of polar printers is that the printer only requires two motors rather than three, which is more energy efficient (Fischer *et al*., 2020; Martel, 2017).

Lastly, SCARA printers are more commonly seen in large-scale industrial manufacture and construction settings rather than bench-top scale type of FDM printers mentioned previously. SCARA printers are designed to move in a way that mimics human movement in order to remove the human element to repetitive processes and actions (Manufactur3D, 2020). SCARA printers offer a greater degree of freedom over other types of FDM printers, since there is no set print bed or building platform *per se*, and the printer is much more mobile than conventional FDM printers. More recently, 3D SCARA printers can be seen in construction sites for 3D printing large-scale structural items such as bridges and buildings (Manufactur3D, 2020; Martel, 2017).

2.3 Rostock 301 3D printer

After considering the various types of FDM printers available, the ‘Geeetech Rostock 301 Mix Colour FDM 3D Printer’ was selected. This printer has a delta configuration which, as outlined previously, was selected due to its ability for complex movement which was believed could be advantageous. However, what makes this 3D printer different from other printers is that it also has the capability to print up to three different coloured plastic filaments simultaneously through three Bowden tubes fed into one nozzle (Fig. 2.2). This 3D printer thus has a mixing function which enables the users to choose a ratio in which to combine three separate colours of the user’s choosing to create a gradient colour effect, or simply be able to seamlessly change between different colours during printing to create a multi-coloured printed model. In

theory, this function could potentially be converted to feed three separate ingredients and combine them before extruding from the print head nozzle, which was the primary reason for evaluating this type of printer specifically.

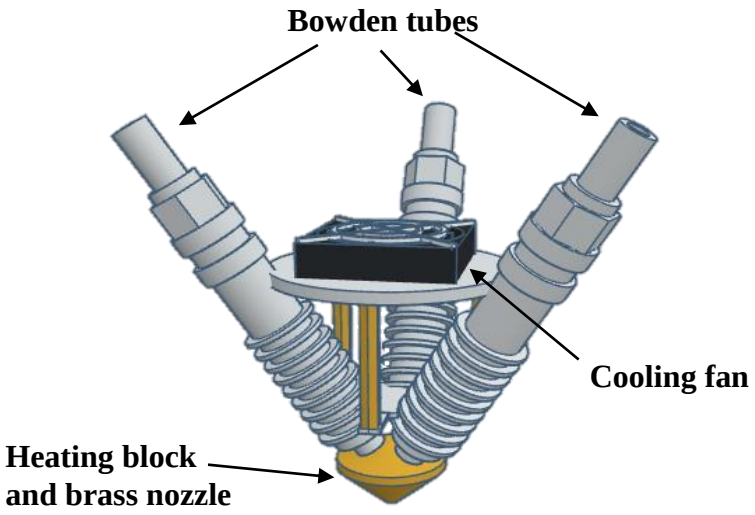

Fig. 2.2. Original Delta print head hotend and nozzle prior to modification.

2.3.1 Modifications

In order to take advantage of the mixing function of this printer, the initial idea was to create three separate streams which would connect at a terminal point before the extruder head. For this to occur, three individual holding vessels (i.e., beakers or similar) would contain the separate ingredients in liquid form (e.g., if the goal was to produce processed cheese, then one vessel could contain water with dissolved emulsifying salt, another contains molten cheese and perhaps a third container holds a liquid fat source or liquid flavouring). These vessels may be heated and stirred by placing the beakers on a heated stirring plate or stirring water bath which has been set up near the printer. The fluids are then pumped to the printer *via* silicone tubes (food-grade) which would be mechanically driven by micro-peristaltic pumps (high dosing anti-corrosion dosing pump purchased online *via* VUYBVY on Amazon.com, Washington, United States), one for each stream of food material. For the purpose of testing the feasibility of combining and printing multiple fluids using a 3D printer, only two pumps were purchased and fitted initially, with the intention of adding a third if the trial was successful. Both micro-peristaltic pumps were fixed to the top plate of the 3D printer in a specially machined stainless steel frame (Fig. 2.3A) which was secured with screws and wing nuts in each corner of the structure to ensure stability during operation.

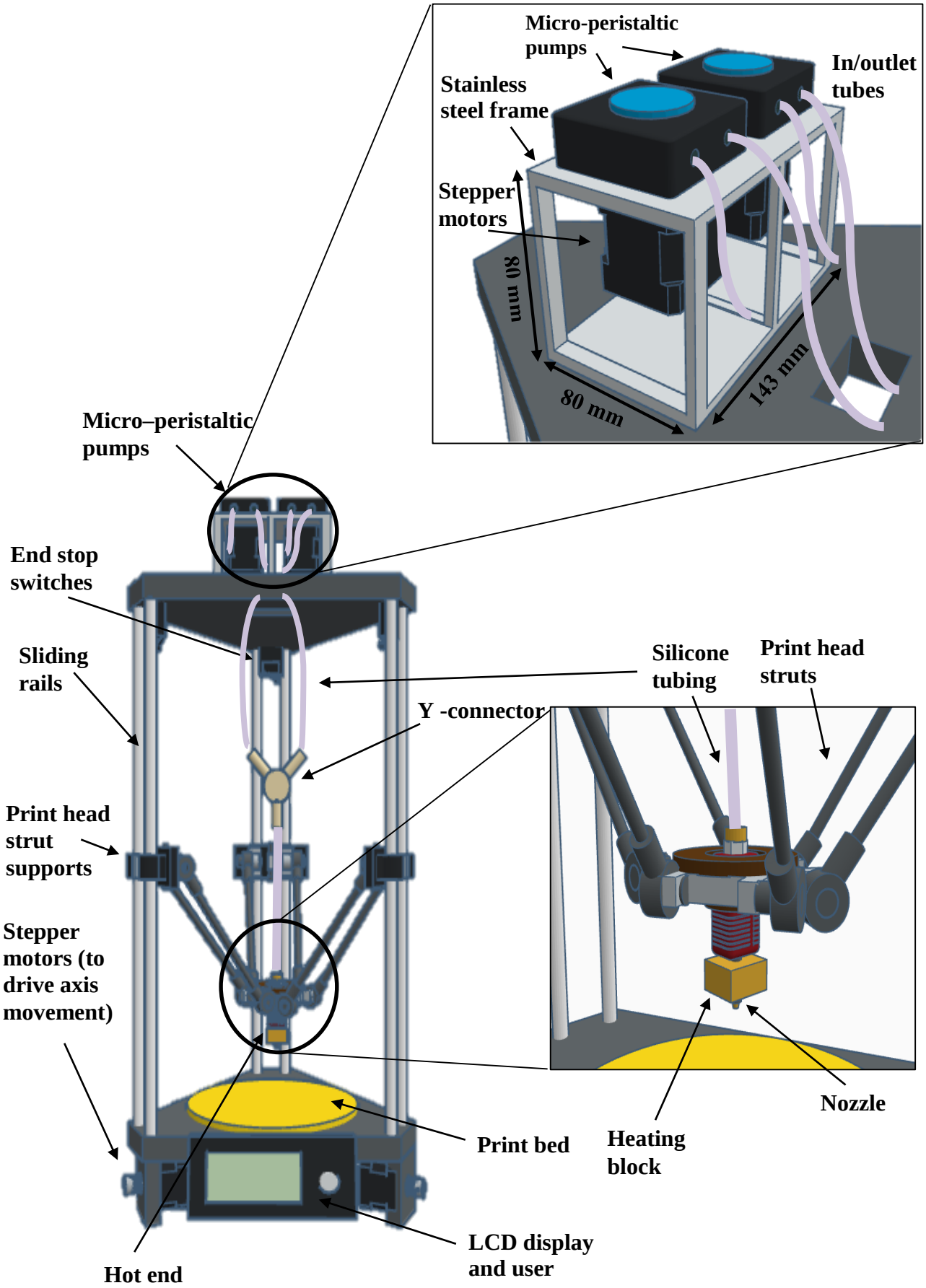

Fig. 2.3. Diagram of Delta 3D printer post-modification. (A) Close-up of micro-peristaltic pumps, stainless steel holder and silicone in/outlet tubes and (B) Close-up of modified print head

The micro-peristaltic pumps are connected to the shaft of the stepper motors (originally supplied with the 3D printer kit) and rotated at a set speed which is determined by the flow rate selected within the software of the printer (Repetier-Host, Hot-World GmbH and Co. KG). The outlet tubes from the individual peristaltic pumps are then joined together using a plastic Y-connector (Fig. 2.4) (double (Y) or triple connector can be used depending on the number of outlet tubes and feed streams) which funnels the fluids into one single stream. To accommodate the combined volume of multiple streams, the internal diameter or bore size of the length of tubing from the connector to the nozzle was increased (for more details on the effect of tubing dimensions on flow rate see Section 2.3.2). The outlet end of the larger silicone tube is attached to the printer nozzle *via* a straight brass male friction fit connector which is screwed into the printer hotend (Fig. 2.3B).

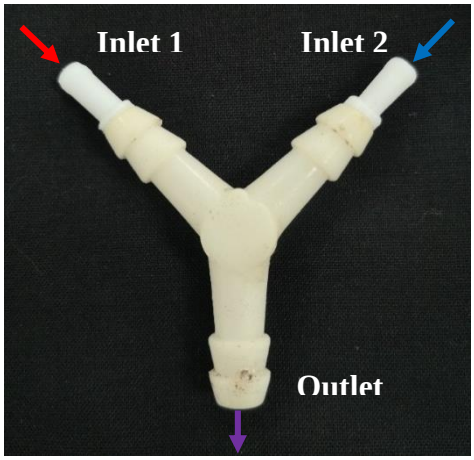

Fig. 2.4. Y-connector used to join silicone tubing from both peristaltic pumps to combine two inlet feeds.

2.3.2 Effect of material viscosity and tubing dimension on flow rate

The ability to predict and manipulate the flow rate of printing materials can be useful in determining the limitations to the types of food material you can successfully 3D-print (i.e., inability to flow/causing obstruction to extrusion). To demonstrate the effect of viscosity on the flow rate of the tubing system fitted on the modified Delta printer, the flow rate of two fluid food materials with differing viscosities were measured. The sections of tubing to be measured were categorised into three separate sections as shown in Figure 2.5: (A) tubing contained within the peristaltic pump, (B) in/outlet

tubing feeding in and out of the peristaltic pump; and (C) tubing from Y-connector to nozzle, where each section listed has a different tubing diameter (Table 2.1).

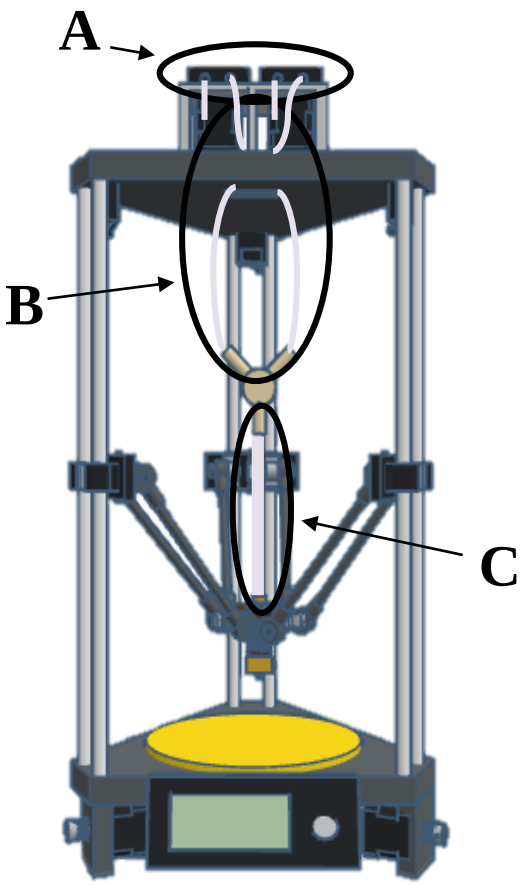

Fig. 2.5. Diagram demonstrating locations of tubing sections A (In/outlet tube), B (Peristaltic pump tubing), and C (Combined flow from Y-connector to nozzle)

Table 2.1. Flow-rate measurements for each section of tubing (A-C) outlined in Fig. 2.5. Tubing dimensions (length and radius (mm) and volume (mm^3)) and flow rate (mm^3/second) used to test the flow rates of calcium lactate (3 % w/v) and sodium alginate (1.75 % w/v) and a combination of the two formulations (in a ratio of 0.05: 0.95 respectively).

Printing material	Material viscosity (Pa.s)	Tubing section	Tubing radius (mm)	Tubing section length (mm)	Tubing section volume (mm^3)	Time (sec)	Flow rate (mm^3/sec)
Calcium Lactate (3 % w/v)	0.7	(A) Within peristaltic pump	1	100	314	1.0	308
		(B) In/outlet tubing	1.5	50	353	0.8	459
Sodium Alginate (1.75 % w/v)	3.0	(A) Within peristaltic pump	1	100	314	1.3	240
		(B) In/outlet tubing	1.5	50	353	1.1	327
Combination	4.1	(C) Y-connector to nozzle	3.2	55	1768	20	88

Sodium alginate (1.75 % w/v) and calcium lactate (3 % w/v) were chosen as the two feed materials, where both had differing viscosity values, 3.00 Pa.s and 0.74 Pa.s, respectively. Sodium alginate and calcium lactate are commonly used ingredients in molecular gastronomy to produce spherical gel beads in a process called spherification. Sodium alginate forms a gel in the presence of divalent ions, such as calcium or magnesium, *via* an ion-exchange reaction between sodium and calcium ions (Yuasa *et al*., 2019). After some initial experimentation, it was found that, when combined in a ratio of 0.95:0.05 (Sodium alginate: calcium lactate), a flowable gel forms with a viscosity of 4.11 Pa.s.

To prepare the tubing for accurate flow rate measurement each area of tubing was marked at a set length using tape, and the volume (mm^3) of these marked sections was calculated (Table 2.1). To determine the flow rate (mm^3/sec) through tubing sections

A and B, either sodium alginate or calcium lactate was pumped at a set rate by the micro-peristaltic pump, and the time taken (in seconds) for the fluid to travel through the set length marked by the tape was noted. To determine the flow rate through tubing section C, where both sodium alginate and calcium lactate combined (dosed at a ratio of 0:95:0:05 or 95% / 5%, respectively, using the mixing function on the printer), the same method of measurement was also used. The flow rate was calculated (Table 2.1).

The flow rate of calcium lactate was faster through the tubing in section A and B than sodium alginate. Unsurprisingly, this suggests that fluids with a lower viscosity flow at a quicker rate (22 % (Section A) and 29 % (Section B) faster) than those with a higher viscosity. Tubing radius was also found to affect flow rate, where fluids flowing through section A tubing (i.e., within the peristaltic pump) (r = 1 mm) were slower than that of section B (r = 1.5 mm). With constant fluid viscosity, the larger the radius of tubing the faster the flow rate. However, when viscosity changes or the solution gels (e.g., when calcium lactate and sodium alginate come into contact within the Y-connector) the flow rate reduces by 81 % and 63 % from the calcium lactate and sodium alginate stream, respectively. Though the tubing radius of section C is more than twice that of section B (to accommodate the incoming flow of dual feeds), this decrease in flowrate shows that the relationship between viscosity and tubing dimension are important to consider. However, due to the squeeze and release (i.e., displacement) movement of the peristaltic pump, a further increase in tubing radius may have a limited effect. Therefore, the only solution to increase flow rate would be to replace the micro-peristaltic pumps with mini-peristaltic pumps with a higher power output to be able to displace materials with a higher viscosity. However, the practicalities would be challenging if tubing dimensions and peristaltic pumps must to be changed to suit every type of food texture. Hence, why quantifying the relationship between tubing dimensions, pump capacity and material viscosity becomes important when initially designing a system with the ability to print food materials with a broad range of viscosities.

The Hagen-Poiseuille (H-P) equation is a fluidic law used to describe the relationship between pressure, fluidic resistance and flowrate (Zhang and Hoshino, 2014). Modified versions of the H-P equation have been used by others to describe and predict the flow behaviour of fluids through narrow orifices like a syringe barrel, nozzle or piping tubes (Kalil and Sun, 2007; Kim *et al*., 2018; Kim *et al*., 2019; Li *et al*., 2016; Schutyser *et al*., 2018). In the original H-P equation (1), Q is the flow rate (m^3/s), ΔP is the pressure difference between inlet and outlet (Pa), r is the internal

tubing radius, μ is the dynamic fluid viscosity (Pa.s) and L is the tubing length (m) (Kim *et al*., 2018).

$$Q = \frac{\pi \Delta P r^2}{8 \mu L} \quad (1)$$

The H-P equation operates on the assumption that the fluid being measured is incompressible, Newtonian, laminar in flow and that the temperature of the fluid remains constant. However, most fluids which are considered suitable for the 3D printing process exhibit non-Newtonian behaviours (i.e., their viscosity is dependent on shear rate), such as that of the calcium lactate and sodium alginate solutions used in the experiment explained previously. For fluids to be considered laminar, the Reynold's number (unitless value) must be less than 2,300 (Sommerfeld, 1908). The Reynolds number of a fluid can be calculated using the following equation (2), where ρ is the density ($kg.m^3$) of the flowing fluid, u is the velocity (m/s) of fluid flow, D is the length (m) through which the fluid will flow in and μ is the viscosity (Pa.s).

$$Re = \frac{\rho u D}{\mu} \quad (2)$$

However, not all fluids are considered laminar and therefore, the original H-P equation (1) must be modified to capture this. For example, Schutyser *et al*. (2018) used a modified version of the H-P equation (3) to include the flow consistency coefficient (m, $Pa.s^n$) (i.e., average viscosity) and flow index (n, (-)) (i.e., deviation of the fluid from Newtonian flow) to calculate the volumetric flow rates (Q, m^3/s) of sodium caseinate dispersions.

$$Q = \frac{n \pi R^3}{1+3n} \left(\frac{R \Delta P}{2mL} \right)^{1/n} \quad (3)$$

Kalil and Sun (2007) also derived a theoretical model similar to this which included the direction of the capillary axis or nozzle tip in order to measure the pneumatic microvalve flow rate of organic tissue. A similar equation with slight variation was used by Li *et al.* (2006) which included calculating shear rates of alginate hydrogels to determine the measurement range suitable for rheological characterisation.

In most cases, these models have certain variables and relationships in common such as flow rate, viscosity and tubing dimensions which are also features of the parameters recorded in the current study. However, the systems mentioned by others operate under the assumption that flow rate is kept constant, and not in a pulsating action exhibited by a peristaltic pump, which is the case in the current study. Other variables

such as tubing flexibility are not taken into consideration by these modified equations, which can add further complexity to the matter. Therefore, the empirical method used to determine flow rate by time taken to travel a set tube length was deemed the most suitable method of calculation for this reason.

2.4 Tronxy X8

A second 3D printer was evaluated in order to compare different printing mechanisms (i.e., peristaltic pump versus syringe type print head). The Tronxy X8 is a basic Cartesian style FDM 3D printer with a build volume of 220 × 220 ×200 mm (l × w × h). This type of 3D printer was selected specifically because of the vertical supports on either side of the printer acting as the z-axis (Fig. 2.6). This is a useful feature which allowed for the additional weight of the new print head design to be supported sufficiently whether the print head is in the home position or travelling to the other end of the x-axis. This particular feature is useful in relation to this project, as some Cartesian printers are designed with only one z-axis support where the x-axis is cantilevered over the print bed instead of equally supported on either side of the printer. This design is normally sufficient for the designed application of that specific 3D printer; however, if modifications are made and additional structures or heavier materials are added to the print head, this could cause the printer to become unbalanced, put undue strain on the stepper motors, affecting the quality of the print overall.

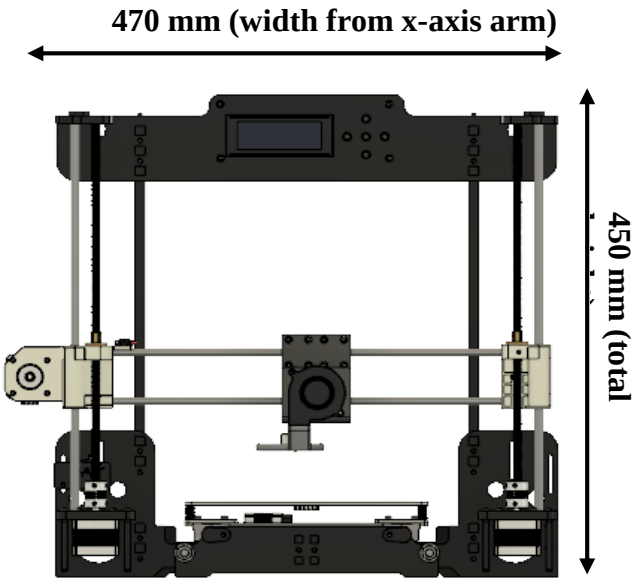

Fig. 2.6. 2D Sketch of Cartesian 3D printer prior to modification (Front view) (Note: unit dimensions are in mm) (Adapted from Valsecchi, 2018).

2.4.1 Modifications

2.4.1.1 Printer syringe and syringe mount

The basis for the design of this 3D food printer came from a previous project involving 3D printing of commercially manufactured processed cheese carried out in UCC by Le Tohic *et al*, (2018). The printer used in that research was a RepRap Pro Ormerod 1, a fully open-source self-replicating RepRap 3D printer. In a similar vein to the current study, Le Tohic *et al*. (2018) modified a plastic printing printer and converted it into a syringe-based 3D food printer by 3D-printing a plastic syringe mount, plunger holder and extrusion motor mount (Fig. 2.7), using the 3D printer itself before conversion.

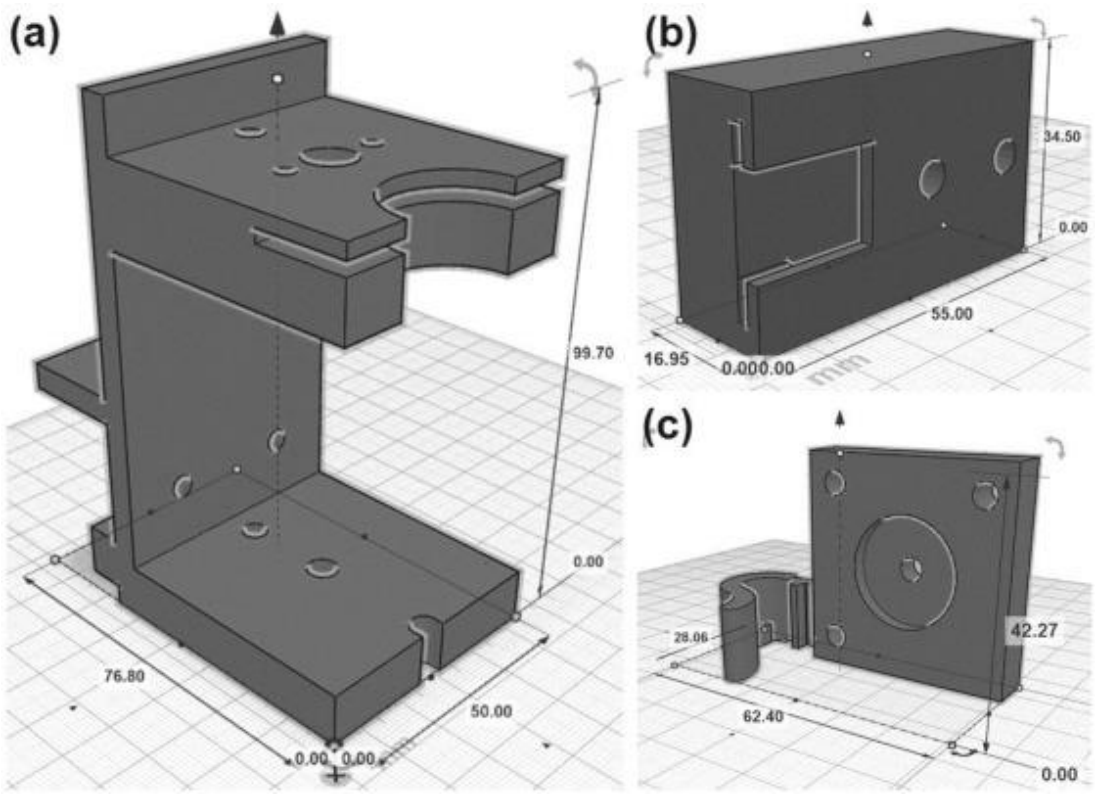

Fig. 2.7. Original design inspiration for modifications made to the Cartesian 3D printer. Computer aided design (CAD) drawings of the extrusion system elements: (a) syringe mount, (b) syringe plunger holder and (c) extrusion motor mount (Obtained with permission from Le Tohic *et al*., 2018).

Figure 2.8 shows a side-by-side comparison of the original printer on the left and the printer post-conversion on the right. One of the modifications made to the 3D printer involved altering the function of the stepper motor which instead of driving plastic filament into the hotend, the stepper motor now operates the flexible driveshaft which rotates in the anti-clockwise/clockwise direction depending on the desired

upwards/downwards motion. The driveshaft is attached to a helical screw which is in turn connected to the syringe plunger housing, which gradually depresses the plunger within the syringe, thereby expressing the food material inside through the nozzle tip and depositing the material on to the print bed. This is the basic operation of the modifications made to the Tronxy X8 printer, with the additional feature of a temperature-controlled syringe to maintain the temperature of the food contained therein. Details of this additional modification will be discussed in Section 2.4.1.2.

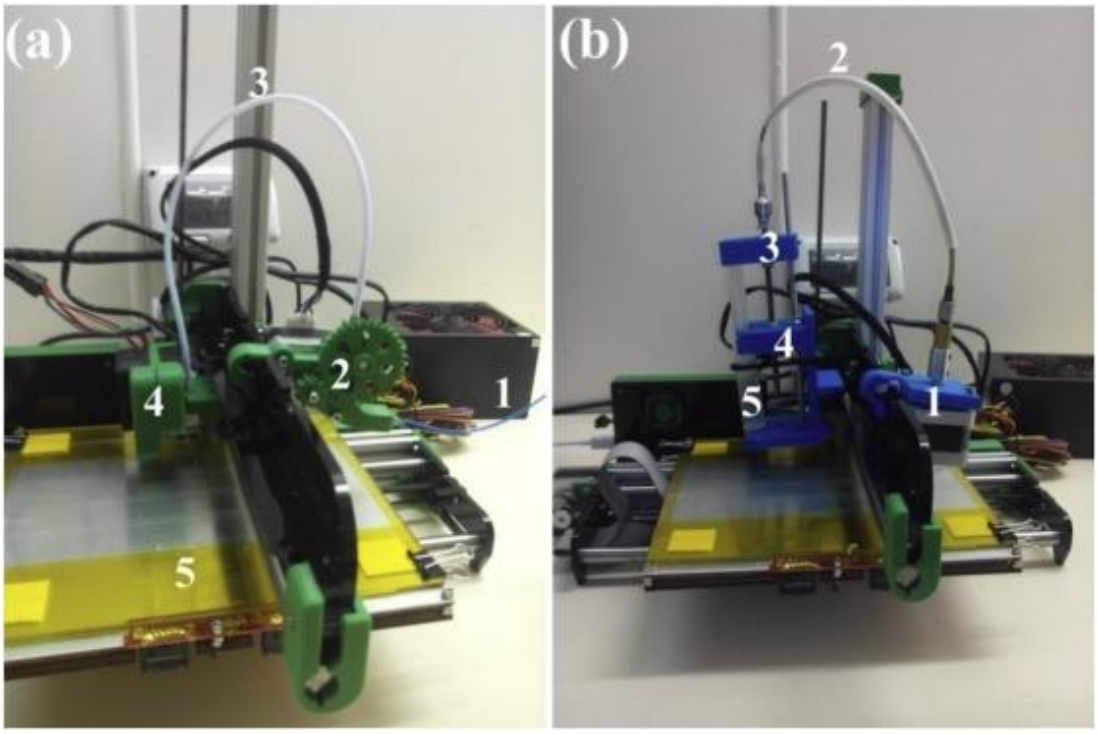

Fig. 2.8. Images of the Reprap Pro Ormerod 1 printer equipped with the plastic printing setup: (a) 1. PLA filament; 2. Extruder drive; 3. Filament Bowden tube; 4. Print head; 5. Print bed, and (b) the syringe-based food printing configuration: 1. Extruder motor mount and motor; 2. Flexible drive shaft; 3. Syringe plunger-holder; 4. Syringe mount; 5. Syringe (Obtained with permission from Le Tohic *et al*., 2018).

The construction of the syringe mechanism used in the current study was similar to that of the Le Tohic model in that the mechanism was separated into two parts, the main syringe mount body and the smaller plunger housing component. The syringe mount in the Le Tohic model was 3D-printed as one complete structure, whereas the syringe mount design for the Tronxy X8 printer was composed of two pieces, a top, and a base which supported the stainless steel syringe in two places to ensure stability during printing (Fig. 2.9). Both the top and base sections of the syringe mount were secured to a thin stainless steel sheet using bolts. The syringe mount design used in the Le Tohic *et al.* (2018) study was primarily constructed from Polylactic Acid (PLA)

plastic, a commonly used thermoplastic polyester filament derived from renewable sources such as starches (Tankilla *et al*., 2017; Wu *et al*., 2021). However, all components relating to the syringe mechanism design in the current study were milled from solid stainless steel using a CNC (Computer Numerical Control) milling machine.

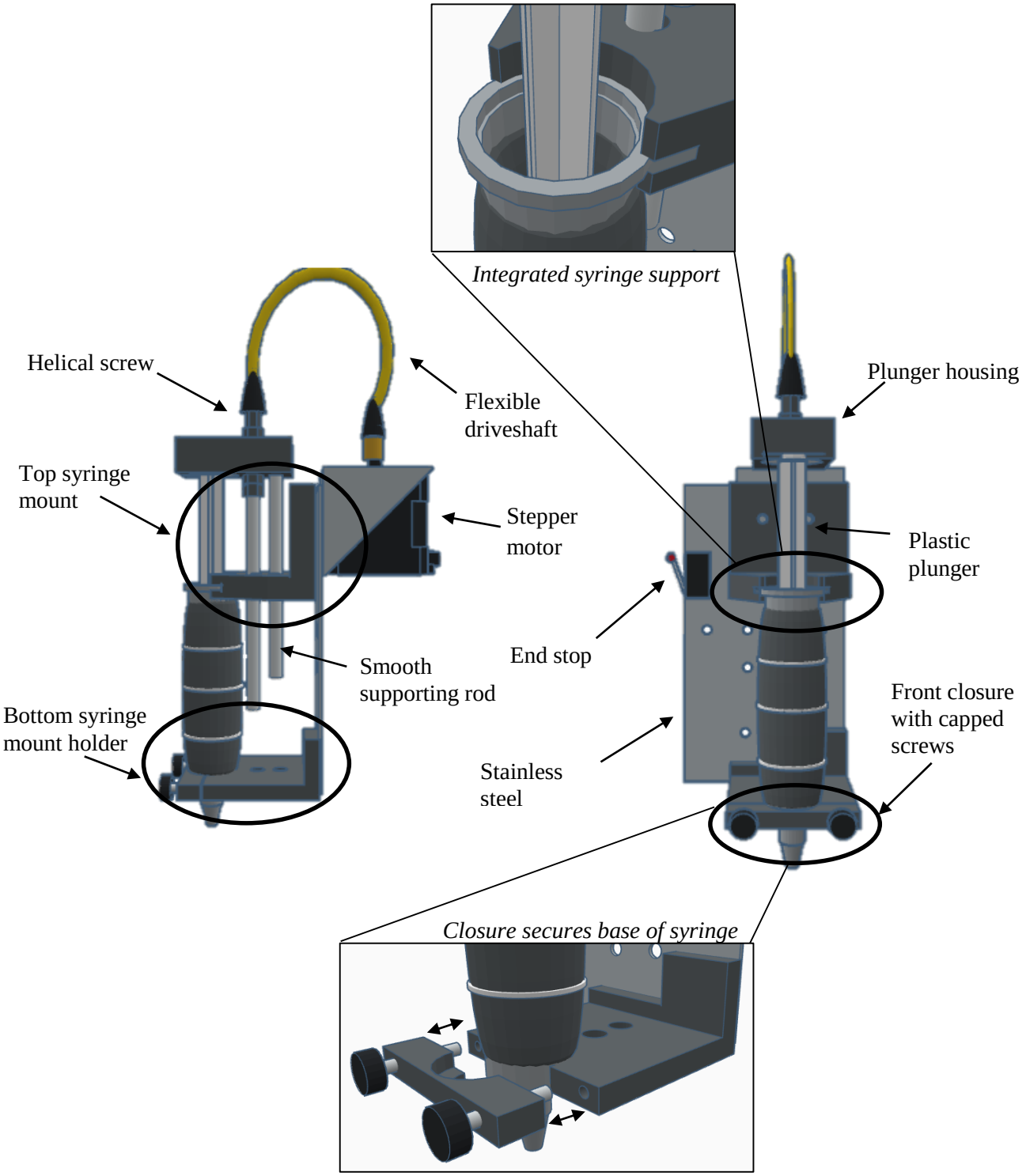

Fig. 2.9. Modified print head for Cartesian 3D printer (Side and front view)

In order to maintain rigidity of the structure, a smooth supporting rod (Fig. 2.9) was fixed to the base of the plunger housing and a hole bored through the top half of the syringe mount to allow the supporting rod to glide upwards and downwards without interference during the depression action of the syringe during printing. A front closure was added to further support the syringe during printing (Fig. 2.9). This closure was also constructed from stainless steel and had two capped screws which were fed through a threaded hole all the way through from front to back of the closure so as the base of the screws could reach the base of the syringe mount. The capped screws were chosen for ease of use and comfort when (un)/screwing the front closure when removing or securing the syringe. The integrated cut-out design of the front closure fit was modelled based on the dimensions of the base of the nozzle and the tip to ensure a snug and secure position during printing.

The stainless steel syringe was also milled using the same CNC machine mentioned previously. Stainless steel was chosen as the primary construction material for a number of different reasons: (1) it could be sterilised easily, (2) has good thermal properties which allowed the contents to be heated *via* a wire heater wrapped around the syringe, and (3) heating/cooling would not affect the structural integrity of the syringe. The syringe barrel was modelled on the dimensions of a 20-ml plastic syringe (Terumo Syringe without needle (Leur lock tip), Terumo Europe, Leuven, Belgium). The plastic plunger was retained from the plastic nozzle to be used in combination with the stainless steel syringe barrel and nozzle. As the rubber end of the plunger tended to degrade over time and was replaced frequently, simply replacing the plastic plunger complete with rubber end was the most convenient option. The syringe barrel itself was milled with a stainless steel rim around the top which was designed to slot neatly into the cut-out integrated in the upper part of the syringe mount (Fig. 2.9). A selection of stainless steel nozzle tips was also milling using the CNC machine. Each tip was threaded at the top, which was designed to be screwed into the tapered end of the syringe barrel, similar in operation to a Luer-lock syringe design.

The modified syringe print head was mounted onto the 3D printer using short screws onto the original print head's slide block linear ball bearings. There are three slide blocks in total, two on the top rail and one on the bottom rail, as seen on the left-hand side in Figure 2.10, which shows the dimensions of the original print head mechanism prior to modification. These slide blocks run along the two x-axis support rails and are fixed in place by the x-axis supports.

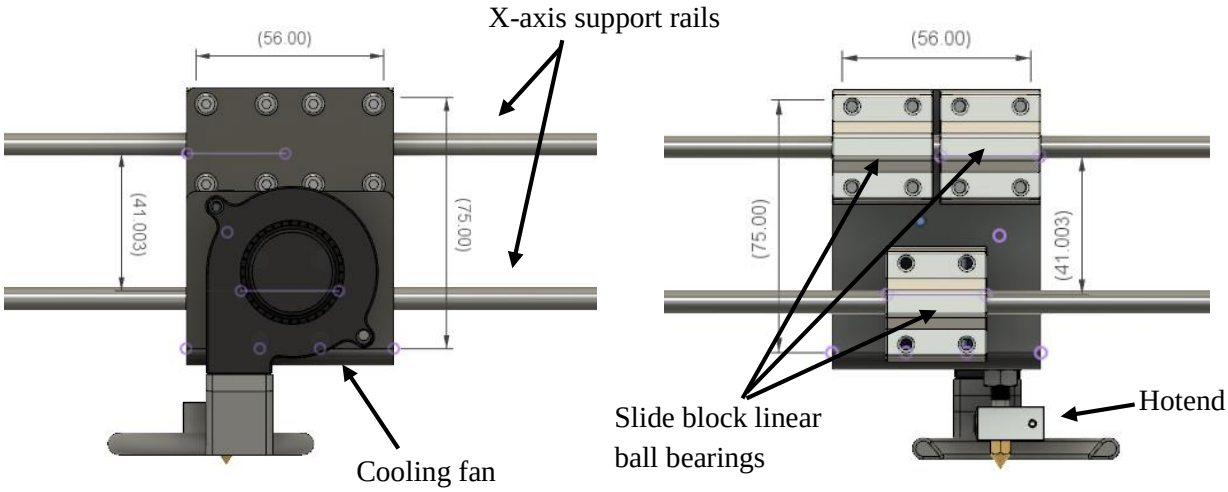

Fig. 2.10. 2D Sketch of the Cartesian 3D printer print head prior to modification (Front and Rear view) (Note: unit dimensions are in mm) (Adapted from Valsecchi, 2018).

2.4.1.2 Heating capacity and temperature control

A useful addition to a 3D food printer is the ability to control and monitor the temperature of the food within the syringe. This feature was added to the 3D food printer design once it was realised that heated foods (such as molten processed cheese) can rapidly lose their thermal energy during printing, leading to inconsistencies between prints. In order to maintain the temperature of the food within the syringe, 34 turns of heating wire made from nichrome (0.5 mm diameter) were wrapped around the length of the syringe barrel to ensure even and broad coverage and dispersion of heat. The heating wires were connected to the main circuit board, where the original hotend heating wires would have been connected prior to modification. The wires and syringe barrel were also wrapped with Kapton tape, a thermally conductive tape made from a 25 μm thick Kapton MT1 polyimide film, which is typically used to insulate wires and reduce the occurrence of electrical fires (Benford *et al*., 1999; Berkebile and Stevenson, 1981). The length of the syringe barrel was also covered in a black exhaust tape to further insulate against heat loss, and this was secured tightly with a pliers using thin flexible wire in three bands. The dimensions of the final syringe barrel and nozzle design are shown in Figure 2.11.

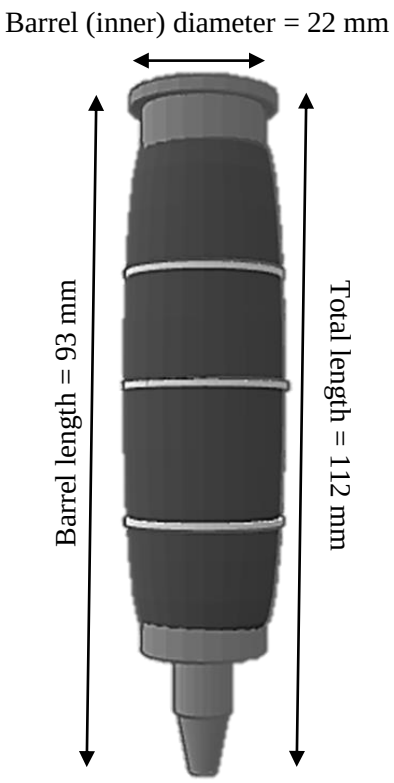

Fig. 2.11. Diagram showing dimensions of modified syringe for 3D food printing use (Note: unit dimensions are in mm)

A preliminary trial was carried out prior to using the stainless steel syringe to test the heating rate and maximum temperature of the system within a reasonable amount of time (approximately 10 minutes). The coil resistance was found to be 14.4 Ω (Ohms) when 14.5 V were applied from the power supply (1 Amp current limit). The temperature of the wire was recorded using a Type-K thermocouple every 10 seconds for 600 seconds (Fig. 2.12), once the current was applied to the wire. It was observed that after 600 seconds (10 minutes) the wire reached 90.6 °C.

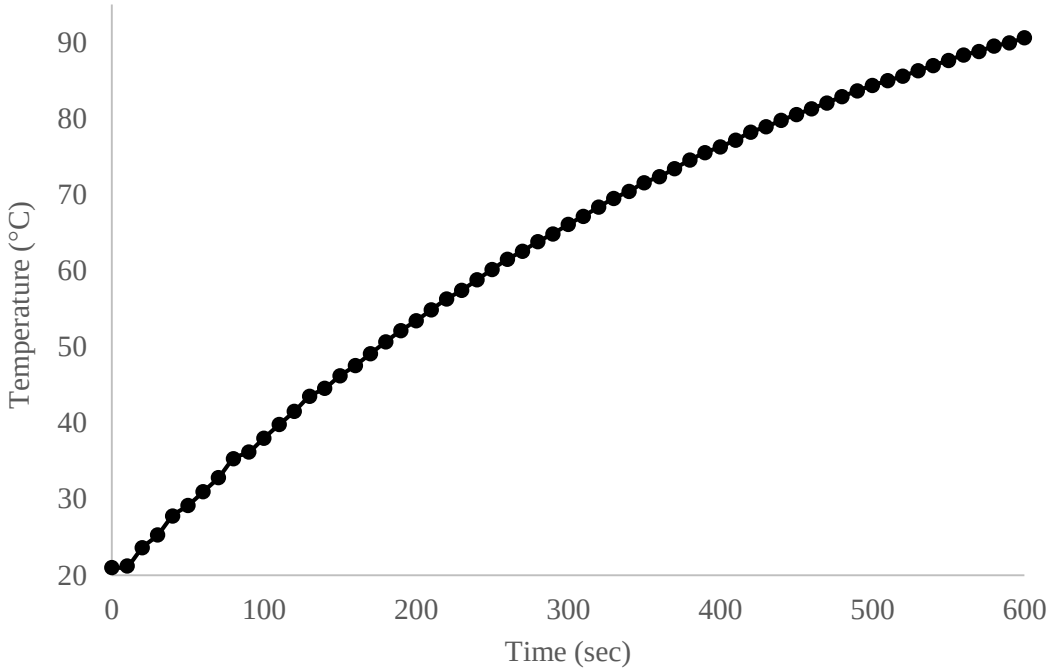

Fig. 2.12. Rate of heating of modified 3D food printer syringe for the Cartesian 3D printer

To ensure the temperature of the barrel was accurate, a thermistor was run between the exhaust tape and the Kapton tape layer. The role of the thermistor was to provide feedback to the printer's controller to intermittently switch on/off the power applied to the nichrome heater wire by the printer's power supply (12V) to maintain the desired temperature of the syringe barrel and, therefore, by extension the contents within the syringe. The thermistor wire was connected to the main circuit board where the original thermistor wire for the hotend would be inserted.

2.5 Comparison between printers

Both the Rostock 301 (Delta printer) and Tronxy X8 (Cartesian printer) function using the same basic principle of FDM 3D printer operation. They both 3D print plastic filament by mechanically feeding a reel of thin solid plastic (commonly used Polylactic Acid (PLA) filament is approximately 1.75 mm in diameter) into an extruder print head and continuously melting this plastic as it comes into contact with the hot-end (i.e., a block of metal which contains the heating element) and then extruding it at a set rate through the tip of the nozzle. However, as mentioned previously, both printers differ in their FDM printer type, as well as the printing application, more specifically post-modification.

In Table 2.2, the differences can be seen between the two printers prior to modification, where the Rostock is a delta configuration, whereas, by comparison, the Tronxy X8 works from a Cartesian configuration. The Delta printer was also the more expensive of the two printers, but this is presumably due to the fact that the delta printer has three extruders, whereas the Cartesian printer only has one extruder. As mentioned in Section 2.2.2, though delta printers operate at much faster printing speeds than a Cartesian printer, printing accuracy can be compromised as the movement of the print head can become turbulent and uneven at higher print speeds.

Table 2.2. Comparison of specifications between both 3D printers prior to modification

	Rostock 301	**Tronxy X8**
FDM printer type	Delta	Cartesian
Purchase cost (€)	340	150
Build volume(mm)	170 × 170 × 870	220 × 220 × 200
Weight (kg)	9.6	8
Number of extruders	3	1

In Table 2.3, the differences between the two printers post-modification are outlined, as well as the advantages and disadvantages to both systems. The most discernible difference between the modifications made to these printers is the delivery system design. In the Delta printer, the printer operates using a peristaltic pump which uses positive displacement to take up the fluid material through the flexible silicone inlet tube and deposit the material through nozzle through the flexible silicone outlet tube. However, in the Cartesian printer, the system is much more rigid in structure and material, whereby the food is manually placed within a stainless steel syringe and the food is then deposited using mechanical pressure from the plunger, which is driven by the stepper motor creating a downward rotational and linear force or torque. Both of these designs enable the printers to 3D-print in different applications, each with their own advantages and limitations. Due to the multi-extruder function of the Delta printer, this would theoretically allow for up to three ingredients to flow and mix simultaneously according to the ratios set by the user using the mixing function.

However, in the case of the Cartesian printer, there is a limit to only one ingredient per print run, due to the syringe containing only one singular chamber to store the food material. With that said, the Cartesian printer can only print a maximum of 20 ml of food material, whereas the maximum volume printable with the Delta printer should theoretically be limited only by the volume of the container that the food is being held in (and of course the maximum build volume of the printer, as mentioned in Table 2.1) Though the Delta printer should in theory be able to build much taller structures than the Cartesian printer, due to the difference in maximum feed volume available, the viscosity of the fluids is limited to very liquid-like food materials due to the power and speed output of the selected peristaltic pumps and stepper motors. As discussed in Section 2.3.2, the length and width of the flexible silicone tubing is an important aspect to consider when trying to control the flow-rate of the food material into account. Due to this factor, the tubing would have to be altered each time a different food was selected to be printed, or the food would require additional ingredients be added to the formulation to ensure optimal viscosity for printing.

Table 2.3. Comparison of both printer design post-modifications

	Delta printer	**Cartesian Printer**
Delivery system	Peristaltic pump driven by stepper motors (food travels through flexible tubes from holding vessel to the nozzle)	Mechanical syringe driven by stepper motor and drive shaft
Max feed volume available (ml)	Unlimited	20
Heating capacity details	Heated print bed	Heated syringe and print bed
Advantages	✓ Larger feed supply volume ✓ Dual/multiple feed sources available ✓ Mixing/ratio feature	✓ Can print thicker pastes ✓ Easy to level bed ✓ Consistent and even heat transfer throughout vessel
Disadvantages	× Limited to low viscosity printing materials × Difficult to level bed × Tube and pump system needs to be modified for each food system × Potential heat loss of food between vessel and nozzle × Increasing print speed impairs printing accuracy	× Limited feed supply volume × Limited to one ingredient printing per print run × Viscosity of pastes printable limited to power output of stepper motor and strength of drive shaft

Part B: Effect of printing parameters and materials on printability

There are a number of parameters to consider before starting to 3D print food, many of which also overlap with the basic fundamentals of 3D printing plastic; namely the nature of the material (e.g., thickness/viscosity, melting temperature etc.), nozzle diameter, distance of the nozzle to the print bed and print speed. In this section, these parameters will be discussed in more detail, accompanied by short descriptions of experiments completed as necessary to illustrate the importance of each parameter, and how they affect the overall printability of 3D-printed structures using cream cheese and tomato ketchup.

2.6 Materials

2.6.1 Cream cheese

Full-fat cream cheese is a spreadable dairy-based product which is high in fat (21 %) and is also a source of dietary protein (5.4 %). The brand of cream cheese used in this study contains pasteurised non-fat cow's milk, cream (dairy fat), citric acid, stabiliser (Locust Bean Gum (LBG)) and salt (0.75 %). Typically cream cheese displays shear-thinning behaviour, or a decrease in apparent viscosity, when shear stress is applied (Ningtyas *et al*., 2017), which makes it an ideal material for 3D printing (Joshi *et al*, 2021). The high fat content in cream cheese allows the product to be spreadable and smooth, whilst the stabiliser (LBG) increases the viscosity of the product and helps maintain structural stability of the product by preventing syneresis (i.e., expulsion of excess whey from casein curds) (Brighenti *et al*., 2020).

2.6.2 Tomato ketchup

Tomato ketchup is also a shear-thinning food substance, but has different rheological characteristics to those of cream cheese due to a much lower fat content found in ketchup (0.1% fat). Ketchup is a tomato-based product containing sugar, vinegar, seasoning and spices; in addition to these base ingredients, it often contains hydrocolloid ingredients in the form of gums and stabilisers. These ingredients are usually added to support the shear-thinning or non-Newtonian fluid [1] characteristic

[1] Non-Newtonian fluids are described as liquids which do not typically follow Newton's law of viscosity (i.e., shear rate and shear stress are directly proportional), where a change in viscosity of non-Newtonian fluids is dependent on applied shear rate. For example, in the case of shear-thinning materials (a category of non-Newtonian fluid), an increase in shear rate leads to a decrease in material viscosity.

that is attributed to the product's signature texture. Hydrocolloids are water-soluble, high molecular weight polysaccharides which, when added to ketchup formulations, become entangled with the tomato base. In this state, they are quite viscous; however, when stress is applied, in the form of pressure from a squeeze of a ketchup bottle or from a vigorous downwards shake of the container, the polymers rearrange into alignment so as to easily slide past each other and become less viscous and more fluid-like. When the force or stress is removed, the polymer molecules return to their entangled state once more (Koocheki *et al*., 2009).

2.7 Methods

2.7.1 Material parameters: Texture and temperature

Printing temperature is an important parameter to consider when selecting a food material to print with. To demonstrate why a certain temperature was chosen for printing, a modified Texture Profile Analysis (TPA) method was carried on a texture analyzer TA-XT2i (Stable Microsystems Ltd., UK) to compare the difference in textural characteristics between printing cream cheese or ketchup at 18°C or 30°C and which is more suitable for consistent printing.

Either ketchup or cream cheese was added to a 20-ml Luer slip syringe ensuring minimal air-pockets were present by gently tapping the syringes on a hard surface. The syringes were wrapped in tinfoil to prevent leakage and stood vertically at refrigerated temperatures (4°C) overnight. One hour prior to analysis, samples were removed from refrigeration and allowed to come to the desired temperatures either at ambient temperature conditions (approx. 20°C) or placed vertically in a water bath set to 30°C. Once target temperatures were reached (checked using a thermocouple inserted in the nozzle), syringes were set up on the modified syringe testing rig shown in Fig. 2.13. The modified rig consisted of a retort stand with a claw clamp holding the syringe barrel close to the base to prevent instability during testing. However, care must be taken that the clamp isn't secured so tightly as to constrict the movement of the syringe plunger within the barrel during the depression action. Before testing commenced, syringe plungers were manually depressed until the 20-ml mark (first measurement mark on barrel) was reached to ensure the start point during measurement was consistent for each sample tested. The syringe nozzle was then inserted in the hole of the base plate whilst being stabilised by the retort stand clamp, and a weight boat placed underneath the base plate to capture the extruded material.

A spirit level was then used to ensure the plunger is parallel with the base of the P75 compression platen. The TPA measurement consisted of a single compression at a rate of 1 mm/sec, where the distance of compression was 60 mm.

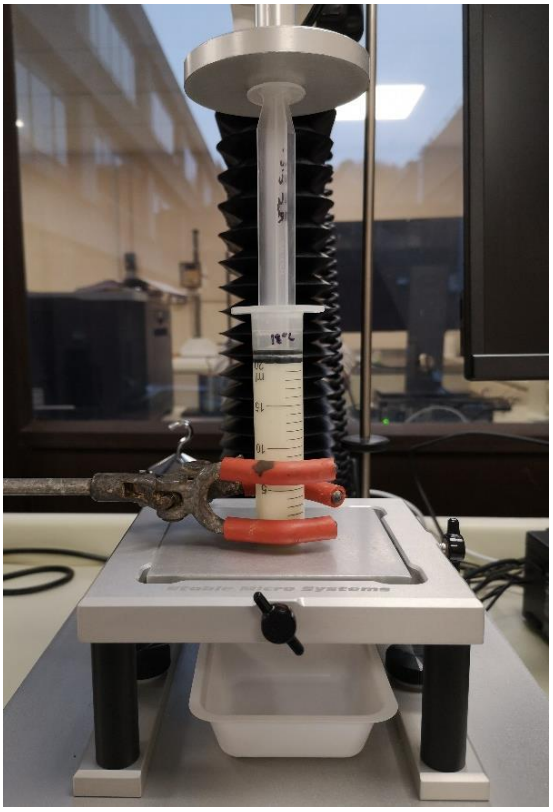

Fig. 2.13. Layout of modified syringe rig using Texture Profile Analysis (TPA) equipment

2.7.2 Material parameters: Viscosity

A rotational viscometer (ViscoQC 100R - Anton Paar Ireland Ltd., Dublin, Ireland) was used to measure the viscosity of cream cheese and ketchup at the temperature at which they would be printed (18°C or 30°C). Samples of equal volume (200 ml) were gently placed into a beaker and allowed to come to temperature in ambient temperature conditions to reach 18°C or placed in a water bath at 30°C for 30 minutes to reach 30°C. Due to the difference in viscosity and texture of both food materials at two different temperatures, two separate spindles were selected to measure viscosity, the RH7 spindle and the RH6 spindle. The conformation of the RH7 spindle allowed the geometry to rotate within thicker samples, such as cream cheese as 18°C, without exceeding excessive rotational torque (i.e., > 100 % torque reading). In comparison, the RH6 spindle has a small resistance disk attached close to the base which enabled the viscometer to measure the viscosity of lower viscosity samples accurately and with sufficient torque (i.e., > 20 % torque reading). The spindle was gently inserted into the

sample and let to rest for a few minutes to avoid major deformation in the sample. The spindle was then allowed to rotate at 20 rpm for 30 seconds while recording the viscosity. This was repeated in triplicate in different areas of the sample and an average viscosity (Pa.s) was calculated (Table 2.4).

Table 2.4. Spindle type used, torque and average viscosity measurements for ketchup and cream

Food material	Spindle	Average torque (%)	Average viscosity (Pa.s)
Ketchup (18°C)	RH6	27	13.64 ± 0.24
Cream cheese (18°C)	RH7	25	34.73 ± 1.17
Ketchup (30°C)	RH7	22	11.02 ± 0.15
Cream cheese (30°C)	RH6	52.7	26.37 ± 2.63

2.7.3 3D-printing parameters

To investigate the effect of printing parameters, such as nozzle diameter and distance to the print bed, on the quality of the resultant print, a series of experiments were conducted using the modified Tronxy X8 3D food printer. Only singular layers were printed to visually display the differences in print quality (i.e., whether there were gaps or differences in width of lines during printing indicating under/over-extrusion etc.). Printing a number of layers would have made it difficult to determine the area printed, and printing a singular layer was believed to be representative of the layers that would have succeeded the first layer. Full-fat cream cheese was used as a printing medium for the following analysis. Images were taken of all completed prints in order to visually compare overall print quality.

2.7.3.1 Nozzle distance to print bed

To determine the optimal distance of the nozzle tip of the printing syringe to the base of the print bed, the printer was instructed to 3D print cream cheese (18°C) in a continuous line (height and width = 2 mm) with four 90° bends (Fig. 2.14) with three different nozzle height settings, 1 mm, 2 mm and 3 mm above the print bed (print speed = 20 mm/s, nozzle diameter = 2 mm). This type of print design was chosen to

demonstrate the print quality of both straight lines and bends during the course of printing. In order to set the range of heights of the nozzle the end-stop switch was manually adjusted to each new height (i.e., 1, 2 and 3 mm above print bed). This step ensured that the correct height was set each time a new print was started.

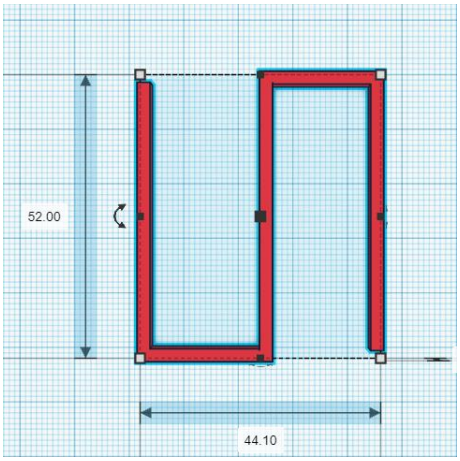

Fig. 2.14. Continuous line design. Line width/height – 2 mm. Total area – 380 mm^2 (Note: dimensional units are in mm)

2.7.3.2 Nozzle diameter

To observe the effect of nozzle diameter on print quality, four nozzles were machined with varying diameters (2, 2.5, 3 and 3.3 mm) to fit the modified syringe for the Cartesian printer. The printer was instructed to print cream cheese (18°C) in two vertical lines (width and height = 2 mm, length = 50 mm) spaced 10 mm apart from each other (print speed = 20 mm/s). This type of print design was selected to demonstrate and visually compare the overall quality of the print in duplicate between the four nozzle sizes. The nozzle diameter setting within the slicing software (CuraEngine, Ultimaker) was also changed to reflect the diameter of the nozzle fitted for each run. ImageJ analysis software (a Java-based image processing programme that can be used for image quantification) was used to find the area of cream cheese line printed using each nozzle size to determine differences in extrusion flow. The printed area was selected as a measureable attribute versus the printed volume as it was found to be difficult to capture an image that was clear enough to objectively measure the height of the printed structures using ImageJ. Perhaps for future studies a more sophisticated measurement can be used to measure the volume of the printed structure or alternatively a larger 3D model can be used which may be easier to measure using computer software.

2.7.3.3 Print speed

Print speed is an important parameter to consider depending on the intricacy of the print and the quality desired. To compare the effect of print speed on print quality, the printer was instructed to print cream cheese (18°C) in the same continuous line design described in Section 2.7.3.1 (nozzle diameter = 2 mm), but using three different speeds (20 mm/s, 40 mm/s and 60 mm/s). This setting was adjusted in the printer software before each print run. ImageJ analysis software was used to find the area of cream cheese shape printed at each speed to determine differences in extrusion flow.

2.8 Results

2.8.1 Effect of different printing material types and temperature on texture and viscosity

Cream cheese and tomato ketchup have very different syringing characteristics overall (Fig. 2.15). When comparing the force (g) needed to compress the syringe plunger over 60 seconds, cream cheese (18°C) required an increasing force to extrude the material, whereas, ketchup (18°C) flowed quite readily from the syringe. This is also reflected in the viscosity values (Table 2.4), where cream cheese (34.73 ± 1.17 Pa.s) was more than twice as viscous as ketchup (13.64 ± 0.24 Pa.s) at 18°C.

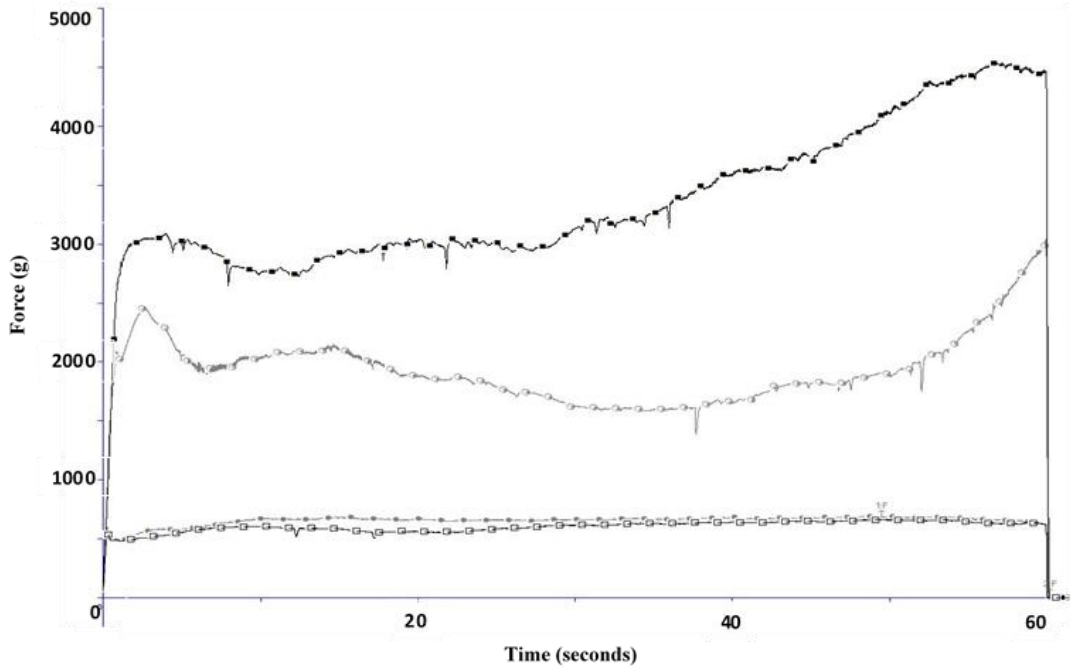

Fig. 2.15. Texture Profile Analysis graph showing force required to extrude Cream cheese (18°C) (■), Cream cheese (30°C) (○), Ketchup (18°C) (●) and Ketchup (30°C) (□) from a syringe

There was also a contrast between the effect of temperature on the forces required to depress the plunger in cream cheese samples. At 30°C, the degree of force needed to push the plunger gradually decreased before increasing slightly at the 40 second mark whereas, at 18°C, the force increased gradually over time. This would suggest that cream cheese at warmer temperatures required less effort to extrude and, therefore, was more fluid-like in consistency. Others have also observed a similar trend where consistency and viscosity are affected when temperature is decreased in chocolate printing (Lanaro *et al.*, 2017; Mantihal *et al.*, 2019). This information can be useful in identifying the ideal temperature range at which printing can occur before the quality and the flow of printing is affected (Lanaro *et al*., 2017).

In terms of the ketchup sample, at warmer temperatures (30°C) the forces required to extrude the sample appeared to follow a similar pattern to that of the 18°C sample. This suggests that temperature has little effect on the flowability of ketchup when extruded.

To demonstrate the differences in printability, the continuous line shape from Fig. 2.14 was printed (at 20 mm/s print speed) using both cream cheese and ketchup, and ImageJ analysis software was used to measure the total area printed each sample and an average was calculated (Fig. 2.16). It can be seen that cream cheese (18°C) produced a print that had a 15 % larger printed area than ketchup (18°C), and a 37 % larger printed area when the temperature was raised to 30°C. This is clearly a consequence of the difference in viscosity between the two materials and its effect on extrusion flow (Fig. 2.16). It was also observed that both cream cheese and ketchup printed at 30°C had 37 % and 14 % larger printed area, respectively, than cream cheese and ketchup printed at 18°C. This result complements the trend in the forces required to depress the syringe in Figure 2.15, which suggests that temperature has a larger effect on the texture and viscosity of cream cheese than ketchup but, also, had a secondary effect on extrusion flow. The suggested reason why this may be the case could be due to the dairy-fat content in cream cheese which would be more susceptible to changes in flow properties when temperature is increased. In contrast, tomato ketchup does not typically contain a source of fat and will be less responsive to changes in thermal energy.

Printing temperature (°C)	18°C	30°C
Cream cheese		
Printed area (mm^2)	1117.64	1766.81
Ketchup		
Printed area (mm^2)	949.54	1108.57

Fig. 2.16. Images of printed continuous line in cream cheese or ketchup at 18°C (top) or 30°C (bottom)

2.8.2 Effect of printing parameters on print quality

2.8.2.1 Nozzle distance to print bed

The distance between the bed and the nozzle can be set automatically in some printers, although others must be set manually. In Fig. 2.17, it can be seen that a difference of 1-2 mm between the print bed and the tip of the nozzle can make quite a large difference to the quality of the print and, therefore, must remain consistent between print runs. The continuous line printed 1 mm distance between nozzle and print bed

was observed to have a printing height of less than 1 mm. The syringe nozzle also tended to drag in the cream cheese during printing leaving an imprint of the nozzle shape. However, at 2 mm distance, the printed shape had much more volume in the z-direction (i.e., achieved the set 2-mm layer height). The print was much more consistent and the nozzle was clean after printing. At 3 mm distance, it was observed that the nozzle was too far away from the print bed for the cream cheese to adhere sufficiently and print consistent lines and bends. The cream cheese tended to drag around the print bed mid-air rather than stick to the print bed and lacked the gentle pressure of the nozzle to aid adhesion to the print bed. Wang *et al*. (2018) reported that, when the nozzle height is above or below the critical nozzle height (or h_c) this can lead to thicker printed layers or delayed deposition during printing, both of which affect the overall quality of the print.

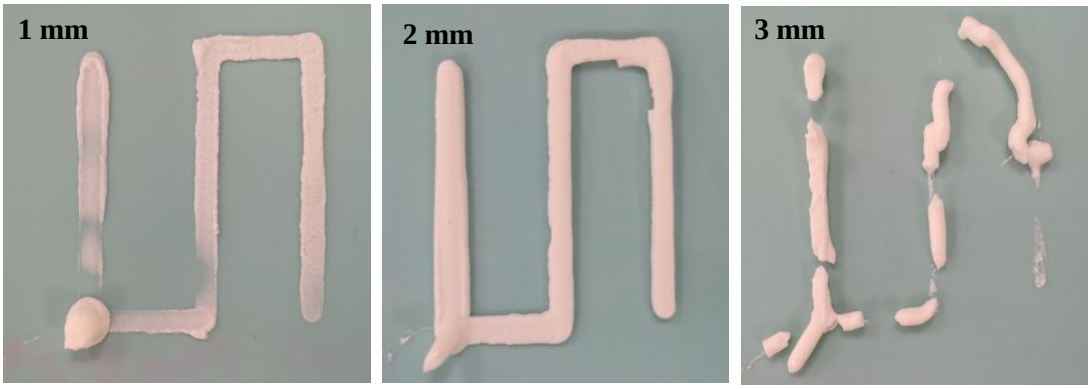

Fig. 2.17. Images of cream cheese 3D-printed in a continuous line shape where the nozzle is 1, 2 or 3 mm away from the print bed (Nozzle diameter = 2 mm)

In conclusion, the 2-mm distance was the most optimal setting for this printing material as it produced the clearest print and didn't interfere with the quality of the print (i.e., no dragging or smearing). It has been suggested by Yang *et al*. (2018) that the nozzle distance, nozzle diameter and layer height are related factors, and in order to print in a suitable manner without affecting print quality, these parameters must have equal values. Hence, 2 mm nozzle distance was inevitably the most suitable option as in the case of the current study where the nozzle diameter and layer height was also set at 2 mm.

2.8.2.2 Nozzle diameter

As well as the distance to the print bed, the diameter of the nozzle used can have an effect on the quality of the print. It can be seen from Fig. 2.18 that the print quality visually remains relatively consistent throughout printing using the nozzle diameters between 2, 2.5 and 3 mm. However, when the same lines were 3D-printed using the

widest nozzle (3.3 mm), this led to a very inconsistent print that had gaps in the structure and appeared to be under-extruded. To quantify the differences in area, ImageJ analysis software was used to measure the area of each printed line and an average was taken (Fig. 2.18). It was observed that, as nozzle size increased, the printed area (mm^2) decreased, which would suggest that the printing software automatically reduced the extrusion rate to ensure the 2 mm width of the model was accurately printed. However, it appears that there is a limit to the maximum diameter of nozzle used without sacrificing quality and consistency of printing. Others have observed the opposite effect where larger nozzles have led to coarser prints, whereas more narrow nozzle diameters have resulted in more intricate and finer printed structures (Krishnaraj *et al*., 2019; Wang *et al*., 2018; Yang *et al*., 2018). It must be noted that most studies observing the effect of nozzle diameter have visually compared the print quality of layered printed 3D-structures such as cylinders and similar shapes and not objectively measured singular layer lines using imaging analysis. Other studies also used a much broader range of nozzle diameters (the narrowest being 0.33 mm (Krishnaraj et al., 2019) and the widest being 2 mm (Wang et al., 2018; Yang et al., 2018)), which allow for a much starker contrast between the difference in print quality of the structures. Unfortunately, narrower nozzles were unavailable to be machined during this study.

	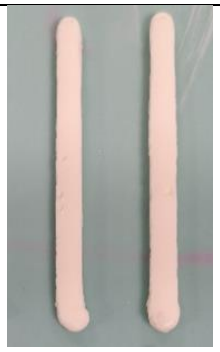			
Nozzle diameter (mm)	**2**	**2.5**	**3**	**3.3**
Average printed area (mm^2)	**205.30**	**199.15**	**168.25**	**122.63**

Fig. 2.18. Images of cream cheese 3D-printed using 2, 2.5, 3 and 3.3 mm wide diameter nozzles. Bottom row of table includes average printed area (mm^2) of each printed line to show change in extruded volume while nozzle diameter is increased.

2.8.2.3 Print speed

Print speed has been shown to negatively impact print quality, where there is an increase in print speed, the width of the printed line tends to decrease (Yang *et al.*, 2018; Zhang *et al*., 2022). This trend was also seen in the present study using cream cheese, where print quality appeared to decrease as print speed increased from 20 mm/s to 40 mm/s and 60 mm/s, especially at the 90° bends of the printed structure (Fig. 2.19). Using ImageJ analysis, it was observed that the average printed area (mm^2) decreased with increasing print speed. If print speed is increased from 20 mm/s to 40 mm/s, 15 % less cream cheese is extruded. However, if print speed is tripled to 60 mm/s, the extruded volume is 20 % less than would be extruded at 20 mm/s. This would suggest that the rate of deposition of cream cheese is not as efficient at faster print speeds, which leads to a compromise in print quality. Others have also confirmed this phenomenon, where high printing speeds (> 30 mm/s) can lead to slower extrusion rates, resulting in dragging of print material and inconsistent prints (Yang *et al*., 2018). Therefore, it is important to consider the intricacy of the design and the desired print quality when selecting a print speed. It must also be noted that the material of the print bed should be considered when testing the various factors affecting print quality. In the case of this particular study a silicone matting is used and may provide more grip than if one was to use parchment paper or a ceramic or glass plate. Surfaces with a smoother consistency may tend to provide less adhesion than those with rougher surfaces. However, for the purpose of experimentation and comparison between printing of materials it is important to be consistent with the conditions of the printing surface. Hence why steps were taken during the preparation step of this research to ensure the silicone matting was allowed to come to room temperature before commencing each experiment.

	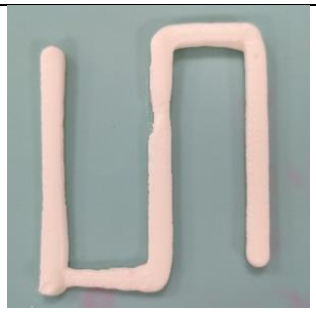		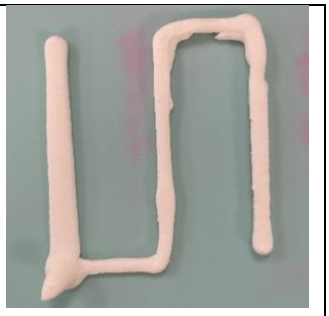
Print speed (mm/s)	**20**	**40**	**60**
Average printed area (mm^2)	**1117.64**	**940.35**	**888.97**

Fig. 2.19. Images of cream cheese 3D-printed at 20, 40 and 60 mm/s to show changes in extrusion volume when print speed is increased (Nozzle diameter = 2 mm).

2.9 Discussion and conclusion

There are many parameters that need to be considered and understood before commencing 3D food printing. As has been discussed in Part A, the design of the 3D printer can be influential in the range of foods which can be 3D-printed successfully. Whether the overall movement of the printer (i.e., delta versus Cartesian) is considered or the extrusion mechanism (i.e., peristaltic pump versus mechanical syringe design), each option can determine whether the printer is capable of printing low or higher viscosity foods. Currently, there are no 3D printers on the market that has the capacity to universally print a multitude of food types (i.e., powders, semi-solids pastes, gels etc.). The closest 3D printer available to this at present is the Mutant V2 which allows the user to use a variety of print head types (i.e., laser cutter, printing syringe etc.) *via* universal tool swapping system (Wham Bam, 2021). Though this innovative solution is primarily aimed towards non-food applications, it is clear that the concept could potentially be useful for transitioning between different types of food printing technologies (e.g., liquid binding printing and FDM printing) without the need for multiple printers. However, until this technology is more readily available, users will have to bear in mind what their desired end-product is select the appropriate printer according to their printing goals.

At an operational level, printing parameters such as print speed and nozzle diameter and positioning has been shown to affect print quality and must be selected with care

and initial experimentation to determine suitable printing conditions for that food type. However, this step may not be necessary for users who have selected to a commercially manufactured 3D food printer. These printers are often sold with food cartridges supplied by the printer manufacturer and have optimal printing settings for that specific food pre-programmed in the printer. This can be useful for users who are inexperienced with the operation of the printer or those who want a simple and convenient user experience. However, for those desiring to create innovative and complex structures, it can be useful to understand how these parameters affect printability.

The characteristics of the food being printed must also be taken into consideration, where the viscosity and texture profiles of the food materials can vary at different temperatures. Therefore, measures to control temperature is strongly recommended and should be implemented (e.g., heating or cooling jackets surrounding the extrusion method or a temperature-controlled water bath) to maintain printing consistency and print quality. With each new food material that is printed, these parameters will need to adjusted accordingly, which can be a major challenge with commercially available 3D food printers that are required to be capable to print a wide range of foods without too much input from the user in order to be user-friendly and convenient. However, as research and development into 3D food printing advances, the technology is becoming increasingly available for 3D food printers to have in-built programmes which can auto-select the settings suitable to print the chosen food to an optimum print quality. Therefore, a thorough understanding of the complex relationship between food rheology and process parameters is essential to further optimise 3D food printer design and operation, which may in turn lead to enhanced user experiences where consumers can conveniently create complex and innovative dishes with ease.

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CHAPTER 3

Parameters affecting the printability of 3D-printed processed cheese

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Parameters affecting the printability of 3D-printed processed cheese

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Declaration

This chapter was written by author MMR and reviewed by their co-authors. MMR co-designed the study, carried out 3D-printing trials, microscopy analysis, Urea PAGE, rheometry work, TPA and imaging measurements and analysed the data. SC provided training and guidance for microscopy analysis. JO performed statistical analysis. A version of this chapter has been published as: **Ross, M.M.**, Crowley, S.V., Crotty, S, Oliveria, J, Morrison, A.P., and Kelly, A.L. (2021) Parameters affecting the printability of 3D-printed processed cheese, *Innovative Food Science and Emerging Technologies*, **72**, 102730.

Abstract

In considering three-dimensional (3D) printing of food materials, fundamental understanding of the "printability" characteristics of different food materials is of vital importance to successfully meet user needs. In this study, a processed cheese formulation was 3D-printed with a modified 3D printer. Both intrinsic factors (i.e., pH and intact casein content) and measureable attributes (i.e., texture, printing accuracy, rheology and microstructure) were analysed. Using rheological methods, the optimal viscosity range (7.55 – 10.94 Pa.s) at which processed cheese will print successfully (i.e., simultaneously flow from the extrusion head uninhibited and build a layered structure) was identified. Processed cheese with a higher pH (5.8) was found to give a printed product that was significantly ($P < 0.05$) softer and less gummy and resilient in texture than the same recipe with a lower pH (5.4 and 5.6). Printed cheese containing exclusively fresh curd was significantly ($P < 0.05$) harder than that containing mild or mature Cheddar cheese. 3D printing at higher temperatures (60°C) led to harder and more resilient cheese than printing at lower temperatures (40°C). Overall, formulation and printing parameters for processed cheese significantly affect properties relating to "printability".

3.1 Introduction

Three-dimensional (3D) food printing has increasingly become a topic of discussion in the food sector in recent years as a disruptive and novel food technology (Dankar *et al*., 2018). 3D printing using traditional materials, such as metal, plastic and other artificial polymers, has been established for many years in other industrial sectors (i.e., automotive, medical and aeronautical) as a valid form of manufacturing. Up until recently, the food sector was a relatively unexplored area for 3D printing in comparison with the rate of progression in other fields (Lille *et al*., 2017). However, the number of studies investigating 3D-printed foods has increased at a steady rate, which has demonstrated the potential 3D printing has to offer to the food industry (Lipton *et al*., 2015; Liu *et al*., 2017; Liu *et al*., 2019).

One common form of 3D food printing, Fused Deposition Modelling (FDM), involves the controlled layering of a flowable food material, like a paste or gel, until the desired 3D structure is built (Wegrzyn *et al*., 2012). There are other types of 3D food printing technologies available involving the binding of powders (such as spray dried cocoa and/or milk powders) and sugar particles; these technologies have been described in more detail elsewhere (Le-Bail *et al*., 2020; Liu *et al*., 2017).

There are several potential advantages for the use of 3D food printers versus traditional food manufacturing methods, such as the ability to customise a consumer's meals according to their dietary needs and preferences (Pérez *et al*., 2019). For example, individuals with dysphagia could potentially benefit from the development of 3D food printers, where the technology has been used to create creative and personalised specially textured foods to suit individual nutritional requirements without discomfort. However, it must be noted that, to the authors' knowledge, very few have carried out physical clinical trials with dysphagia patients (Dick *et al*., 2020; Dick *et al*., 2021; Kouzani *et al*., 2017). 3D printers may also be used to create different geometries and textures and which may be difficult or impossible to create by hand or by conventional manufacturing operations (Sun *et al*., 2015).

Similar to 3D printing of traditional materials (i.e., plastic, metals etc.), there are many challenges associated with printing of food, one of which is obtaining the ideal consistency of printing material. According to Lille *et al.* (2017), the viscosity of a suitable food material for 3D printing should be low enough to permit extrusion through a nozzle, yet high enough to give a cohesive and stable structure when layered on a print bed. An increasing amount of published work has attempted to understand

the link between shearing forces through 3D printing and textural changes to food stuffs (Dick, Bhandari and Prakash, 2019; Lee *et al*., 2019; Pérez *et al*., 2019; Zhu *et al*., 2019). 3D printing parameters are also important elements to consider in determining the printability of certain foodstuffs. Derossi *et al*. (2017) found that the dimensions and microstructural properties of a 3D-printed fruit-based snack were affected by the flow rate of the material, which is an adjustable setting in the slicing software (i.e., computer software used in most printing processes which plays an active role in converting a 3D model into g-code or instructional code for the printer) of the printer, dictating the volume of material extruded over a period of time. It was observed that low flow rates resulted in an unstable and disrupted internal structure, whereas increased flow produced thicker structures with merged layers and decreased porosity.

Formulation of each 3D-printed food material is also of vital consideration when 3D printing stable structures with high degrees of accuracy. The addition of a structure-building ingredient is important when considering the stability during and post-printing. Proteins which develop strong gel networks can be ideal additions to printing formulations, as seen in many studies involving 3D printing dairy ingredient-based structures. For example, Liu *et al*. (2019) reported that Milk Protein Concentrate (MPC) powder had a strengthening property when combined with sodium caseinate dispersions. This was achieved by increasing protein-protein interactions through inter- and intra- molecule disulphide bonds formed by sulfhydryl-disulphide interchange or sulfhydryl oxidation reactions; the ideal total protein content of the mixture was reported to be between 400-450 g/L. However, there appears to be a limit to the quantity of ingredients with a structural-aiding capacity that can be added to a formulation before negatively impacting the extrudability and printing quality of the food material. In the same study, formulations with a total protein content of more than 500 g/L showed compromised printing quality due to the lack of ductile strength of the printing material during printing.

Similar results have also been found in other dairy-based formulations, where Skim Milk Powder (SMP) (composed of 53% carbohydrate (primarily lactose), 35% protein and 0.6% fat) with a solids content of 50% produced viscous, but printable pastes. However, increasing the solids content to 60% resulted in extremely viscous and unprintable pastes, despite an increase in nozzle diameter from 0.41 mm to 1.19 mm (Lille *et al.*, 2018). In the same study, lactose-free Semi-Skimmed Milk Powder (SSMP) (containing 38% carbohydrates, 37% protein and 15% fat) with a solids

content of 60% was also found to exhibit excellent 3D printing qualities. It was suggested that the higher fat content in the lactose-free SSMP was the primary reason for improved printing characteristics, where the lipids in the formulation act as a lubricant, thereby improving flow during printing.

The objective of this study was to build on the work of Le Tohic *et al*. (2018), who showed that commercial processed cheese can be 3D-printed using a modified 3D printer. In that study, commercial processed cheese was printed at high and low printing speeds, and compared to melted and untreated (i.e., non-heated/extruded) processed cheese for texture, colour and microstructural changes. In the current study, a formulated processed cheese recipe was used in place of commercial processed cheese, and both intrinsic factors (i.e., pH and intact casein content) and measurable attributes (i.e., texture, printing accuracy, rheology and microstructure) were analysed. The role of emulsifying salt (ES) (in this case Tri-Sodium Citrate (TSC), the most commonly used ES in processed cheese manufacture) concentration and moisture was also briefly studied in the preliminary trials of this work in order to optimise a workable food material to 3D print successfully (i.e., build a layered structure). According to Shimp, (1985), ESs, such as TSC, as calcium-chelating agents, disperse and solubilise casein, thereby enabling the emulsification of fat in order to create a homogenous process cheese mixture. TSC is commonly used in sliced processed cheese applications where the body of cheese must ideally be robust to mechanical cutting and maintain its shape without crumbling or deforming (Purna *et al*., 2006), a suitable property required for structure-building during 3D food printing.

3.2 Materials and Methods

3.2.1 Materials

Two batches of Cheddar cheese used in the processed cheese recipes were obtained from Carbery Group Ltd. One batch was aged for 1-2 months and the other batch for 6-8 months, to give two different levels of intact structural protein, casein, as this decreases during ripening due to proteolysis. Alkali (3.12 M caustic solution – 15% sodium hydroxide and 15% potassium hydroxide mixture) used to adjust pH was obtained from Water Technology Ltd. (Ireland). A 3.12 M Hydrochloric Acid (HCl) solution (Sigma-Aldrich, USA) was used to adjust the fresh curd processed cheese recipe to pH 5.6. Trisodium Citrate (TSC), two fluorescent dyes, Nile Red and Fast Green FCF, and Polyethylene Glycol were purchased from Sigma-Aldrich (Arklow, Co.Wicklow, Ireland).

3.2.2 Methods

3.2.2.1 Printer modifications

A Tronxy X8 (Shenzhen Tronxy Technology Co., Ltd., China) 3D printer was used in this project, altered to print paste-like foods rather than plastic filament. This was achieved through modifications to the original extrusion system, by replacing the hot-end assembly with a custom-made stainless steel syringe mount and syringe barrel (for more information on heating mechanism of the syringe, please refer to Chapter 1) (Fig. 3.1). The original print head operated by feeding plastic filament (Fig. 3.1B) into the hot end (Fig. 3.1C) through the rotation of the stepper motor (Fig 3.1A). However, in order to print processed cheese, the entire print head had to be remodelled to accommodate a syringe-based mechanism. An stainless steel syringe mount (Fig. 3.1J) was manufactured to hold the syringe (Fig. 3.1I), and the original print head stepper motor (Fig. 3.1A) was reattached to the rear of the syringe mount, as shown in the right hand figure. In order to drive the helical screw (Fig. 3.1H) connected to the plunger housing (Fig. 3.1G), the stepper motor rotates a flexible drive shaft (Fig. 3.1F) which is connected to the helical screw driving the plunger mechanism, thereby pushing the plunger housing downwards (Fig. 3.1G) (or upwards) depending on the direction of travel (i.e., (anti)/clockwise).

The printing temperature, flow rate (i.e., volume of fluid which passes through the nozzle per unit of time), printing speed (i.e., amount of printed material extruded over a given time, typically measured in mm/s), and layer height were controlled through the printing software (Repetier-Host, Hot-World GmbH and Co. KG).

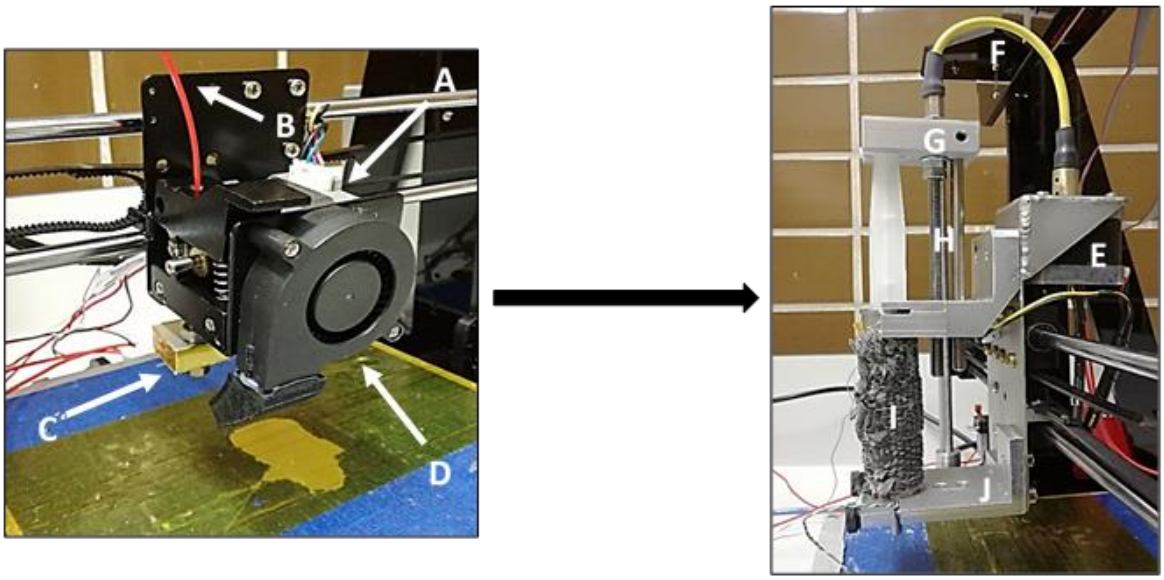

Fig. 3.1. A - Stepper Motor, B - Polylactic Acid (PLA) Filament, C - Hotend, D - Hotend Fan, E - Stepper Motor, F - Flexible Drive Shaft, G - Plunger Housing, H – Helical Screw, I- Syringe Barrel with Insulation, J - Syringe mount

3.2.2.2 Compositional analysis

Three production runs of processed cheese were tested in order to eliminate the impact of batch-to-batch variation on the printing process. Moisture, fat, protein, and ash content of all Cheddar samples were analysed in triplicate. Carbohydrate content was calculated by difference. Compositional analysis of the eight recipes is shown in table 3.1. Each recipe is hereafter designated by a code (A – H; Table 3.1)

Table 3.1. Composition of eight experimental formulations, including a control recipe using fresh curd. (NB: Ratios used for the maturity part of the study, i.e., 75:25 refers to 75% 1-2 month old cheddar and 25% 6-8 month old cheddar). Carbohydrates were calculated by difference. Each sample were analysed in triplicate for n = 3 batches

Code	Recipe	Fat %	Protein %	Ash %	Moisture %	Carbohydrate %	pH
A	pH 5.4	17.69 ± 0.21	22.2 ± 0.1	4.36 ± 0.26	47.25 ± 1.27	8.45	5.4
B	pH 5.6	18.14 ± 0.07	22.4 ± 0.4	4.29 ± 0.23	47.13 ± 0.11	8.09	5.6
C	pH 5.8	17.78 ± 0.13	21.7 ± 0.4	4.25 ± 0.15	47.80 ± 0.62	8.43	5.8
D	Maturity 25:75	17.62 ± 0.30	22.6 ± 0.3	4.48 ± 0.03	45.60 ± 0.45	9.72	5.6
E	Maturity 50:50	17.68 ± 0.08	22.3 ± 0.5	4.37 ± 0.03	46.54 ± 0.91	9.07	5.6
F	Maturity 75:25	17.98 ± 0.86	22.0 ± 0.1	4.09 ± 0.05	46.34 ± 0.7	9.55	5.6
G	Maturity 100:0	18.28 ± 0.09	21.3 ± 0.4	4.51 ± 0.15	46.83 ± 0.62	9.06	5.6
H	Maturity 100:0 (Fresh curd)	18.40 ± 0.35	20.3 ± 0.6	4.81 ± 0.18	48.08 ± 0.42	8.41	5.6

3.2.2.3 Electrophoretic analysis

Polyacrylamide gel electrophoresis was performed on the raw Cheddar samples (i.e., fresh curd, 1-2 month and 6-8 month old Cheddar cheese) to determine the peak volumes of individual bands, thereby quantifying the amount of structural protein (i.e., caseins) within each sample (Fig. 3.2 - Left). Electrophoresis in polyacrylamide gels was performed using a Protean II xi vertical slab-gel unit (Bio-Rad Laboratories Ltd., Watford, Herts., UK) according to the method of Andrews (1983) with modifications. Gels were stained directly by method of Blakesley and Boezi (1977) and destained in deionized water.

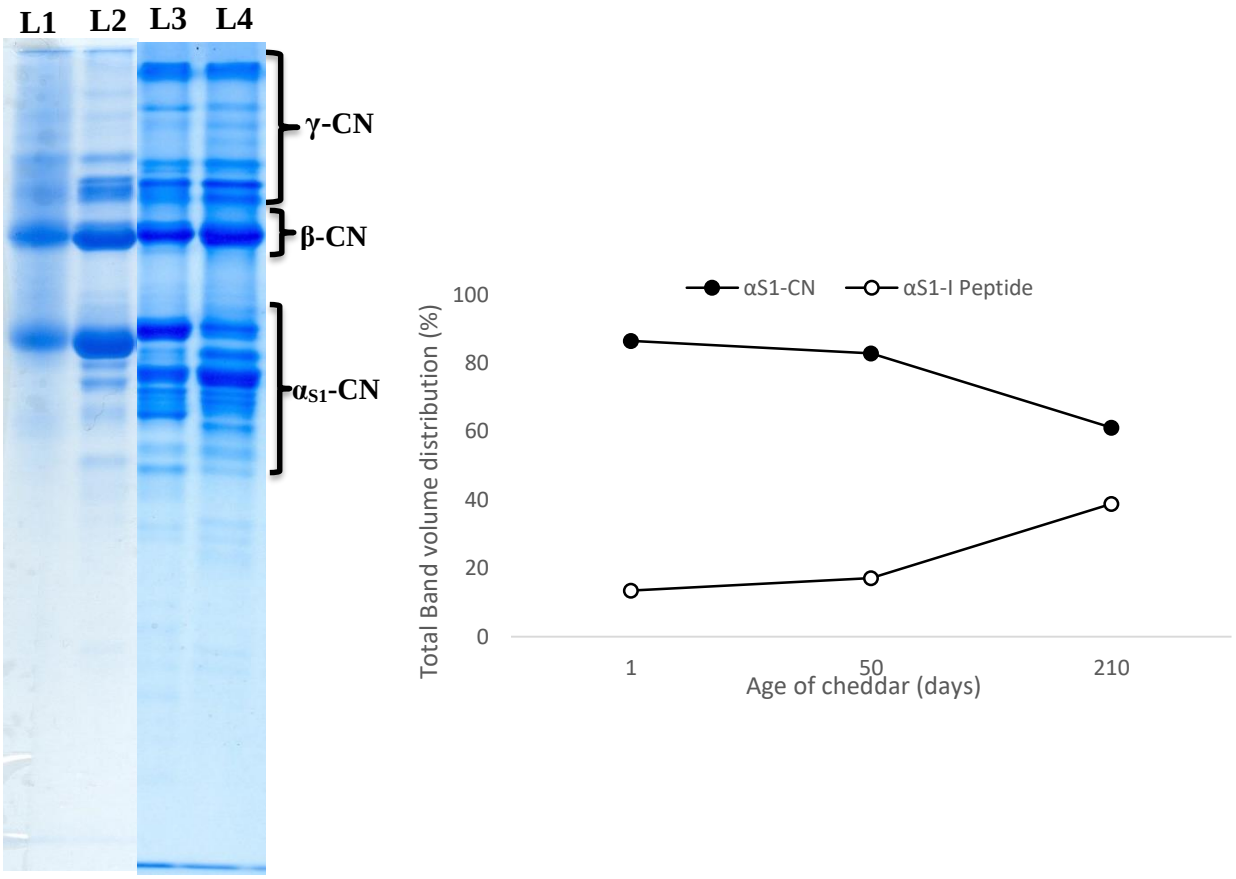

Fig. 3.2 (Left) Urea-PAGE electrophoretogram of cheddar cheese. L1 – sodium caseinate standard, L2 – fresh cheddar curd, L3 – 1-2 month old cheddar and L4 - 6-8 month cheddar (note bands from different gels are presented). (Right) Breakdown of α_{S1}-CN and production of α_{S1}-CN-I in cheddar cheese during ripening

3.2.2.4 Initial processed cheese formulation development

Some preliminary research into various processed cheese recipes from the literature was completed before selecting the final core ingredients. An adapted recipe was generated based on two sources (Kapoor and Metzger., 2005; Berger *et al*., 1998) and simplified into three core ingredients (Cheddar cheese, trisodium citrate (TSC) and water). With these basic ingredients, eight variations of processed cheese recipes were chosen. Three recipes varied in pH (i.e., pH 5.4 (A) (natural pH), 5.6 (B) and 5.8 (C)), where a 15% sodium hydroxide and 15% potassium hydroxide alkali solution was added to give the desired pH. The remaining five recipes differed in structural protein content, where the ratio of young and mature Cheddar cheese was varied to establish a range of high and low structural protein contents (i.e., 1-2-month old Cheddar: 6-8-month old Cheddar –25:75 (D), 50:50 (E), 75:25 (F), 100:0 (G) and the control – fresh curd (H)) (Note: 100% 6-8 month old cheddar recipe was not included as the mixture was found to be far too liquid-like to be printed). These five recipes were all adjusted to pH 5.6 using alkali/acid solutions as required. A comparison sample using fresh cheese curd was used a control to compare the effects of intact protein content on the internal structure, printing accuracy and texture. The formulation for each recipe (per 100 g) are shown in Table 3.2.

Table 3.2. Ingredient formulation of each processed cheese recipe (per 100 g)

	Recipe Code							
Ingredients	A	B	C	D	E	F	G	H
1 – 2 month old cheddar/*fresh curd (g)	58.13	58.13	58.13	19.38	38.75	58.13	77.5	77.5*
6 – 8 month old cheddar (g)	19.38	19.38	19.38	58.13	38.75	19.38	0	0
TSC (g)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Water (g)	20.0	19.82	19.52	19.82	19.9	19.89	19.8	19.76
3.12 HCl/Caustic Sol[n] (g)	0	0.17	0.47	0.1	0.1	0.17	0.2	0.24

Moisture levels and TSC content of the original processed cheese recipe (i.e., Recipe B/F) were chosen based on preliminary experiments, with samples that varied in moisture (55%, 50% and 47% total moisture content) and TSC (1.5, 2 and 2.5% TSC) content were printed into grids and cylinders (see Section 3.2.2.7 for expanded detail of each process). Texture Profile Analysis (TPA) was conducted on the printed cylinders according to the method outlined in Section 3.2.2.8. Textural attributes (i.e.,

adhesiveness, cohesiveness, springiness, hardness etc.) were compared between each sample.

3.2.2.5 Rheological profiles of processed cheese formulations

Rheological profiles for each recipe were generated using the viscosity data measured during each run (Fig. 3.3). This data reflected the typical viscosity profiles for each individual recipe and were used as a guide to predict how the sample would print (Section 3.3.4).

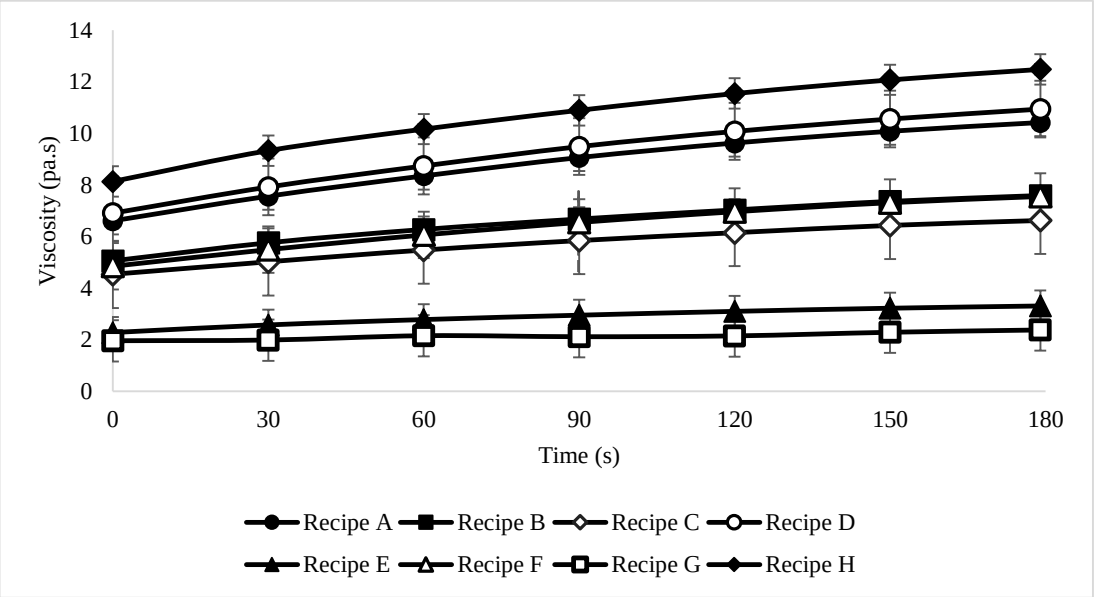

Fig. 3.3. Rheological profile of each processed cheese recipe. Table shows viscosity (Pa.s) of each processed cheese sample at the latter end of the processed cheese making cycle, where the mixture was held at 65°C for 3 minutes.

For initial small scale processed cheese production, an AR-G2 rheometer (TA Instruments, New Castle, USA) with a Starch Pasting Cell (SPC) attachment was used for its heating and shearing function, making it ideal in mimicking the traditional manufacture of processed cheese. The raw ingredients (grated cheese, TSC, water and alkali/acid solution (if required)) were added to the cylindrical stainless steel barrel of the rheometer (capacity 30 g) at room temperature, and the mixture was heated to 80 °C (5 °C min^{-1}) from room temperature (25 °C) and held at 80 °C for a total of five minutes. The mixture was then cooled to 65 °C (5 °C min^{-1}) and held for 3 minutes to allow the mixture to thicken and become less liquid-like for printing. The mixture was sheared at a constant rate of 7.5 s^{-1} during the entire process, and the approximate time taken to complete this cycle was 22 minutes.

3.2.2.6 Preparation of processed cheese for printing

Two methods were used for preparation of samples of processed cheese, one on a small scale for characterisation of formulation rheology, and one on a larger scale for preparation of batches for printing. For the former purpose, the starch-pasting cell (SPC) geometry on the Rheometer proved to be comparable when producing small processed cheese batches. Then, to print multiple samples in quick succession, a larger heating and shearing unit was required to produce a large volume of processed cheese mixture (i.e., 200 g versus 30 g batches); a Thermomix (Vorwerk UK LTD, UK) food processor was thus used to mimic the heating and shearing effect of the traditional processed cheese production cooker and the SPC on the rheometer.

Eight processed cheese recipes intended for 3D printing were produced in 200 g batches using the Thermomix. The processed cheese ingredients were mixed at rotational speed 1 (1.67 s^{-1}) at room temperature until 80 °C was reached, and then held for five minutes to ensure the cheese was melted and the mixture fully homogenous. The mixture was then cooled to 65 °C (at 1.67 s^{-1}), held at this temperature for 3 minutes followed by an immediate transfer to the syringe barrel (approximately 75% filled) for printing. Processed cheese recipes intended to be used as controls (i.e., poured/non-printed) to evaluate the specific effect of printing were immediately poured into cylindrical silicone moulds, stored in an airtight container, and allowed to set at 4°C overnight before analysis the following day.

3.2.2.7 3D printing process of processed cheese (cylinder and grid structure)

The shapes intended for printing were created using Tinkercad (Autodesk, California, United States), a free online Computer Aided Design (CAD) software used to model 3D designs. Twenty millimetre cylinders with an aspect ratio of 1 (Fig. 3.4A) were printed with each recipe in order to assess differences in texture and microscopic structure. Forty-millimetre squared grid structures (Fig. 3.4B) were printed to determine printing accuracy in conjunction with ImageJ analysis.

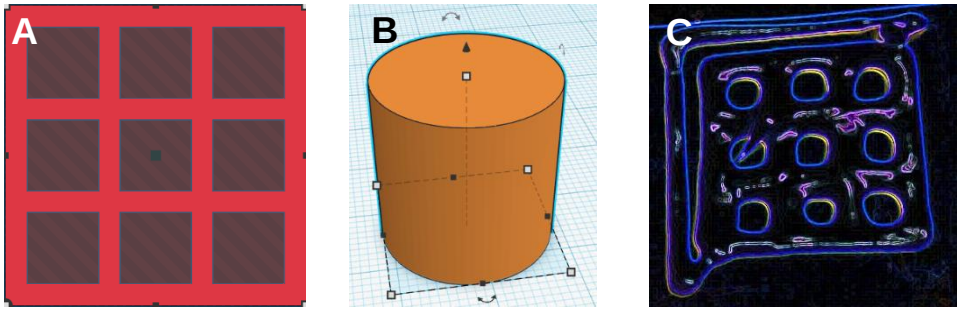

Fig. 3.4. A = CAD drawing of 40 mm square grid structure, B = CAD drawing of of 20 mm cylinder (AR = 1), C = Example of 'Find Edges' function of ImageJ analysis software. This function enables the user to measure the perimeter of the edges with increased accuracy in comparison to measurements using the original image without this function.

Immediately after the cheese mixture was cooled to 65°C in the Thermomix, the syringe was filled to approximately 75% of the barrel volume, and then mounted onto the printer. To print the 20 mm cylinder, a 2 mm nozzle was used with 50%-grid infill at a print speed of 20 mm/s. When printing the 40-mm square grid structure, the print speed was set to 20 mm/s, with no infill and the flow rate set to 50%. A single skirt line was added 5 mm away from the grid structure to avoid printing inconsistencies and broken lines during the initial print. All processed cheese recipes were printed between 40 - 45°C, where the mixture was allowed to cool for a few seconds during filling of the syringe. During these trials, the heated barrel was set to the temperature of the cheese mixture as it was filled into the barrel.

Trials were also repeated using Recipe D at higher temperatures (65°C) to determine the effect of printing temperature on the texture and microstructure of the sample. These printed samples were compared to the poured controls into silicone moulds. All printed structures were allowed to cool to room temperature and subsequently stored in an airtight container (to avoid moisture loss) under refrigerated conditions (4°C) overnight before analysis the following day.

3.2.2.8 Texture Profile Analysis of processed cheese

TPA was conducted on the processed cheese cylinders immediately after their removal from refrigeration conditions (4°C), using a texture analyzer TA-XT2i (Stable Microsystems Ltd, UK), as described by O'Mahony *et al*. (2008). The TPA measurement consisted of two compression-decompression cycles, separated by a time interval of 5 s, at a rate of 1 mm/s, using a cylindrical aluminium geometry (diameter of 35 mm). The probe compressed the sample to 25% (5 mm) of its initial height (20 mm) before decompression. The TPA indicators recorded were hardness, adhesiveness, springiness, cohesiveness and resilience, and at least triplicate measurements were performed for each formulation (i.e., three printed cylinders and three poured cylinders per formulation).

3.2.2.9 Determination of printing accuracy using image analysis

ImageJ is a Java-based image processing programme that can be used for image quantification and descriptive analysis across a broad range of sample types originating from various disciplines (Harris *et al*., 2018; Scheuer *et al*., 2015). Using ImageJ, the image of the grid was enhanced in order to accurately measure the perimeters of the gaps (i.e., non-printed areas) and the printed grid itself. This was done by selecting the 'Process > Find Edges' function; this function enables the use of a Sobel edge detector to highlight sharp changes intensity in the active image (Fig. 3.4C) (Ferreira and Rasband, 2012).

The 40-mm^2 grid structures were then measured using the 'Analyse > Measure' function of the software in order to determine the area of cheese printed. In order to measure the area accurately in the software, the width of the printed grid was measured prior to analysis and the distance value entered in the 'Analyse > Set Scale' function as the 'known distance'. Using the 'Polygon' shape measuring selection, a line was drawn around the perimeter of the grid structure and the area calculated using the 'Analyse > Measure' function. The areas of each 'non-printed' areas (i.e., the nine gaps shown in dark striped grey in Fig. 3.4A) were then measured using the same function and the sum of the area of the nine 'gaps' was calculated. In order to calculate the area of printed cheese (i.e., the solid light grey area shown in Fig. 3.4A), the total area of the nine 'gaps' was subtracted from the total area of the squared structure (including the non-printed area or 'gaps'). To determine the accuracy of the printed grid, the area value measured was compared to the area of the original CAD design measurements and expressed as a percentage (Table 3.3).

Table 3.3. Printing accuracy of 40 mm^2 grids of all experimental batches where values closest to 1.0 represent absolute precision (Value representing original CAD dimensions = 1.0) (* Though gap accuracy is highest in this category, the sample printed an incomplete grid – leading to large unprinted areas contributing to a larger gap area value)

Sample Code	Total printed area/grid accuracy	Total non-printed area/gap accuracy
A	1.6	0.3
B	1.7	0.3
C	1.8	0.3
D	1.6	0.4
E	1.7	0.3
F	1.6	0.3
G	1.8	0.2
H	(1.4)*	(0.4)*

3.2.2.10 Microscopic imaging of processed cheese samples

Confocal Laser Scanning Microscopy (CLSM; Olympus FV1000 confocal laser scanning inverted microscope, Olympus Corporation, Japan) was used to view the structure of each processed cheese recipe (both control and printed samples) in closer detail. Each cheese sample was cut to the approximate dimensions of 5 x 5 x 2 mm using a sharp scalpel before being carefully positioned in a 35 mm diameter glass bottomed dish with a 0.17mm coverslip base. A liquid mixture of Nile Red and Fast Green FCF fluorescent dyes were prepared to stain the fat and protein phases respectively in each sample. This was achieved by preparing a mixture of 200 µl Fast Green FSF aqueous solution (0.1 g/L) and 600 µl of Nile Red in Polyethylene Glycol (0.1 g/L). Fluorescent dye mix (50 µl) was applied to the surface of the sample before covering with the accompanying plastic lid, and allowing the sample to absorb the dye for 10 minutes at 4 °C before imaging. To achieve excitation of both fluorescent dyes,

argon (488 nm) and helium/neon (633 nm) lasers were used to elicit fluorescence of Nile Red and Fast Green FCF, respectively, at their excitation wavelengths (Auty *et al*., 2001).

Scanning Electron Microscopy (SEM; Jeol JSM 5510 Scanning Electron Microscope, Jeol Ltd., Japan) was also used to visualise the microstructure of each processed cheese recipe (both control/poured and printed samples) in more detail. The samples were prepared using an adaption of SEM preparation methods from Tamime *et al*. (1990) and Černíková *et al*. (2017). Each recipe was 3D-printed the day before the preparation stage for SEM and refrigerated overnight. The morning of preparation, the samples were cut into narrow three-dimensional rectangles of the approximate dimensions 1 x 1 x 15 mm using a sharp scalpel or blade. The samples were then fixed in 0.2M Sodium Cacodylate Trihydrate buffer containing 25% v/v Electron Microscopy-grade glutaraldehyde for 2 hours as a primary fixative, followed by a buffer wash (0.1M Cacodylate buffer) for 15 minutes. The samples were subsequently dehydrated in a gradient (30, 50, 70, 90 and 100%) ethanol series for 15 minutes each. The final absolute ethanol step was repeated for a total of three changes. The samples were then defatted in chloroform (three changes of chloroform, 15 minutes for each change), and returned to the absolute ethanol for 15 minutes. The samples were then frozen and fractured in liquid nitrogen and dried using Tetramethylsilane (TMS) for 15 minutes, before allowing the TMS to evaporate from the sample containers in the fume hood. When the samples were entirely dry, they were mounted on labelled aluminium stubs using double-sided carbon tape and sputter-coated with 5 nm layer of of gold/palladium (80:20) using a Quorum Q150 RES Sputter Coating System (Quorum Technologies, UK). The samples are then held in a desiccator until they were examined at 5 kV using a Jeol JSM 5510 Scanning Electron Microscope (Jeol Ltd., Japan).

3.2.2.11 Statistical analysis

All recipes were printed in cylindrical and grid structures in triplicate, and analytical measurements were carried out at least in duplicate. Statistical analysis for this study was conducted in IBM SPSS Statistics Version 26 (IBM Corporation, US). Statistical analysis was carried out separately for the TPA measurements of both the pH and structural protein content datasets. Two-way Analysis of Variance (ANOVA) was carried out, with post-hoc analysis using the Tukey HSD test to compare treatments and assess the impact of changes in the product and process factors (i.e., printed or

poured, pH, structural protein content). Differences were deemed statistically significance for $p < 0.05$ (5% or lower probability of the null hypothesis).

A Factor Analysis was performed on all datasets pooled together. As can be seen in the description of the methods, a large number of individual quality measurements were taken. A Factor Analysis (FA) is a variable reduction technique that replaces n-individual measurements (the responses, or output variables) by a few multilinear combinations of these, which have the feature of a maximum orthogonality between them, so as to capture as much information as possible from the individual output variables into a limited number of higher-level variable compositions, or Factors.

FA is commonly used for quality charts to provide integrated assessments of a large variety of individual quality measurements in a much more user-friendly way than considering each of them individually in turn. It also avoids redundant information arising from correlations between individual measurements or clusters of them. The physical meaning of one of these multilinear combinations is extracted from the clustering of individual measurements provided by those that have high loading factors with the respective Factor (loadings being the individual correlation of each quality measurement and each Factor). The first dimension, or Factor, corresponds to the multilinear combination able to explain the highest percentage of the total variability of the data (if all quality measurements could be correlated together by a simple linear combination and none of them had any errors of measurement, this Factor would explain 100% of the variance of the data and it would capture the entirety of information provided by the n-different measurements). The 2^{nd} Factor will explain the greater percentage possible of the remaining variance of the data not explained by the 1^{st} and that has a maximum possible orthogonality with it, and so forth. The number of Factors that captures the entirety of the data depends on a number of criteria, but one usually expects that all Factors selected to represent the data, when combined, would explain at least 80% of the variance of the data of all n-quality measurements. Calculations and graphical representations were performed using Statistica 13.0 (TIBCO, California, USA).

3.3 Results and Discussion

3.3.1 Influence of TSC level and moisture content on printing accuracy and texture of 3D-printed processed cheese

During the initial formulation process, three TSC contents (1.5, 2, and 2% TSC) and three total moisture contents (47, 50 and 55% total moisture) were used as modifications of Recipe F to determine the optimum texture and printing accuracy for a standard recipe.

There was no statistically significant difference ($p > 0.05$) between the values measured for each textural parameter of the three TSC samples. However, there was a difference in printing accuracy between the grid samples with different TSC levels, where successful samples were selected by visually assessing for a lack of sample spreading during printing, and evenly printed lines with no gaps. Processed cheese containing 2.5% TSC (Fig. S3.1C) printed most evenly with minimal spreading, compared to 1.5% TSC (Fig. S3.1A), which produced a liquid-like and less precise print due to spreading; therefore, 2.5% TSC was selected for the final recipe. Shirashoji *et al.* (2006) found that, when heated from 5 to 85 °C, the storage modulus (G') of processed cheese mixtures with higher TSC concentrations (e.g., 2.75% TSC) tended to also have higher G' values compared to those with lower TSC concentrations (e.g., 1.5 and 0.25% TSC). Due to a larger quantity of the emulsifying salt present in the mixture, a larger amount of calcium was chelated allowing for fat emulsification and casein dispersion to be improved during the cooking phase and resulted in more solid-like, reinforced processed cheese structure (Shirashoji *et al*., 2006).

The effect of total moisture content on printability was then evaluated. Processed cheese samples (Recipe F) with 55%, 50% and 47% total moisture (the potential printability of processed cheese with 44% total moisture was also investigated, but the mixture was deemed too viscous to print) were 3D-printed into grids using the same methods mentioned previously. On visual assessment of the printed grid structures, the print clarity and lack of spreading of the lowest moisture sample (47% total moisture) (Fig. S3.1D) was deemed a more precise print than the highest moisture sample (Fig. S3.1F). For this reason, processed cheese samples with 47% total moisture were 3D-printed for the remaining portion of the study.

3.3.2 Effect of 3D Printing on microstructure of 3D-printed Processed Cheese

Scanning Electron Microscopy (SEM) and Confocal Scanning Laser Microscopy (CSLM) were used to evaluate the effect of printing on the microstructure of processed cheese. In Figure 3.5, the SEM micrographs show an example of the microstructure of two processed cheese recipes (Recipe A and F). Figures 3.5A and 3.5B show the microstructure of the poured processed cheese recipes and Figures 3.5C and 3.5D show the printed version of the corresponding recipes.

The microstructural differences between poured and printed samples varied between recipes. Certain poured samples (Fig. 3.5A) displayed distinctly spherical fat globules, whereas other poured samples (Fig. 3.5B) exhibit slight pooling and partial coalescence of the lipid fraction. These minor microstructural deviations within poured samples could be due to differences in recipe in terms of pH and structural protein content; processed cheese recipes with a higher pH or higher ratio of mature Cheddar to immature Cheddar could lead to increased fat pooling and reduced firmness in the final product (Marchesseau *et al.*, 2000; Purna *et al*., 2006). In comparison to the poured samples, the printed samples (Fig. 3.5C and 3.5D) appear to also have partially coalesced fat globules, but to a greater extent. This could be due to the longer heating times and shearing forces whilst the sample is extruded through the nozzle during printing, enabling the fat globules to interact and merge together, but not to such an extent that the fat is fully coalesced.

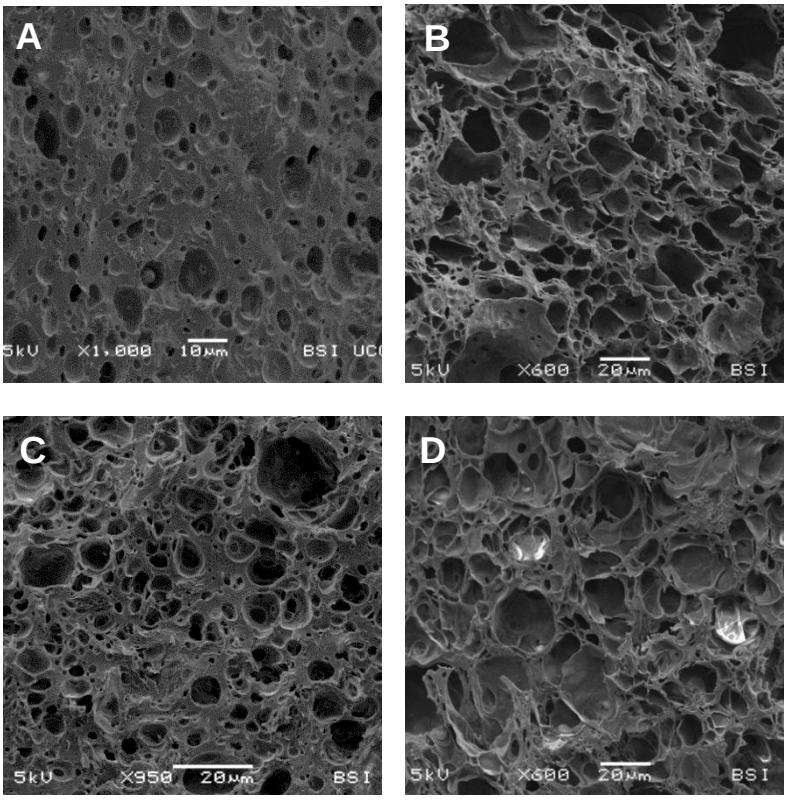

Fig. 3.5. SEM Micrographs: A = Poured recipe A (Magnification x 1,000), B = Poured recipe F (Magnification x 950), C = Printed recipe A (Magnification x 600), D = Printed recipe F (Magnification x600)

Figure 3.6 shows micrographs of the same processed cheese recipes (Recipe A and F) using CSLM to further evaluate the microstructure of the printed versus poured processed cheese samples. The fat globules, seen in red, in the poured sample (Fig. 3.6A and 3.6B) showed a similar trend to those in the SEM micrographs; they appear partially coalesced to an extent, but not to the same magnitude as the printed samples (Fig. 3.6C and 3.6D), where the lipid fraction appears to have a higher rate of interaction and change following printing. Due to limited magnification in CSLM micrographs (x 40 magnification) compared to the SEM micrographs (from x 600 to x 1000 magnification), fat globules appear larger in collective images in Figure 3.5 than in Figure 3.6. However, it appears SEM images capture the coalescing effect of 3D printing more so than CLSM, yet the combination of both microscopy techniques allows for a more comprehensive illustration of microstructural differences (both the surface level and more in depth view) between poured and printed processed cheese samples

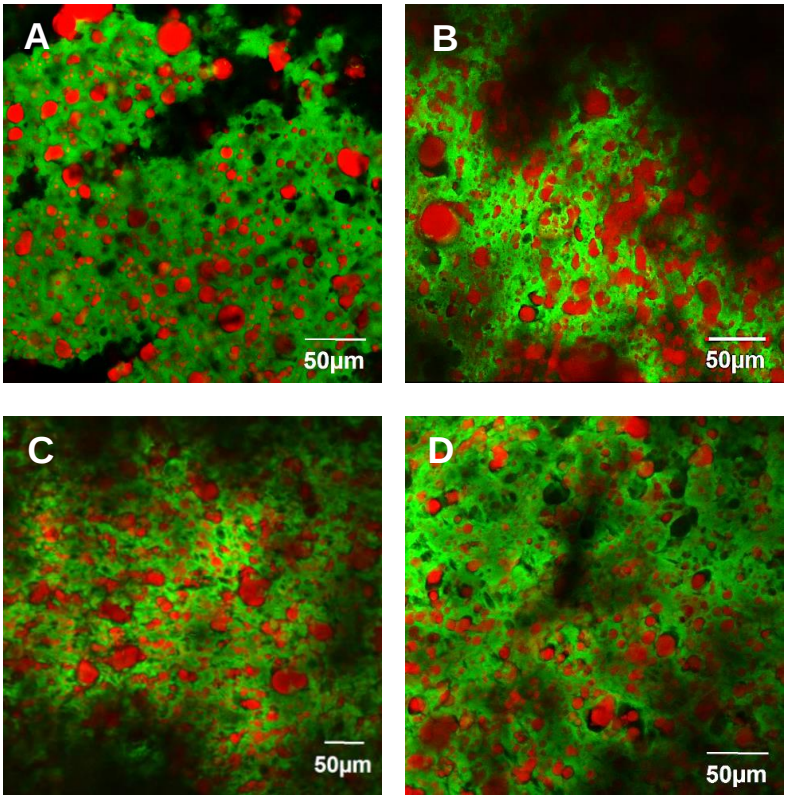

Fig. 3.6. CSLM Micrographs - A = Poured recipe A, B = Poured recipe F, C = Printed recipe A, and D = Printed recipe F (Images were taken using a 40x objective) (Red (Nile red stain) = Lipids, Green (Fast Green FCF) = Protein)

3.3.3 Change in structural protein content during Cheddar cheese aging

Urea-PAGE electrophoretograms of the raw Cheddar cheeses added in various quantities to the eight recipes are shown in Fig. 3.2 (left). It was observed that α_{S1}-CN was intact in fresh curd samples, but gradually reduced in volume during ripening. It is generally accepted that exogenously added chymosin contributes to the breakdown of α_{S1}-CN, where the Phe_{23}-Phe_{24} or Phe_{24}-Phe_{25} bond is readily hydrolysed to produce fragment α_{S1}-CN-I (α_{S1}-CN f24/25-199) (Guerreiro *et al.*, 2013; McSweeney *et al.*, 1993; and Fox., 1979). As shown in Fig. 3.2 (right), there was an inverse relationship between the parent α_{S1}-CN and α_{S1}-CN-I peptide during the period of aging in Cheddar cheese, with a gradual decrease in the parent band volume overtime whilst simultaneously increasing α_{S1}-CN-I peptide band volume. The higher levels of α_{S1}-CN-I in more mature samples suggest that there is a reduction in structural protein (i.e., parent α_{S1}-CN), therefore producing a softer, more brittle Cheddar with improved meltability. Conversely, younger Cheddar samples appear to contain less breakdown

peptides of the parent protein and more intact α_{S1}-CN, producing a harder Cheddar with poor meltability, though more suited to slicing applications than more mature Cheddar, which has a crumblier body (Creamer and Olsen, 1982; Fenelon and Guinee, 2000; McMahon *et al*., 2014).

3.3.4 Effect of pH and structural protein content on the printing accuracy of 3D-printed processed cheese

To compare the printing accuracy of each recipe, and therefore, the impact of each parameter (i.e., pH and structural protein content), the area values of the printed grids were compared to the dimensions of the original CAD drawing (Fig. 3.4A) and expressed as a percentage (Table 3.3). Measurements of each printed grid were performed by ImageJ analysis software using the method described in Section 3.2.2.9. It should be noted that grid accuracy values deviating from a value of 1.0 indicate a loss of accuracy; therefore, values close to 1.0 indicate high accuracy. It should also be noted that the accuracy values of Recipe H are unrealistic (non-printed area accuracy value– 0.4, printed area accuracy value – 1.4), in comparison to the other sample values and should be omitted. This is due to the sample printing an incomplete grid (Fig. 3.7A), therefore leading to large unprinted areas and contributing to a larger than normal area value. The high final viscosity (Fig. 3.3) of Recipe H (η = 12.48 Pa.s) and poor meltability due to a lack of mature Cheddar content in comparison to the other recipes may have led to poor print bed adhesion leading to dragging and poor printing accuracy.

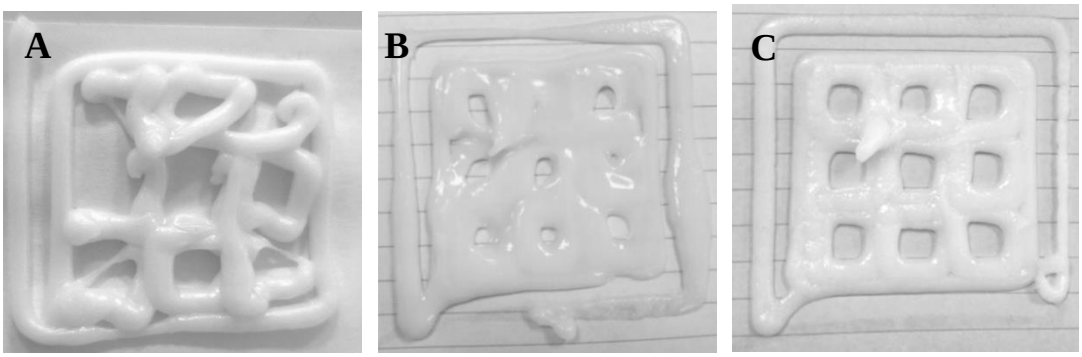

Fig. 3.7 (A-C). 3D-printed 40 mm 2 grid: A, Recipe H: Fresh curd control (incomplete grid squares, dragging of mixture causing distorted lines); B, Recipe G: 100% 1-2 month old cheddar (spreading, suboptimal printing precision); C, Recipe D: 25% 1-2 month old cheddar and 75% 6-8 month old cheddar (accurate, clean lines, very little spreading, optimal printing precision).

Sample pH had no significant effect ($p > 0.05$) on printing accuracy of Recipe A, B and C, where the accuracy values between the three pH ranges was negligible (between 1.23 - 2.78% difference in non-printed areas and 0.3 – 10.93% difference in printed areas). On visual assessment (Fig. 3.8A-C), however, it was noted that processed cheese samples with a higher pH (Recipe C - pH 5.8) resulted in printed grids which had a soft, sticky consistency which contributed to the mixture spreading post-print (Fig. 3.8C). Marchesseau *et al.* (1997) reported that processed cheese produced at higher pH (pH 5.7 – 6.1) showed protein particles of a "string-like" quality, leading to a reduction in structural stability. Sołowiej (2007) also observed reduced firmness in processed cheese samples of pH 5.5 and above and concluded that increasing pH leads to an increase in casein hydration due to a decrease in protein-protein interaction.

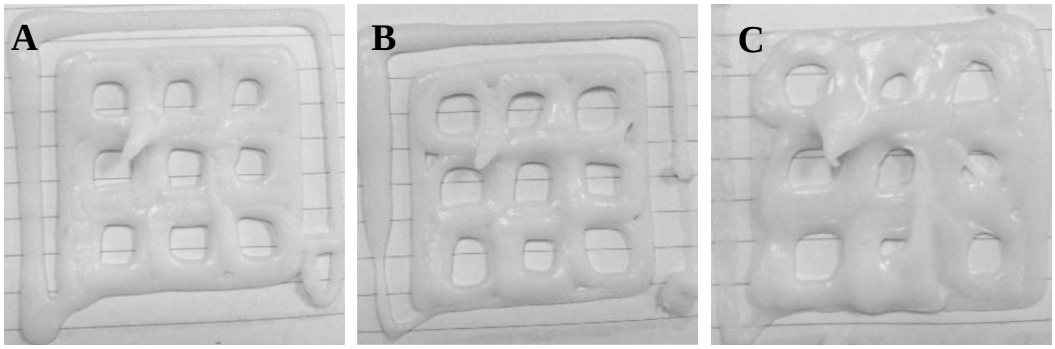

Fig. 3.8 (A-C). 3D-printed 40 mm^2 grid, A (Recipe A: pH 5.4), B (Recipe B: pH 5.6), and C (Recipe C: pH 5.8).

In addition, there was no statistically significant difference ($p > 0.05$) between the accuracy values for the processed cheese recipes with varying structural protein content (Recipe D, E, F, G and H). However, from the accuracy values calculated, and from visual comparison between samples, Recipe G was the poorest performing in terms of printing accuracy. This recipe, which contained only 1-2 month old Cheddar as its cheese source, had the lowest gap (0.2 printing accuracy value) and printed grid area accuracy (1.8 printing accuracy value) due to spreading during printing (Fig. 3.7B). This may be due to the higher structural protein content in the younger cheese, making it difficult for the cheese to flow with a smooth consistency through the nozzle onto the print bed and, therefore, spreading occurred during printing.

It was also observed that Recipe D, which contains 25% 1-2 month old and 75% 6-8 month old Cheddar, performed best in terms of printing accuracy (Fig. 3.7C), showing the highest total gap area (0.4 printing accuracy value) and grid printing accuracy (1.6 printing accuracy value) closest to absolute accuracy (= 1.0) out of all eight

formulations. During printing, there was very little spreading of the processed cheese mixture, which appeared to flow smoothly through the print nozzle during printing. This may have been due to the presence of a higher quantity of mature Cheddar in Recipe D, allowing the processed cheese mixture to flow in an uninhibited manner due to lower structural protein content and better meltability than recipes with larger quantities of younger cheese. However, the addition of small quantities of young cheese in Recipe D allowed the formation of a solid grid structure with very little spreading into gap area, leading to a cleaner, more accurate print.

Wang *et al*. (2011) and Fox *et al*. (2004) reported on the impact of ripening on Cheddar cheese before manufacture for processed cheese, noting changes in texture of young Cheddar during ripening, where the sample becomes softer, less rubbery and smoother as the cheese ages. Fox *et al*. (2000) also noted that processed cheese mixtures intended to produce as blocks with good sliceability requires predominantly young Cheddar (75-90% intact casein) in their recipe, whereas a recipe with predominantly mature Cheddar (60-75% intact casein) is more suited to cheese spreads.

During ripening, a 'softening' effect is observed in the texture of Cheddar cheese; these textural changes can be attributed in significant part to progressive proteolysis. In parallel, throughout ripening, a shift in calcium occurs as the Cheddar pH decreases due to increasing lactic acid content, and the reduction in cheese hardness can also be partly attributed to a reduction in levels of colloidal calcium phosphate (CCP) (Lucey *et al*., 2005; O'Mahony *et al*., 2006). These microstructural changes impact important functional properties such as melting, where mature Cheddar, which has less intact protein content and reduced CCP, exhibits better meltability than that of a younger cheese with larger quantities of intact protein, due to lack of proteolysis as a consequence of limited aging of the cheese (Kapoor and Metzger., 2008). Brickley *et al*. (2007) also suggested that a reduction in firmness and increase in flowability in processed cheese recipes can be linked to a reduction in the levels of intact casein in the Cheddar cheese during ripening, thereby resulting in a reduction in the number of casein–casein interactions in the cheese matrix during processed cheese manufacture.

As previously mentioned in Section 3.2.2.5, rheological profiles (Fig. 3.3) were used as a guide to predict how each processed cheese recipe would perform during printing based on their final viscosity values. From the viscosity data shown in Fig. 3.3 for each recipe, recipe G had the lowest final viscosity value (η = 2.14 Pa.s). This sample was also one of the poorest performing recipes in terms of printing accuracy due to

spreading of the mixture during printing and its thin consistency (Fig. 3.7B). In contrast, Recipe H displayed the highest final viscosity values (η = 12.48 Pa.s), yet also displayed poor printing accuracy due to the mixture dragging during printing, leading to large gaps and inconsistent prints (Fig. 3.7A). Therefore, it cannot be assumed that viscosity and printing accuracy are directly proportional. However, it could be suggested that there are a range of final viscosities for which processed cheese mixtures would be considered acceptable in terms of accurate printing. Recipes A, B, D and F all have final viscosity ranges between 7.55 and 10.94 Pa.s and, both from visual assessment and values of printing accuracy (Table 3.3), are deemed to yield a reasonable print. Any value that falls outside this range is considered suboptimal in terms of printing accuracy, due to line gaps, spreading or dragging during printing. Liu *et al*. (2019) also found, using a range of rheological methods (e.g., dynamic rheology and creep-recovery test), that a spectrum can be determined which allows a mixture of sodium caseinate and milk protein concentrate to print within the optimum printing precision range (e.g., when printed, flows uninhibited from the extrusion nozzle, but also holds a 3D structure without collapsing or spreading). Identifying this range for each food material also allows us to understand the function of each ingredient when added, omitted or when quantities are changed within the mixture, as was the case in a study involving the addition of whey protein (WP) powder to konjac flour (Yuwei *et al*., 2021). The authors found that when adding WP powder at levels from 0% - 30% (w/w) to the konjac flour mixture, the addition of the milk protein up to 20% had a significant effect in printing performance in comparison to the control (no WP added) and lower concentrations of WP addition (i.e., better structural hold post-printing and printed layers were “cleaner”).

3.3.5 Effect of pH and structural protein content on textural properties of 3D-printed processed cheese

Texture Profile Analysis (TPA) analysis was performed for each printed and poured processed cheese recipe sample using the methodology described in Section 3.2.2.8; for ease and clarity of discussion, results for pH and structural protein content are separated into two tables (Table 3.4 (pH) and Table 3.5 (structural protein content)).

3.3.5.1 Effect of pH

There was a statistically significant difference ($p < 0.05$) in hardness, gumminess, chewiness and resilience of recipe C in comparison to both Recipes A and B (Table 3.4). This confirms that altering the pH of the processed cheese formulation significantly affects the texture. Recipe C, which had pH 5.8 (i.e., the highest pH within the pH range tested), was found to be softer, less gummy, less chewy and less resilient than processed cheese samples of Recipe A and B, at pH 5.4 and pH 5.6, respectively. It was also found that Recipe C was significantly less cohesive ($P < 0.05$) than Recipe A, but there was no significant difference between cheese with Recipe C and Recipe B. These results compliment the grid analysis work mentioned in Section 3.3.4, where grids printed using Recipe C were observed to be sticky, soft and generally difficult to print accurately without spreading or dragging.

Adhesiveness of samples appeared to be the sole textural characteristic affected by the action of printing (in the pH dataset). Poured samples were found to be significantly ($P <0.05$) less adhesive than printed samples. There are few studies in this area suggesting why this may be the case. Le Tohic *et al*. (2018) reported a decrease in adhesiveness in 3D-printed samples in comparison to the control, where it was suggested that increased levels of surface oil were expressed during extrusion of the printed samples which in turn acted as a lubricant preventing adhesion to the TPA probe during testing.

Table 3.4. TPA measurements of samples with varying pH level (5.4, 5.6 and 5.8) (poured and printed). Superscript letters indicate homogenous groups in a Tukey-HSD test at 95% confidence level. Those with differing superscript letters are statistically significantly different ($p < 0.05$).

	Hardness (N)		**Adhesiveness (N.s)**		**Springiness (-)**		**Cohesiveness (-)**	
Sample	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*
A	7.94 ± 0.48 ^{b}	8.45 ± 0.31^{b}	-0.13 ± 0.19 ^{a}	-0.62 ± 0.47 ^{b}	0.84 ± 0.03 ^{a}	0.85 ± 0.02 ^{a}	0.80 ± 0.01 ^{b}	0.78 ± 0.02 ^{b}
B	8.28 ± 1.35 ^{b}	9.05 ± 1.16 ^{b}	-0.25 ± 0.13 ^{a}	-0.80 ± 0.31 ^{b}	0.82 ± 0.04 ^{a}	0.82 ± -0.01 ^{a}	0.79 ± 0.01 ^{ab}	0.77 ± 0.01 ^{ab}
C	5.97 ± 0.64 ^{a}	6.40 ± 0.36 ^{a}	-0.17 ± 0.28 ^{a}	-0.83 ± 0.49 ^{b}	0.81 ± 0.05 ^{a}	0.81 ± 0.03 ^{a}	0.77 ± 0.01 ^{a}	0.76 ± 0.01 ^{a}

	Gumminess (-)		**Chewiness (-)**		**Resilience (-)**	
Sample	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*
A	6.35 ± 0.45 ^{b}	6.60 ± 0.31 ^{b}	5.32 ± 0.39 ^{b}	5.60 ± 0.34 ^{b}	0.36 ± 0.01 ^{b}	0.35 ± 0.02 ^{b}
B	6.53 ± 0.98 ^{b}	7.01 ± 0.96 ^{b}	5.36 ± 0.69 ^{b}	5.76 ± 0.80 ^{b}	0.37 ± 0.02 ^{b}	0.35 ± 0.003 ^{b}
C	4.61 ± 0.49 ^{a}	4.86 ± 0.29 ^{a}	3.77 ± 0.61 ^{a}	3.93 ± 0.26 ^{a}	0.34 ± 0.01 ^{a}	0.32 ± 0.01 ^{a}

3.3.5.2 Impact of structural protein content

With regards to the structural protein content data (Table 3.5), differences in cheese maturity (and hence structural protein content) result in statistically significant differences ($P < 0.05$) in hardness, gumminess and chewiness of processed cheese Recipes D, E, F and H (no significant effect on G). Recipe H, which contains 100% fresh curd, was found to be significantly harder than Recipes D (25% young Cheddar, 75% mature Cheddar), F (75% young Cheddar, 25% mature Cheddar) and G (100% mature Cheddar). It was also observed that processed cheese made using fresh curd (Recipe H) was also chewier and gummier than Recipes D and G. This is most likely due to the dense, fibrous protein network (i.e., greater levels of structural protein) of the fresh curd (see S3.2A in Supplementary Material) making up Recipe H. The lack of softening in texture in comparison to Recipes E-G, which contain varying ratios of young and mature Cheddar, could be due to the lack of proteolysis within the fresh curd due to limited ripening. The lack of proteolysis products can be seen in an absence of bands occurring in the lower sections of the Urea-PAGE gel of the fresh Cheddar curd (Fig. 3.2 (left, L2)). This profile is typical of a day 1 fresh curd sample, where there is limited breakdown of α_{S1}-CN (Fenelon and Guinee, 2000; McMahon *et al*., 2014).

Where most textural parameters within this dataset was affected by structural protein content, the only textural parameter affected by the action of printing was cohesiveness, where it was found that poured samples were more cohesive than printed samples. This may be due to the disrupted structure in the printed samples, where the processed cheese mixture was extruded into a ten-layered structure, thereby allowing ten potentially weakened points to be more susceptible to the compressive forces of the TPA probe. In contrast, in the poured samples, the processed cheese mixtures were allowed to settle evenly into one solid and even mass within the silicone mould in preparation for texture analysis. The lack of separate layers within the poured samples may allow the structure to withstand compressive forces, and therefore resist deformation and rupturing.

Table 3.5. TPA measurements of samples with varying structural protein content (poured and printed). Superscript letters indicate homogenous groups in a Tukey-HSD test at 95% confidence level. Those with differing superscript letters are statistically significantly different ($p < 0.05$).

	Hardness (N)		**Adhesiveness (N.s)**		**Springiness (-)**		**Cohesiveness (-)**	
Sample	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*
D	7.23 ± 3.14 [a]	7.85 ± 1.73 [a]	-0.24 ± 0.21 [a]	-0.52 ± 0.52 [a]	0.83 ± 0.02 [a]	0.82 ± 0.01 [a]	0.79 ± 0.01 [a]	0.77 ± 0.01 [b]
E	7.36 ± 0.02 [a]	7.40 ± 1.55 [a]	-0.48 ± 0.36 [a]	-0.33 ± 0.24 [a]	0.82 ± 0.05 [a]	0.82 ± 0.03 [a]	0.78 ± 0.0004 [a]	0.77 ± 0.01 [b]
F	7.85 ± 3.26 [a]	7.91 ± 1.94 [a]	-0.20 ± 0.03 [a]	-0.44 ± 0.39 [a]	0.84 ± 0.01 [a]	0.83 ± 0.02 [a]	0.78 ± 0.01 [a]	0.77 ± 0.02 [b]
G	5.38 ± 1.96 [a]	6.20 ± 2.15 [a]	-0.02 ± 0.02 [a]	-0.51 ± 0.18 [a]	0.82 ± 0.05 [a]	0.80 ± 0.03 [a]	0.76 ± 0.03 [a]	0.75 ± 0.04 [b]
H	10.98 ± 0.66 [b]	11.79± 1.02 [b]	-0.22 ± 0.24 [a]	-0.08 ± 0.06 [a]	0.85 ± 0.01 [a]	0.84 ± 0.004 [a]	0.79 ± 0.004 [a]	0.77 ± 0.01 [b]

	Gumminess (-)		**Chewiness (-)**		**Resilience (-)**	
Sample	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*
D	5.69 ± 2.52 [a]	6.06 ± 1.45 [a]	4.77 ± 2.20 [a]	4.94 ± 1.17 [a]	0.35 ± 0.04 [a]	0.35 ± 0.01 [a]
E	5.79 ± 0.01 [ab]	5.70 ± 1.26 [ab]	4.83 ± 0.25 [ab]	4.64 ± 0.91 [a]	0.35 ± 0.003 [a]	0.35 ± 0.01 [a]
F	6.16 ± 2.64 [ab]	6.08 ± 1.63 [ab]	5.21 ± 2.27 [ab]	5.08 ± 1.48 [a]	0.35 ± 0.04 [a]	0.34 ± 0.01 [a]
G	4.12 ± 1.63 [a]	4.67 ± 1.80 [a]	3.44 ± 1.50 [a]	3.75 ± 1.54 [a]	0.31 ± 0.04 [a]	0.33 ± 0.04 [a]
H	8.69 ± 0.50 [b]	9.09 ± 0.74 [b]	7.38 ± 0.47 [b]	7.60 ± 0.66 [b]	0.39 ± 0.004 [a]	0.35 ± 0.01 [a]

3.3.6 Factor analysis

A quality chart showing the scores of Factors 1 and 2 of the Factor Analysis of all TPA measurements provides an integrated view of the differences between the samples, and is shown in Figures 3.9 and 3.10 for the data set with varying pH and with varying maturity, respectively; the points shown are the averages of three replicate measurements. It is noted that the factor scores in both graphs are from the same Factor Analysis and therefore can be compared. These factors are a multilinear combination of standardised values of the individual measurements, which implies that a score of 0 means an average value, a score of 1 a value above average by one standard deviation, a score of -1 a value below average by one standard deviation, etc. If the distribution of values was normal, 95% of observations would be within -2 and 2. The quality charts represent 2 graphs in each case, with Factor 2 in the y-axis of both, giving the 3D visualization of the Factor space in a projected plane form. The clustering of the TPA variables in the Factor Analysis is shown in Table 3.6, where it can be seen that just 3 Factors explain 92% of the variance of the data generated by the 7 different measurements for all samples (and 3 repeats in each case) and are therefore essentially capturing all of the TPA information of all samples (totaling 336 individual data points). It can also be seen that Factor 1 clusters measurements that quantify attributes of compression, whereas Factor 2 is essentially adhesion behaviour and Factor 3 relates to cohesion of the material. Thus, the terms "compression", "adhesion" and "cohesion" will be loosely used to designate Factors 1, 2 and 3, respectively.

Table 3.6. Loadings of the Factor Analysis of the entire set of data showing the clustering of individual TPA measurements in the factors extracted with Varimax normalised rotation. The dominant TPA measurements in each factor are highlighted in bold.

	Factor 1	**Factor 2**	**Factor 3**
Hardness	**0.963**	-0.088	0.237
Adhesiveness	-0.085	**0.988**	0.056
Springiness	0.235	0.190	**0.834**
Cohesiveness	0.376	-0.155	**0.839**
Gumminess	**0.952**	-0.093	0.282
Chewiness	**0.929**	-0.066	0.344
Resilience	**0.701**	0.113	0.570
Variance explained	48.4%	15.3%	28.3%
Cumulative variance explained	48.4%	63.7%	92.0%

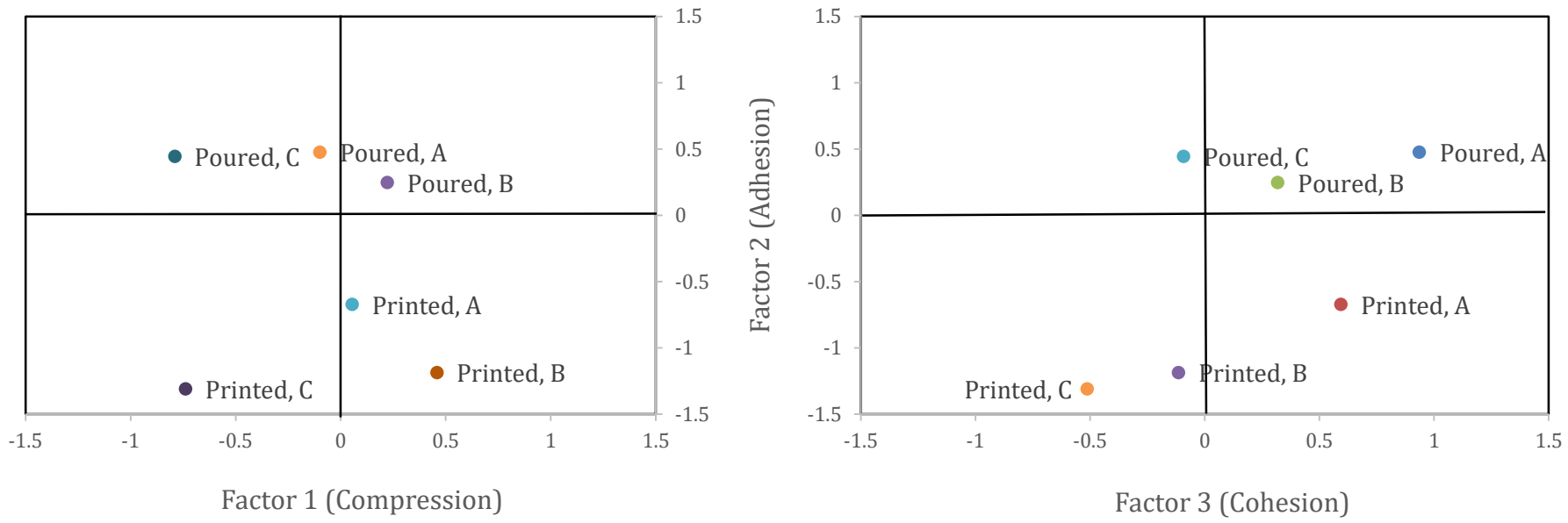

Fig. 3.9. Quality chart with the factor scores of the samples (averages of 3 independent repeat measurements) of the pH dataset (Recipes A-C)

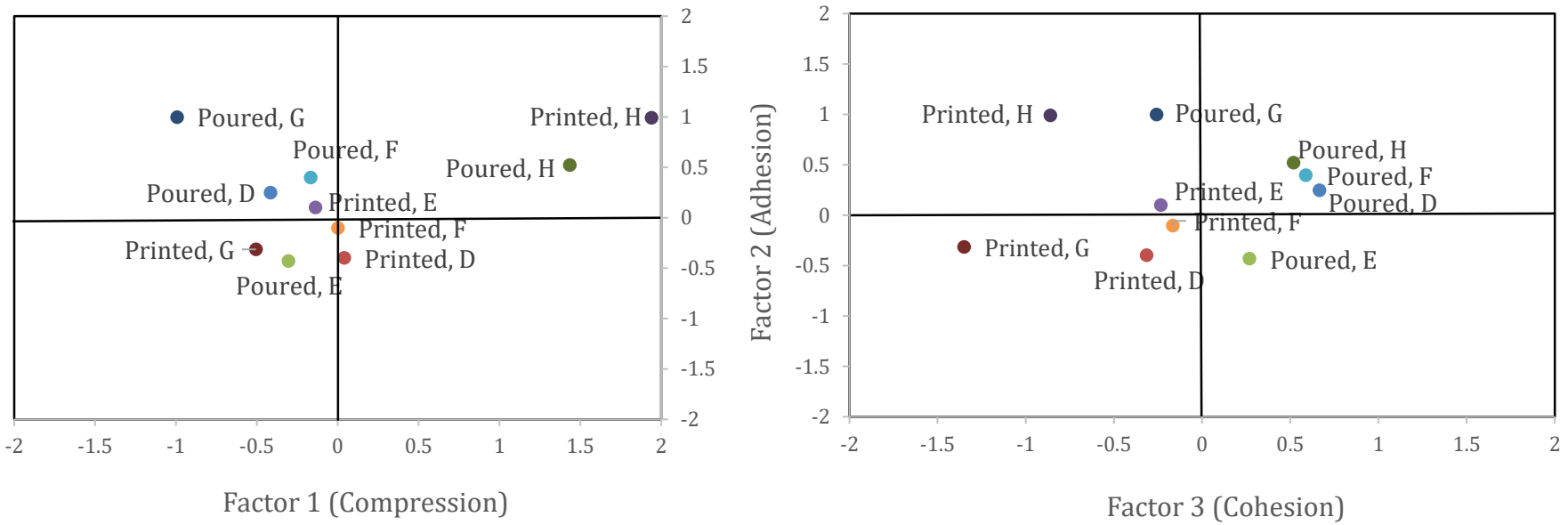

Fig. 3.10. Quality chart with the factor scores of the samples (averages of 3 independent repeat measurements) of the structural protein content dataset (Recipes D-H)

Figure 3.9 shows a clear difference between printed samples and the control, with the former having negative scores in adhesion and the latter positive ones, as already noted as factor 2 is essentially dominated by adhesiveness. One can also see a clear effect of pH in the scores of factor 1 that quantify compression behaviour, with the low pH at 5.4 at the center, the pH of 5.6 with high compression scores and the pH of 5.8 with the low compression scores, whether for printed or control samples.

Figure 3.10 shows less clear differences between samples, with the exception of a significantly higher compression score of samples H, whether printed or control. It should be noted that these are scores of a same factor analysis as Figure 3.9 and that the plots have different scales; sample H had a significantly higher compression score than all others, which means that the fresh curd itself gives a completely different compression behaviour to any of the matured cheese samples, whatever its maturity. It can also be seen that, for the samples where maturity varied, the printed ones tend to generally have high cohesion scores whereas, printed ones have low scores. This is especially true for samples G and H.

The joint analysis of all data allows also to compare the magnitude of the effect of pH with that of maturity in the texture quality factors. A main effects ANOVA was applied to the whole set of data and results are shown in Table 3.7. It is noted that, in a main effects ANOVA, the error includes not only the white noise (variability between repeat measurements), but also all eventual interactive effects (quantifications of whether the impact of one variable depends on the values of another), but calculating interactive effects would have required a full factorial design. In any case, the results are very clear; printing did not affect the compression scores, with pH and maturity both causing statistically significant differences, and with maturity having the most significant impact. This is primarily due to the significantly lower compression score for samples of pH 5.8 and significantly higher score for sample H. On the other hand, printing had a statistically significant impact on adhesion and cohesion, where the effects of pH and of maturity were not significant. In both cases, the outcome was a significantly lower score for the printed samples.

Table 3.8. Main effects Analysis of Variance of the totality of the data. SS are the raw sums of squares and p-values the probability of the null hypothesis (that the variable has no effect)

	Compression		**Adhesion**		**Cohesion**	
Main effects	SS	p-value	SS	p-value	SS	p-value
Printing	0.928	0.196	**6.295**	**0.010**	**6.534**	**0.007**
pH	**3.796**	**0.038**	0.505	0.749	3.482	0.132
Maturity	**21.071**	**0.000**	5.491	0.297	4.350	0.394
Error	20.878		33.752		31.843	

3.3.7 Effect of printing temperature on textural properties and microstructure of 3D-printed processed cheese

The microstructure of processed cheese from Recipe D printed at a higher printing temperature (65°C) compared to the same recipe printed at 40°C was also investigated. TPA was carried out as described previously (See Section 3.2.2.8) to compare the potential differences in texture of the two samples (Table 3.8). The processed cheese samples printed at higher temperatures were harder, less adhesive, gummier and springier than samples printed at lower temperatures. This result is similar to that of Lee *et al*. (1981), where it was found that the firmness of processed cheese increased with increased cooking temperature. The results of an independent T-test show that there is relatively little statistical significance to the textural differences outlined above, with the exception of the resilience of the samples ($P < 0.05$). Processed cheese samples (Recipe D) printed at the higher temperature (65°C) were found to be more resilient than those printed at a lower temperature (40°C), which correlates with the findings of Lee *et al*. (1981).

Table 3.8. TPA measurements of Recipe D 3D-printed at 40°C or 65°C. Superscript letters indicate homogenous groups in a Tukey-HSD test at 95% confidence level. Those with differing superscript letters are statistically significantly different ($p < 0.05$).

Sample	**Hard-ness**	**Adhesive-ness**	**Springi-ness**	**Cohesive-ness**	**Gummi-ness**	**Chewi-ness**	**Resilience**
Units	*N*	*N.s*	-	-	-	-	-
40°C	7.91 ± 1.59 [a]	-0.44 ± 0.32 [a]	0.83 ± 0.02 [a]	0.77 ± 0.01 [a]	6.08 ± 1.33 [a]	5.08 ± 1.21 [a]	0.34 ± 0.01 [a]
65°C	10.56 ± 0.30 [a]	-0.30 ± 0.19 [a]	0.87 ± 0.01 [a]	0.79 ± 0.01 [a]	8.35 ± 0.30 [a]	7.29 ± 0.32 [a]	0.38 ± 0.01 [b]

From SEM micrographs (Fig. 3.11A and B), it was observed that the sample printed at 40 °C (Fig. 3.11A) showed partially coalesced fat globules interspersed amongst a dense protein matrix. However, in the sample printed at 65 °C (Fig. 3.11B), the fat droplets are more numerous, are closer in proximity, and appear to have much higher incidence of coalescence. The higher printing temperatures would seem to accelerate the coalescing effect on the fat globules in comparison to the sample printed at lower temperatures. Kapoor and Metzger (2008) reported that processing conditions such as cook time, cooking temperature, extent of agitation during cooking and rate of cooling of the mixture can have a significant effect on the functional properties of processed cheese. Processing temperatures affect the physical state of the lipid fraction within the processed cheese matrix, where increased temperatures liquefy milk fat, potentially leading to coalescence and formation of large fat globules.

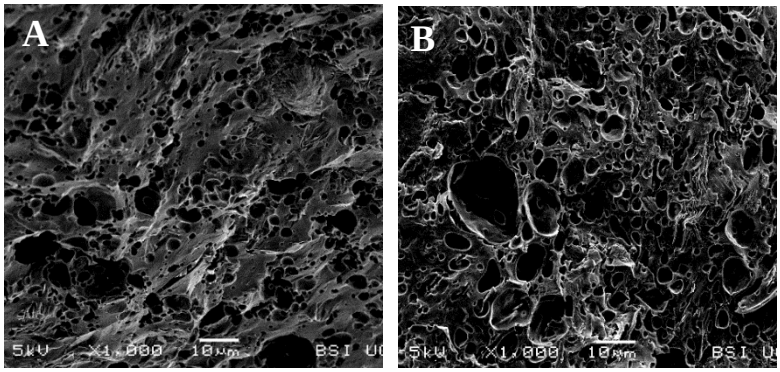

Fig. 3.11. SEM Micrographs showing Fig. 16A – recipe D, 3D-printed at 40°C (Magnification x 1,000) and Fig. 16B – recipe D, 3D-printed at 65°C (Magnification x 1,000)

3.4 Conclusion

There are multiple interrelated factors to take into consideration when 3D printing a food product. In the case of processed cheese, pH and structural protein content are key components in creating 'printable' recipes. These parameters can be optimised depending on users' preference for texture and printing accuracy subject to the desired product's attributes in terms of application. Rheological profiles are also useful when considering the printability of a potential processed cheese recipe; in this particular case, processed cheese mixtures with viscosity between 7.55 – 10.94 Pa.s were the most suitable for printing, meaning that the resulting mixtures were acceptable in terms of printing accuracy due to optimal texture and flow. Temperature is also important in terms of critical processing factors which can affect 'printability' in terms of optimum texture and printing accuracy; at higher printing temperatures (65°C), the structure becomes harder and more resilient to compressive forces, where the coalescing effect of fat droplets is accelerated and there is an increase in droplet size, thereby dominating the overall structural composition, in comparison to fat droplets in samples printed at lower temperatures (40°C). In conclusion, 3D food printing is a growing area of research and discussion. Understanding the fundamental effects of 3D printing on food systems, such as processed cheese, is important in order to progress to 3D printing more complex and intricate structures. Only then perhaps will 3D food printing become more of a common technique within the food sector.

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Supplementary material

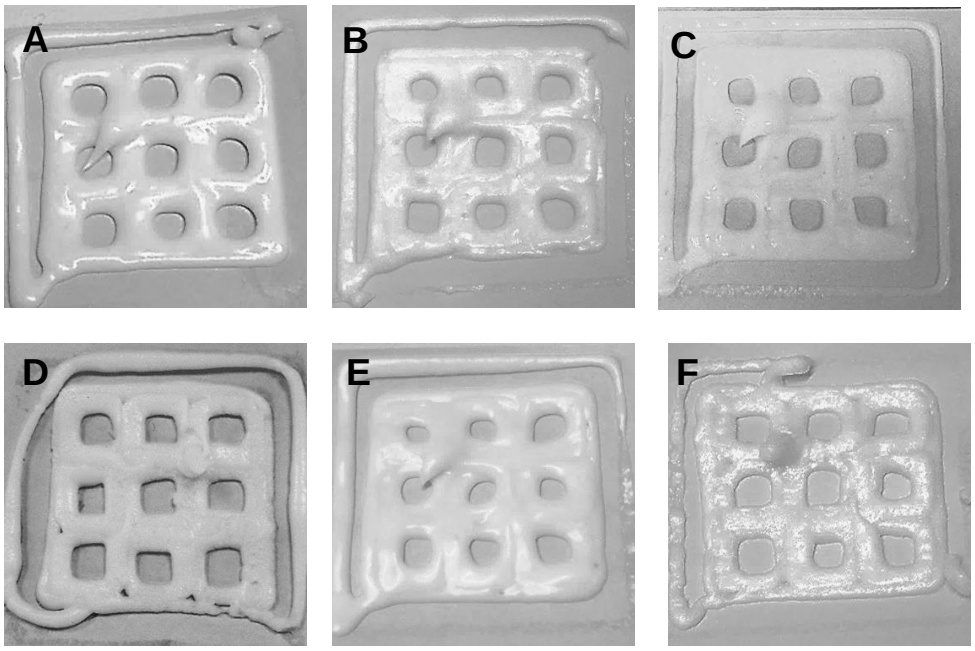

Fig. S3.1A-F. 40 mm 2 printed grid structures with varying TSC contents (at 55% total moisture content), A = 1.5% TSC, B = 2% TSC, C = 2.5% TSC and with varying total moisture contents (with 2.5% TSC), D = 47% Total moisture, E = 50% Total moisture and F = 55% Total moisture

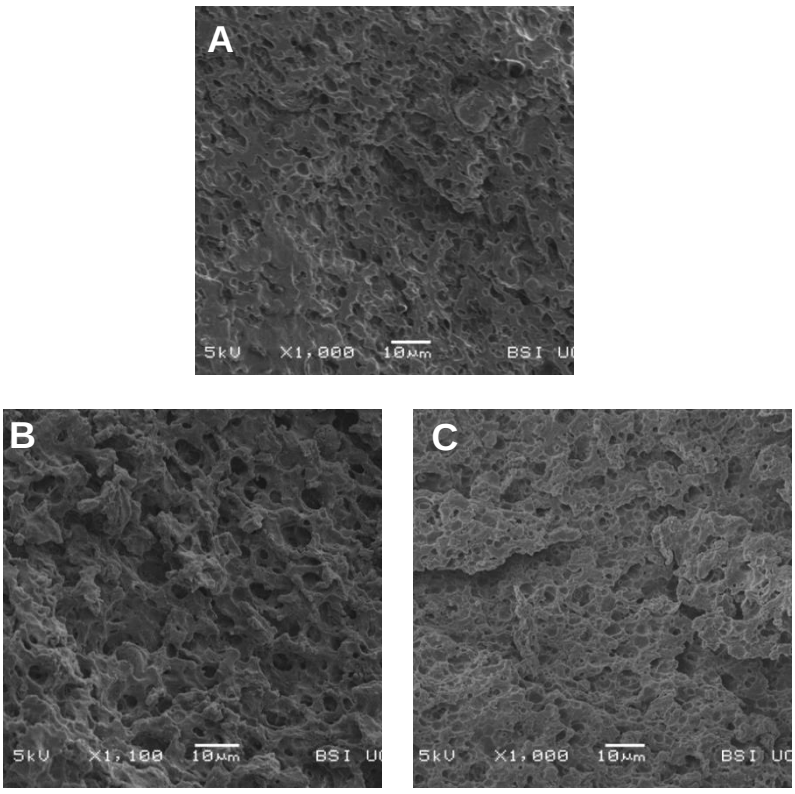

Fig. S3.2A-C. SEM micrographs of cheddar cheese (A – Fresh curd, B – 1-2 month old cheddar and C – 6-8 month old cheddar)

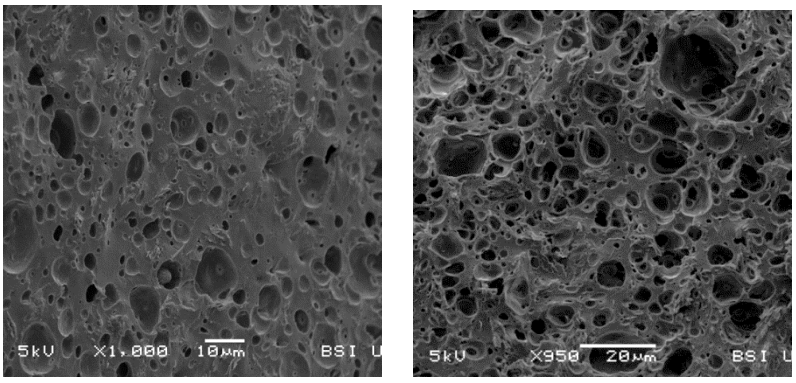

Fig. S3.3. SEM Micrographs of (Left – Recipe A poured samples showed defined uninterrupted fat globules interspersed between continuous protein network, Right – Recipe A printed samples showed partial coalescence of fat globules due to additional heating and shearing forces occurring during printing)

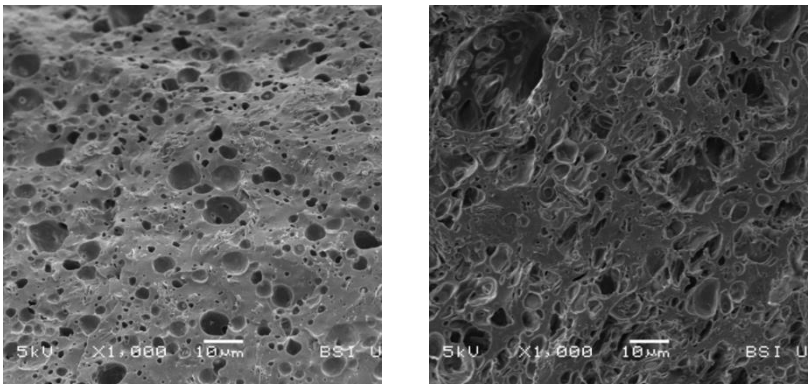

Fig. S3.4. SEM Micrographs of (Left – Recipe B poured samples showed spherical fat globules uninterrupted by printing, Right – Recipe B printed samples showed irregularly shaped fat globules which appear to be partially coalesced due to heating and shearing during printing)

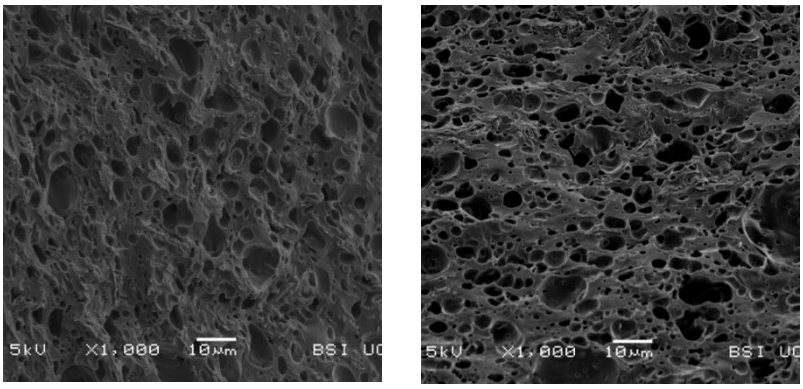

Fig. S3.5. SEM Micrographs of (Left – Recipe C poured samples appear to have fat globules of varying sizes but mostly spherical in shape, Right – Recipe C printed samples show fat globules with irregular shapes and appears to be partially coalesced)

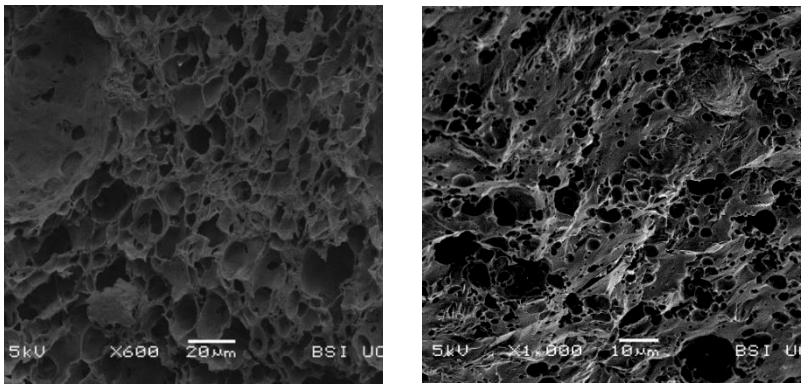

Fig. S3.6. SEM Micrographs of (Left – Recipe D poured samples appear to have a reduced protein network density between the fat portion of the sample, Right – Recipe D printed samples appear to contain fat globules of a smaller size in comparison to poured samples, yet partial coalescence is still occurring more predominantly in printed samples than poured samples due to prolonged heating and shearing during printing)

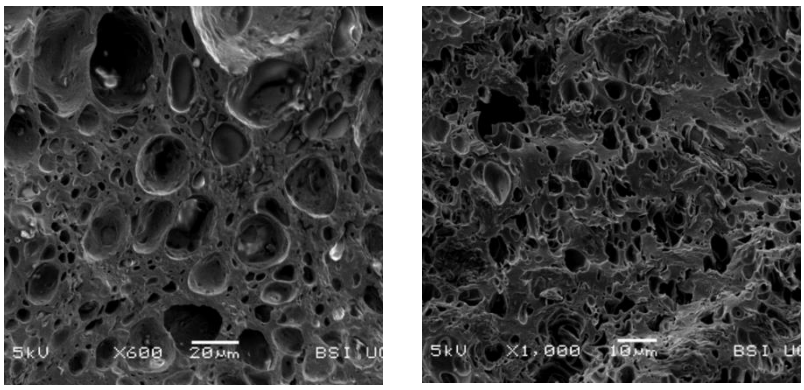

Fig. S3.7. SEM Micrographs of (Left – Recipe E poured samples contain mostly large spherical fat globules interspersed between a reasonably dense protein network, Right – Recipe E printed samples appear to have a lipid fraction which are irregular in shape and are much smaller in size than those appearing in poured samples)

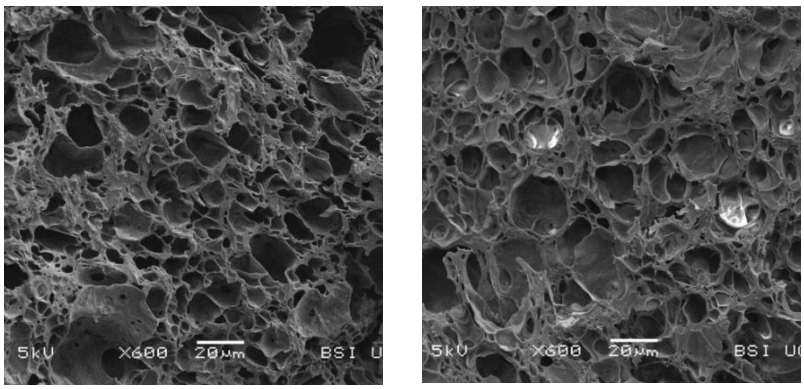

Fig. S3.8. SEM Micrographs of (Left – Recipe F poured samples appear to have a reduced protein network surrounding the irregularly shaped fat pockets within the processed cheese sample, Right – Recipe F printed samples appear to have a similar protein network distribution to poured samples, yet the fat pockets seem to be larger in size)

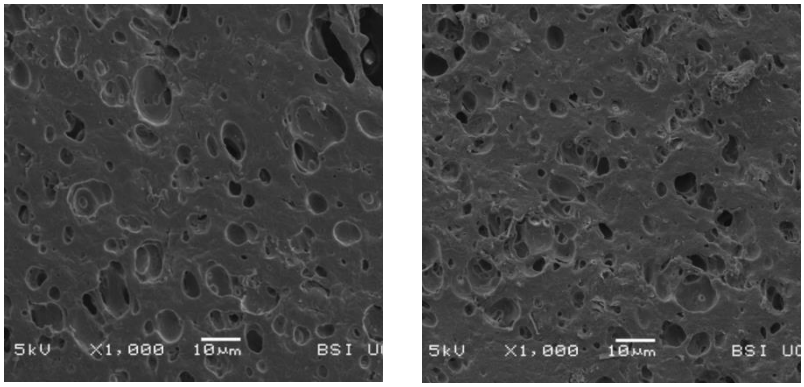

Fig. S3.9. SEM Micrographs of (Left – Recipe G poured samples have defined fat globules surrounded by a dense protein network, Right – Recipe G printed samples have less defined and smaller fat globules that appear to be partially coalesced, presumably due to extended heating and shearing forces during printing in comparison to samples which are poured)

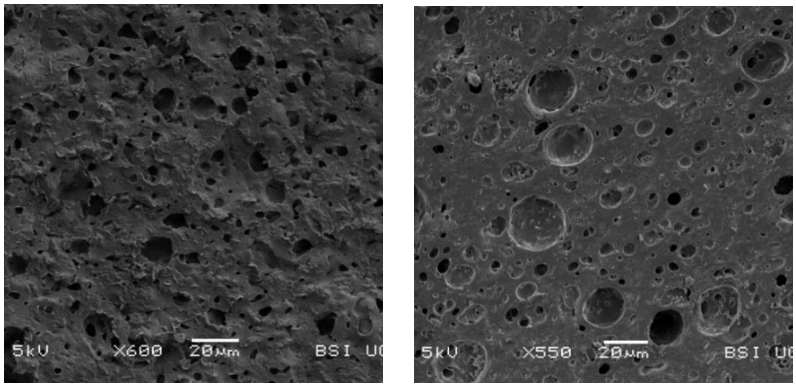

Fig. S3.10. SEM Micrographs of (Left – Recipe H poured samples have varying sizes and shapes of fat globules surrounded by a dense protein network, Right – Recipe H Printed samples appear more regularly shaped fat globules than samples which are poured)

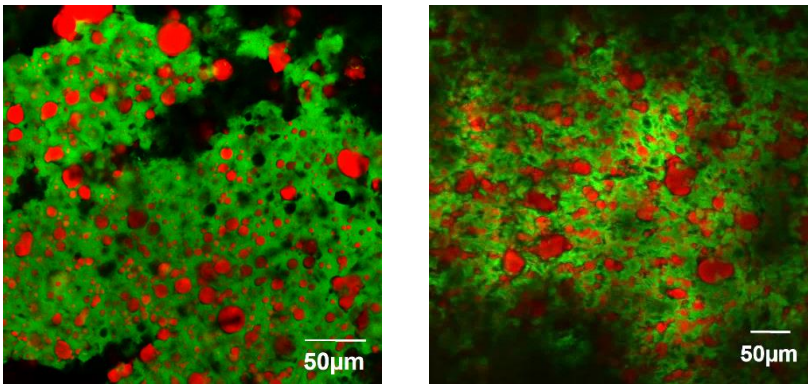

Fig. S3.11. SLM Micrographs of (Magnification x40) (Left – Recipe A poured samples appear to contain regular, spherically shaped fat globules (red) surrounded by a dense protein network (green), Right – Recipe A printed samples have fat pockets which are less defined than those which are poured. The fat portions of the samples are partially coalesced due to extended periods of heating and shearing during printing in comparison to poured samples)

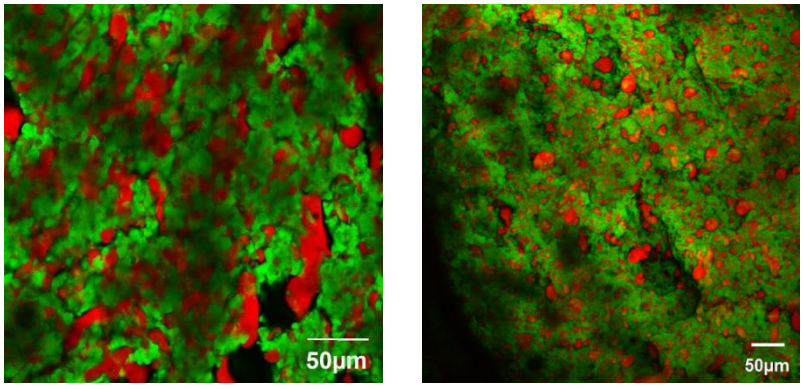

Fig. S3.12. CSLM Micrographs of (Magnification x40) (Left – Recipe B poured samples have fat globules which appear to be pooled and extended, Right – Recipe B printed samples appear to contain fat globules which are less stretched in appearance than poured samples. Fat appears to be smaller in size and there is less interaction between individual fat pockets)

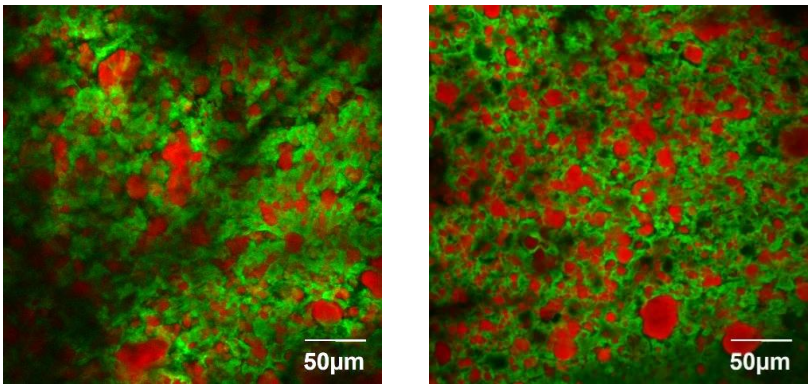

Fig. S3.13. CSLM Micrographs of (Magnification x40) (Left – Recipe C poured samples appear to contain a small number of partially coalesced fat globules interspersed with smaller, irregularly shaped fat pools, Right – Recipe C printed samples appear to more numerous in quantity and densely grouped with very little protein network interspersed between them)

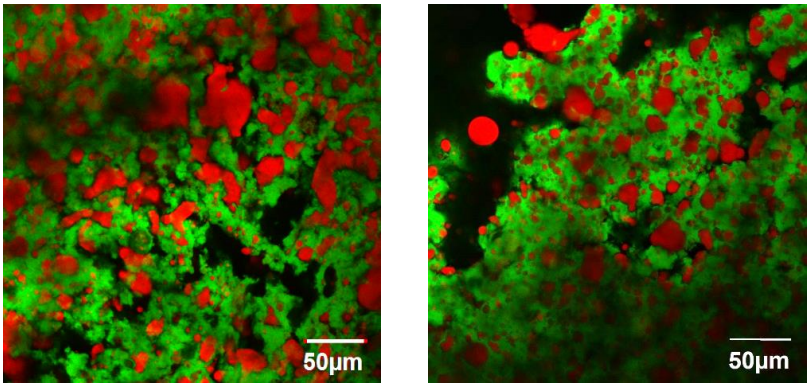

Fig. S3.14. CSLM Micrographs of (Magnification x40) (Left – Recipe D poured samples show mostly large, irregularly shaped fat globules due to partially coalesced smaller fat globules, Right – Recipe D printed samples show more interspersed fat globules of varying sizes)

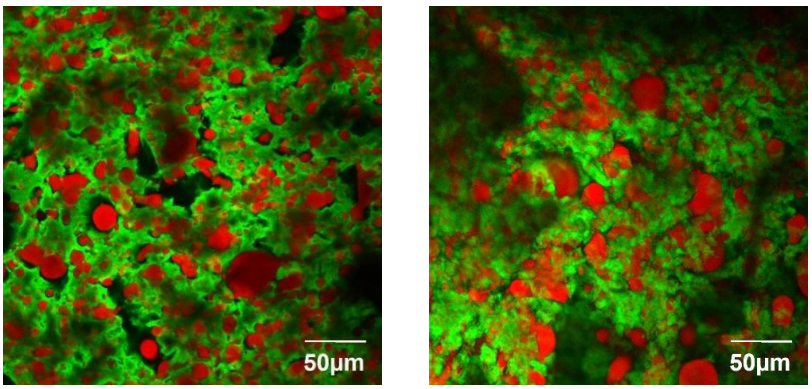

Fig. S3.15. CSLM Micrographs of (Magnification x40) (Left – Recipe E poured samples appear to contain a mixture of spherical and irregularly shaped fat globules, where some of the larger fat globules are interacting to form larger coalesced globules, Right – Recipe E printed samples appear to also contain fat globules of varying sizes and shapes)

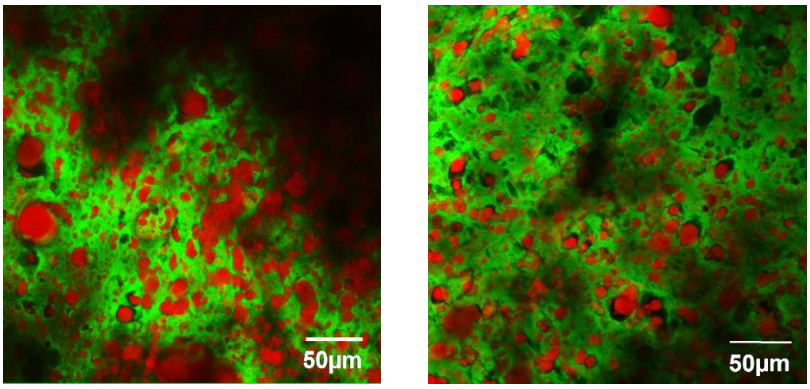

Fig. S3.16. CSLM Micrographs of (Magnification x40) (Left – Recipe F poured samples contain a mixture of large and small fat globules, but most appear to be spherical and regular in shape, Right – Recipe F printed samples contain mostly small fat globules which are widely interspersed between the protein network)

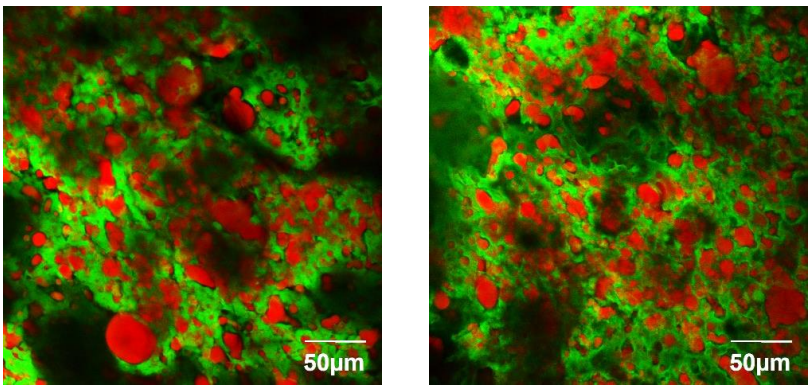

Fig. S3.17. CSLM Micrographs of (Magnification x40) (Left – Recipe G poured samples contain elongated fat pools which vary in size, Right – Recipe G printed samples contain fat globules which are more regular in shape and smaller size than poured samples)

CHAPTER 4

Application of micellar casein concentrate in 3D-printed food structures

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Declaration

This chapter was written by the author MMR and reviewed by their co-authors. MMR co-designed the study, and carried out 3D-printing experiments, and TPA and spectrophotometric measurements. MMR also performed statistical analysis on the data. A version of this chapter has been submitted to *Innovative Food Science and Emerging Technologies* and is currently under review.

Abstract

3D food printing has been touted as a useful tool to create innovative textures and eating experiences. A comprehensive understanding of the effect of internal (e.g., calcium supplementation/chelation) and external (e.g., printed geometry and dissolution temperature) factors on the functionality of 3D-printed Micellar Casein Concentrate (MCC) is therefore, advantageous for the development of potential 3D-printed dairy-based product applications. Calcium supplementation or chelation was found to effect melting behaviour and textural attributes of 3D-printed MCC. Printed samples with higher concentrations of calcium chloride (5 mM) had slower rates of dissolution and solubility than printed control or samples with lower concentrations (1 mM). This trend was augmented at higher dissolution temperatures. Samples printed in a porous 3D-shape with high surface area: volume ratio dissolved at a faster rate than those with low surface area: volume ratio. Overall, highly concentrated MCC systems were demonstrated to be suitable base-materials for 3D-printing snack-sized dairy products.

4.1 Introduction

The demand for three-dimensional printing (3D printing) has increased in recent times. According to global market growth trends for 3D printing, in 2020 there was a 21% increase in market value in comparison to 2019, which translated to an estimated value of $12.6 billion (Everett, 2021). Individuals have been able to take advantage of the customisability of 3D printing, becoming both the "producer and consumer simultaneously" (Bravi *et al*., 2017). It has been suggested that there is a current shift from mass production to more personalised manufacturing, which could improve the consumer's purchasing and user experience by tailoring products to the individual's exact needs and not the other way around (Siacor *et al*., 2021).

For 3D food printing to become accessible and user-friendly for consumers, a fundamental understanding of the impact of formulation on a product's functional properties is essential (Wilms *et al*., 2021). Dairy-based ingredients, such as milk protein concentrate, milk powders, milk protein isolate, whey protein isolate and sodium caseinate, have become increasingly utilised as primary ingredients in 3D food printing research (Lee *et al*., 2020; Lille *et al*., 2018; Liu *et al*., 2018a; Schutyser *et al.,* 2018; Uribe-Alverez *et al*., 2021). However, there are a limited number of studies reporting the use of the casein-based protein ingredient Micellar Casein Concentrate (MCC) as a potential 3D printing material. MCC, also known as native phosphocaseinate, is the dried retentate fraction of micro-filtrated (0.1-0.2 μm pore size) skim milk (Carr and Golding, 2016). After being subjected to a diafiltration treatment, the majority of serum proteins (up to 95%) are removed from skim milk in the permeate stream. Meanwhile, a casein micelle-enriched material remains in the retentate stream, along with some lactose, minerals and a small amount of serum proteins (Beckman *et al*., 2010; Carter *et al*., 2021; Nelson and Barbano, 2005; Saboya and Maubois, 2000; Maubois, 2002). Spray-dried MCC can have poor functional properties (i.e., solubility and rehydration) and may require significant time and appropriate temperature and stirring conditions to ensure sufficient hydration is achieved (Jeantet *et al.,* 2009; Richard *et al*., 2013; Zhang *et al*., 2018). Some studies attribute this poor reconstitution ability to hydrophobic interactions and the slow release of casein micelles into solution from powder particles (Mimouni *et al*., 2009; Schokker *et al*., 2011). Potential commercial applications of MCC include high protein-beverages,

meal substitutes, coffee whiteners and gym supplements (Beckman *et al*., 2010; Chen *et al*., 2019).

In one such study involving MCC, Daffner *et al*. (2021a) recently explored the optimal aggregation rate of cold acidified (pH 5.4 – 4.8) casein-whey protein suspensions *via* a pH-temperature route, where MCC and whey protein isolate were used as the source of protein in each instance. It was found that formulations with a lower pH and higher concentration of protein were associated with an increased aggregation rate and the authors proposed that aggregation rate may be a good indicator of optimal printability. Daffner *et al*. (2021b) also examined the effect of milk fat in a similar casein-whey protein suspension on printability, reporting that the addition of cream was a suitable fat source to be included within the formulation for 3D food printing under certain pH-temperature conditions.

There are few studies of highly concentrated micellar casein suspensions; however, Amelia and Barbano (2013) successfully produced an 18% protein liquid MCC which they claim has a long shelf-life duration up to 16 weeks under refrigerated conditions. The authors attributed this longer shelf-life to the removal of low-molecular weight compounds such as lactose and non-protein nitrogen during the manufacturing process which involves ultrafiltration of skim milk followed by a three-stage microfiltration process. Others, such as Lu *et al*. (2016), have studied the cold gelation properties of high concentrated micellar casein concentrate systems (23% total protein), where thermo-reversible behaviour was demonstrated when solutions of MCC mixed with cream formed a gel at low temperatures (5°C) and converted to a liquid state when heated to 50°C. This phenomenon was said to be due to extensive dissociation of the casein micelle at cooler temperatures, allowing for the necessary (hydrogen) bonding interactions (Lu *et al*., 2016). In the same study, it was also observed that certain factors had an influence on the rheological characteristics of the MCC and cream mixture. For example, the addition of sodium citrate and high levels of calcium increased the cold gelation temperature; however, lowering the pH from neutral pH to pH 6.6 reduced the cold gelation temperature. Suspensions with a pH above pH 6.7 appeared to gel at higher temperatures (> 26°C), which was attributed to the increase of α_S- and β-CN dissociating from casein micelles, thereby increasing the number of protein strands available to entangle and

interact with different particles and leading to the eventual formation of a gel (Lu *et al*., 2016).

To the authors' knowledge, there are no studies conducted at the time of writing involving 3D printing of MCC as the sole dairy-based ingredient in a printable formulation. The impact of calcium activity on casein micelle integrity in MCC systems has been investigated (Hussain *et al*., 2011; Schokker *et al*., 2011; Schuck *et al*., 2002), but not in terms of its effect on printability (e.g., texture and rheological characteristics). The aim of this project was to expand on the research currently undergoing in the 3D printing dairy ingredients space and to study the influence of various factors on the functional properties of 3D-printed MCC, such as the influence of calcium supplementation (i.e., addition of a range of CaCl2 concentrations) and calcium chelation (i.e., addition of a range of Tri-sodium Citrate, or TSC concentrations) on texture, protein solubility and dissolution rate. Other experimental parameters such as the geometry and melting temperature of the printed object were examined to explore 3D food printing as a method to enhance the dissolution rate of liquid MCC concentrates. Fast-melting MCC gels may present an alternative to slow-dissolving powders (Amelia and Barbano, 2013) and could potentially offer "melt-in-mouth" properties at low temperatures (< 40°C). As MCC is a high-protein, neutral tasting ingredient that has structure-forming properties of potential use in 3D food printing, the objective of this study was to increase our understanding of how it might be applied effectively.

4.2 Materials and Methods

4.2.1 Materials

Micellar Casein Concentrate (MCC 88) was obtained from Milei GmbH (Leutkirch im Allgäu, Germany). The MCC contained 88.8% protein, 4.2% moisture, 1.3% fat, 0.8% lactose and 8% ash. A 1 M Hydrochloric (HCl) acid or 1 M Sodium Hydroxide (NaOH) solution (both from Sigma-Aldrich, Arklow, Co.Wicklow, Ireland) was used to adjust the pH of the MCC solutions to a constant pH (See Section 2.2 for more details). Tri-sodium citrate (TSC) and calcium chloride ($CaCl_2$) (anhydrous, granular) were purchased from Sigma-Aldrich (Arklow, Co.Wicklow, Ireland). A Bicinchoninic Acid (BCA) kit and powdered Bovine Serum Albumin were also purchased from Sigma-Aldrich (Arklow Co.Wicklow, Ireland).

4.2.2 Methods

4.2.2.1 3D Printer Modifications

A Tronxy X8 (Shenzhen Tronxy Technology Co., Ltd., China) 3D printer was used in this project. The print original print head was modified to 3D print food by replacing the hot-end assembly with a custom-made stainless steel syringe mount and syringe barrel. The modified print head was also fitted with heater and thermistor wires to allow the insulated syringe to be heated in a controlled manner. For more information on these modifications, see Ross *et al.* (2021).

4.2.2.2 Preparation of MCC for printing

To prepare MCC solutions for 3D printing, TSC or calcium chloride was added to a beaker of deionised water to make up the solution concentrations mentioned in Section 4.3.1 Solutions were stirred until particles were completely dissolved and then covered. A control solution was included which did not contain any TSC or calcium chloride to compare the effect of calcium chelation/supplementation on the MCC mixture. The beakers were then heated in a water bath at 50°C for approximately 15 - 20 minutes, or until a temperature of 50°C was reached. As the final mixture of 30% w/v total solids was quite viscous and paste-like, it was found to be difficult to take an accurate pH reading. Therefore, to ensure consistent and accurate pH readings, only a partial amount of the total quantity of MCC powder was added where the mixture was still liquid (approx. 5% w/v MCC solution) and the pH of each solution measured using a pH meter (Mettler Toldedo, Columbus, Ohio, United States). 1 M HCl or 1 M NaOH was added drop-wise using a Pasteur pipette to make sure each solution was consistent with the control sample pH (pH 6.8 ± 0.05). The remainder of MCC powder was added gradually to make up the solutions to a final concentration of 30% w/v total solids. The solutions were mixed thoroughly after each addition of powder to ensure no lumps of undissolved powder remained. The beakers were then covered once again and returned to the 50°C water bath for 1-2 hour(s) to ensure the powder was suitably rehydrated.

For printing, the solutions were filled into a modified 20-ml syringe (Terumo Syringe without needle (Leur lock tip), Terumo Europe, Leuven, Belgium) with an extended pipe nozzle which was used to accurately fill the stainless steel printing syringe to ensure no gaps or air pockets were present. The solutions were then 3D-printed at 40°C at a print

speed of 20 mm/s using a 2 mm (diameter) nozzle. Each 3D-printed shape (e.g., disk, cylinder, grid etc.) also included a single skirt line, which was added 5 mm away from the structures to act as a priming step to avoid printing inconsistencies and broken lines during the initial print. Printing parameters, such as infill percentage and flow rate for each 3D-printed shape, will be discussed in more detail in subsequent sections.

4.2.2.3 Rheological melting profiles

An AR-G2 rheometer (TA Instruments, Leatherhead, Surrey, UK) with a Peltier plate temperature system was used to determine the melting profiles for each of the MCC solutions. A stainless steel cross-hatched parallel plate (d = 40 mm) was used in combination with a cross-hatched Peltier plate surface attachment to ensure a secure grip on the sample during melting. Each MCC sample was 3D-printed into 3 mm thick disks (d = 40 mm) with 75% grid infill. Samples were 3D-printed at least in triplicate to measure and control for variability between individual samples. 3D-printed disks were refrigerated for approximately one hour prior to testing to increase structural integrity during handling and positioning of the samples during analysis. The 3D-printed MCC disks were gently loaded onto the cross-hatched Peltier plate attachment and a 2 N axial force was applied to grip the sample, after which the sample was allowed rest for five minutes to minimise any compression effects of the applied force on the structure. The exposed edges of the sample (i.e., area of the sample not covered by the parallel plate) were removed using a sharp scalpel and liquid paraffin oil lightly applied using a dropper to the edges to prevent moisture evaporation. A solvent trap was also used to further prevent moisture loss in the sample.

Prior to conducting the dynamic temperature sweep test, the Linear Viscoelastic Region (LVR) of the sample was determined using a dynamic strain sweep and frequency sweep test. The strain sweep test was conducted between a range of 0.01 and 100 rad/s, where a strain value of 1% was found to be within the LVR. A frequency sweep test was then conducted within a frequency range of 0.1 and 20 rad/s in 1 rad/s increments to determine an appropriate frequency range. Thus, a dynamic temperature sweep test was performed with an angular frequency of 1 Hz and a strain of 1%, starting at 20°C and heating to 80°C at a rate of 5°C/min. A conditioning step was carried out before the temperature ramp for 5 minutes at 20°C to ensure the sample was equilibrated to the starting temperature of the temperature ramp to avoid thermal shock of the sample.

The loss tangent, tan δ, was recorded and is described as the ratio between the viscous modulus (G") and the storage modulus (G'), where tan δ_{max}, represents the point at which the sample exhibits the greatest flowability (Foegeding *et al*., 2011). To describe the melting behaviours of each of the MCC samples (control and samples with various concentrations of TSC/calcium chloride), tan δ was plotted against temperature to generate a rheological profile.

4.2.2.4 Texture Profile Analysis

Each MCC sample was 3D-printed in triplicate into cylinders (d = 20 mm, AR = 1) with 50%-line infill. Samples were refrigerated at 4°C for one-hour post-printing, before being removed for a further hour to equilibrate samples to room temperature for accurate testing. TPA was conducted on the MCC cylinders using a texture analyzer TA-XT2i (Stable Microsystems Ltd, UK) with a 5 kg load cell, as described by O'Mahony *et al*. (2008). The TPA measurement consisted of two compression-decompression cycles, separated by a time interval of 5 s, at a rate of 1 mm/s, using a cylindrical aluminium geometry (diameter of 35 mm). The probe compressed the sample to 25% (5 mm) of its initial height (20 mm) before decompression. The TPA indicators recorded were hardness, adhesiveness, springiness (omitted from results as values were equivalent and not statistically significant), cohesiveness and resilience, and at least triplicate measurements were performed for each formulation.

4.2.2.5 Turbidity measurements

Turbidimetry has been shown to be an appropriate tool in describing the rehydration characteristics of various milk-protein powders by identifying the particle size distribution and colloidal suspension stability (Crisp *et al*., 2007; Gaiani *et al*., 2007; Hussain *et al*., 2011). Turbidimetry was utilised in this study to determine the effect of temperature, calcium supplementation and structural geometry on dissolution rate of 3D-printed MCC samples. This was achieved by measuring the absorbance of aliquots of the dissolution solution over a set period of time.

To compare the effect of temperature on the dissolution rate of 3D-printed samples, a set volume of water (300 ml of deionised water in 600 ml volume beakers) was adjusted to 21°C (ambient) or 37°C (i.e., approximating body temperature); the latter was intended to simulate the approximate rate of dissolution in the mouth (i.e., 'melt-in-the-mouth'

sensation). Each beaker of water was magnetically stirred using a stirring box and magnetic stirrers. Deionised water was brought to the higher dissolution temperature (37°C) using a heated water bath with magnetic stirring box underneath.

To compare the effect of the geometry (i.e., exposed surface area and volume) of the 3D-printed structure on dissolution rate, two models were chosen, a hemisphere (1 face) and a porous lattice structure (multiple faces) (Fig 4.1A and B). The hemisphere (d = 40 mm, h = 20 mm) (Fig. 4.2A) shape was 3D-printed with 40% grid infill, whereas, the lattice (l = 40 mm, w = 40 mm, h = 10 mm) (Fig. 4.2B) shape was 3D-printed with no infill, but at a 90% flow rate to avoid spreading of material due to over extrusion of material (Note: the addition of structure-enhancing additives such as hydrocolloids could have improved the accuracy and quality of the printing material, however for the purpose of this study the properties of MCC alone were intended to be explored without the aid of other additives). The dimensions, infill percentage and flow rate were chosen so that both shapes would be comparable in terms of roughly equal weight (Table 4.1).

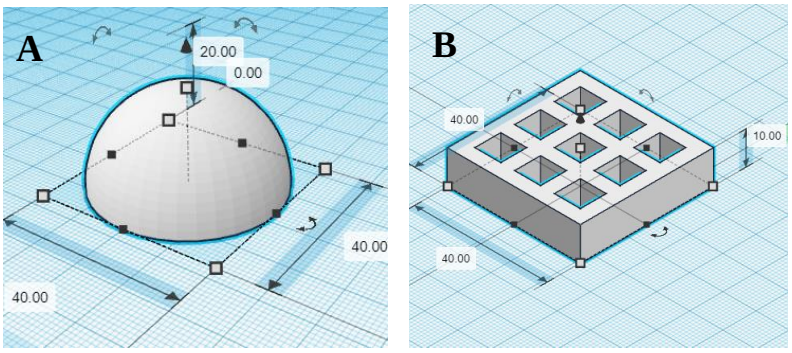

Fig. 4.1A and B. 3D-model dimensions of (A) hemisphere and (B) lattice (Note: dimensions are in mm)

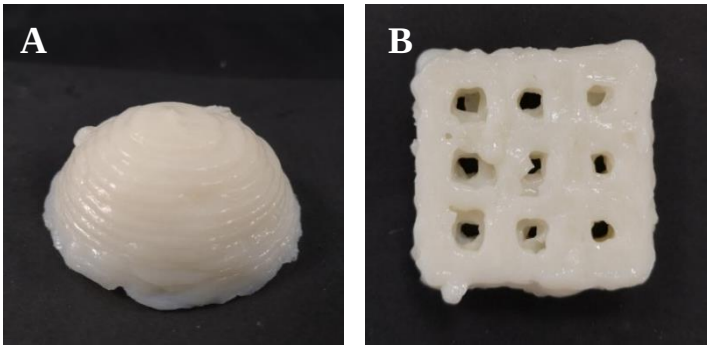

Fig. 4.2A and B. Comparison of effect of geometry on melting rate and solubility for (A) hemisphere and (B) lattice.

Table 4.1. Surface area, volume, and surface area: volume ratio of both 3D-printed MCC shapes (all values mentioned are theoretical with the exception of average weight, which was measured after each print, and an overall average taken for each printed shape).

Shapes	**Surface area**	**Volume**	**Surface area: Volume ratio**	**Average weight**
Units	mm^2	mm^3	mm^{-1}	*g*
Hemisphere	2512	16747	0.15	15.42 ±0.71
Lattice	6528	10240	0.64	15.99 ±0.87

Samples were refrigerated (4°C) for one-hour post-printing before being weighed to ensure samples were similar in weight to reduce inconsistencies in dissolution rate. Samples were then allowed rest at room temperature for one further hour to equilibrate to ambient temperature, and then gently dropped into the beaker of stirred water and a stopwatch was started. Aliquots (2 ml) were taken from the beaker at set time intervals and displaced into 2-ml Eppendorf tubes (Eppendorf, Hamburg, Germany) and centrifuged (Eppendorf Centrifuge 5418R, Eppendorf, Hamburg, Germany) at 2,800 rpm (700 *g*) for 10 minutes at room temperature (21°C). The supernatant was removed from the Eppendorfs and deposited into 1-ml polystyrene semi-micro cuvettes (Sarstedt AG and Co., Nümbrecht, Germany). Absorbance of samples were read in triplicate using a Varian Cary 300 UV-Vis Bio Spectrophotometer (Varian, Inc., Palo Alto, CA, USA) as transmission of light (λ = 700 nm) through a 1-cm path length (de Kort *et al*., 2011; Mizuno and Lucey, 2005). An average absorbance value was calculated for each sample and converted into turbidity values using the calculation described by Herri *et al*. (1999) and Pitkowski *et al*. (2008).

4.2.2.6 Protein solubility

Bicinchoninic Acid (BCA) assays were employed to quantify the amount of dissolved protein, and therefore, simultaneously indirectly calculate the quantity of undissolved protein by subtraction. Aliquots of samples used for measuring absorbance (see Section 4.2.2.5) were transferred to Eppendorf tubes once turbidity values were obtained, and frozen until the day prior to BCA testing, where they were defrosted over a period of 24

hrs at refrigerated temperatures (4°C). Samples were allowed to come to room temperature and thoroughly vortexed before being diluted with deionised water (1 in 4 dilution) and vortexed once again. The samples and BCA working solution were prepared and loaded into a 96-well flat-bottomed clear assay plates (Grenier CELLSTAR 96-well plates, Grenier Bio-one, Frickenhausen, Germany) according to the method used by Smith *et al*. (1985). Samples were loaded at least in duplicate and a dilution series (0 – 2.0 mg/ml protein) of 1% w/v Bovine Serum Albumin (BSA) was used as the protein standard to create the standard curve. Plates were covered with a lid to prevent evaporation and incubated at 37°C for one hour. Plates were then read with a microplate reader (Varioskan Flash 3001, Thermo Fisher Scientific) at a wavelength of 570 nm.

4.2.2.7 Statistical Analysis

Statistical analysis for this study was conducted in IBM SPSS Statistics Version 26 (IBM Corporation, US). One-way Analysis of Variance (ANOVA) was carried out, with *post-hoc* analysis using the Tukey HSD test to compare the impact of calcium supplementation or calcium chelation on the textural characteristics of the product. Differences were deemed statistically significant if $p < 0.05$ (5% or lower probability of the null hypothesis).

4.3 Results and Discussion

4.3.1 Preliminary experimentation

A range of concentrations of MCC suspensions (17-30 % w/v MCC powder) were produced before identifying that 30% MCC was the most suitable concentration in terms of printing consistency by manually syringing each MCC solution. The necessary printing consistency was considered a sample that was liquid enough to flow through the nozzle uninhibited, yet solid enough to form self-supporting layers once deposited onto a flat surface. The MCC suspensions (17-30 % w/v) were also tested across a pH range between pH 6.0 and pH 9.0 to determine whether each solution could pass the initial manual syringing test before being deemed suitable for use in the 3D food printer. Mixtures with a more alkaline pH ($pH \geq 8.0$) were found to be brittle and dry and tended to clog the syringe nozzle. According to Sinaga *et al*. (2017), increasing pH (up to pH 8.5) leads to an increase in negatively charged casein micelles, which results in repulsive charges

between the supramolecules (i.e., complex multi-molecular structures). This contributes to a more open and loose casein micelle structure that swells and increases in size at an alkaline pH. Above pH 8.5, due to this shift into more alkaline conditions, casein micelles tend to decrease in size, in addition to serum calcium phosphate being precipitated into the serum phase, leading to low levels of ionic calcium and non-micellar calcium. These conditions lead to casein micelle dissociation (i.e., breakage of hydrophobic bonds amongst casein micelles) and also a reduction in milk whiteness and turbidity values (Odagiri and Nikerson 1965; Sinaga *et al*., 2017).

Suspensions with higher MCC concentrations (22 – 30 % w/v MCC powder) at natural pH (pH 6.7-6.8) were considered the most printable when manually syringed. The suspensions could easily be syringed and remain in a solid layered structure once extruded through the syringe nozzle. MCC suspensions at a more acidic pH (pH 6.0) were too liquid to form a solid self-supporting structure once extruded. It has been observed that decreasing pH leads to a decrease in net charge of casein micelles and solubilisation of Colloidal Calcium Phosphate (CCP) into solution. Loss of CCP leads to casein micelle shrinkage and dissociation (Dalgleish and Law, 1988; Sinaga *et al*., 2017). However, as MCC samples were at pH ≥ 6.0 and well above the isoelectric point (pH 4.6) of casein, the dissociation effect of the pH decrease was minimal.

Once the concentration and pH of MCC suspensions were optimised (30% w/v MCC powder at natural pH), the impact of tri-Sodium Citrate (TSC) and calcium chloride ($CaCl_2$) were investigated. Concentrations of 5, 10 and 15 mM TSC were examined, as well as 1, 2.5, 5, 10, 15, 20 mM $CaCl_2$. It was observed that solutions containing TSC caused a thickening of the solution, which has been observed by others to be caused by a reduction in CCP through calcium chelation, resulting in the dissociation of casein micelles (Kaliappan and Lucey, 2011; Lu *et al*., 2015). It was also observed that suspensions formulated with more than 20 mM $CaCl_2$ were too soft and brittle to handle manual manipulation (i.e., movement and manual handling of the sample for texture and rheological analysis).

Table 4.2. TPA measurements of 3D-printed MCC cylinders with either calcium chelators/supplementation added, plus the control (without any additions). Superscript letters indicate significant differences in a Tukey-HSD test at 95% confidence level. (Note: The measure for springiness was not included as values were not statistically significant)

Sample	**Hardness**	**Adhesiveness**	**Cohesiveness**	**Gumminess**	**Chewiness**	**Resilience**
Units	*N*	*N.sec*	-	-	-	-
Control	1.83 ± 0.32^{a}	$-0.11 \pm 4 \times 10^{-2\ bc}$	$0.009 \pm 9 \times 10^{-5\ b}$	$0.02 \pm 3 \times 10^{-5\ abd}$	$0.0002 \pm 1 \times 10^{-7\ ac}$	$0.01 \pm 5 \times 10^{-4\ c}$
5 mM TSC	4.30 ± 0.30^{d}	$-0.001 \pm 5 \times 10^{-4\ b}$	$0.008 \pm 3 \times 10^{-4\ b}$	$0.04 \pm 5 \times 10^{-5\ c}$	$0.0005 \pm 2 \times 10^{-7\ d}$	$0.007 \pm 7 \times 10^{-3\ b}$
10 mM TSC	4.18 ± 0.31^{d}	$-0.003 \pm 2 \times 10^{-3\ b}$	$0.009 \pm 1 \times 10^{-4\ b}$	$0.04 \pm 7 \times 10^{-5\ c}$	$0.0003 \pm 2 \times 10^{-8\ a}$	$0.01 \pm 2 \times 10^{-4\ b}$
1 mM $CaCl_2$	1.87 ± 0.27^{a}	$-0.19 \pm 2 \times 10^{-1\ c}$	$0.009 \pm 1 \times 10^{-4\ b}$	$0.02 \pm 3 \times 10^{-5\ ad}$	$0.0002 \pm 1 \times 10^{-7\ ac}$	$0.005 \pm 2 \times 10^{-4\ ac}$
2.5 mM $CaCl_2$	1.54 ± 0.40^{a}	$-0.37 \pm 2 \times 10^{-1\ a}$	$0.008 \pm 2 \times 10^{-4\ b}$	$0.01 \pm 1 \times 10^{-4\ d}$	$0.0002 \pm 3 \times 10^{-7\ bc}$	$0.004 \pm 5 \times 10^{-4\ a}$
5 mM $CaCl_2$	2.05 ± 0.44^{ab}	$-0.01 \pm 9 \times 10^{-3\ b}$	$0.008 \pm 1 \times 10^{-4\ b}$	$0.02 \pm 6 \times 10^{-5\ ad}$	$0.0002 \pm 2 \times 10^{-8\ bc}$	$0.01 \pm 3 \times 10^{-4\ c}$
10 mM $CaCl_2$	2.65 ± 0.49^{bc}	$-0.01 \pm 7 \times 10^{-3\ b}$	$0.007 \pm 2 \times 10^{-4\ a}$	$0.02 \pm 1 \times 10^{-4\ ab}$	$0.0002 \pm 7 \times 10^{-9\ bc}$	$0.004 \pm 3 \times 10^{-4\ a}$
20 mM $CaCl_2$	2.95 ± 0.27^{c}	$-0.005 \pm 4 \times 10^{-3\ b}$	$0.007 \pm 7 \times 10^{-4\ a}$	$0.02 \pm 2 \times 10^{-4\ ab}$	$0.0002 \pm 1 \times 10^{-6\ ac}$	$0.004 \pm 8 \times 10^{-4\ a}$

4.3.2 *Effect of calcium supplementation or chelation on textural properties of 3D-printed MCC*

To gauge the effect of calcium supplementation or chelation on the textural characteristics of 3D-printed MCC cylinders (printed according to the method outlined in Section 4.2.2.2.) a number of properties were measured using a texture profile analyser (Table 4.2). A subset of samples was chosen to represent both types of sample containing TSC (5 or 10 mM TSC) and $CaCl_2$ (1, 2.5, 5, 10 and 20 mM $CaCl_2$).

Printed samples containing calcium chelating salt (5 or 10 mM TSC) were found to be significantly harder, gummier and more resilient ($P < 0.05$) than control samples and those containing calcium chloride. Lu *et al.* (2016) reports that the addition of TSC to highly concentrated MCC suspensions led to dissociation of the casein micelles due to a reduced influence of calcium-mediated interactions between micelles. As a result of dissociation, the primary casein particles can be more densely packed together, possibly contributing to a firmer texture. It was also noted that samples containing higher $CaCl_2$ concentration ($\geq$ 10 mM $CaCl_2$) were significantly ($P < 0.05$) harder than those with lower concentrations (1-5 mM $CaCl_2$). This could potentially be due to the increase in calcium activity as more calcium is added to the formulation, thereby, increasing the incidence of calcium crosslinking and creating a stronger network and resulting in increased firmness (Lin *et al*., 2020). A correlation matrix (Table S4.1 and Fig. S4.1) is provided in the supplementary material to illustrate statistical relationships between the experimental variables from this study, which also includes the relationship between calcium addition and TPA indicators.

4.3.3 *Effect of calcium supplementation or chelation on melting behaviour of 3D-printed MCC*

To compare the effect of calcium supplementation or chelation on 3D-printed MCC disks (d = 40 mm) (Fig. 4.3A and B), samples were subjected to a temperature ramp (21 - 80°C) and the rate of deformation of structure (i.e., melting) was measured (Fig. 4.4). The control sample was the least meltable and most solid-like in comparison with the samples containing calcium chloride or calcium chelators. This trend was apparent at the initial rise in temperature until approximately 40°C, before melting slightly. It was also observed that, at lower melting temperatures (i.e., 21°C - 40°C), samples supplemented with calcium concentrations $\geq$ 10 mM $CaCl_2$ had tan δ values (0.23 –

0.27) almost double that of other samples (i.e., the control, samples ≤ 5 mM $CaCl_2$ and samples containing TSC), which would indicate that these samples were softer and less elastic. According to other sources, tan δ values of < 1.0 indicates that the material is solid-like (as the ratio of G' is greater than G" (i.e., G' > G")) (Álverez-Castillo *et al*., 2021; Masbernat *et al*., 2021), so in this case all samples were solid-like (all tan δ values < 1.0), while some exhibited more solid-like characteristics than others. Wang *et al*. (2018) noted that, in gelled 5% w/w Milk Protein Concentrate (MPC) samples acidified using glucono-δ-lactone (GDL), there was an indirect relationship between calcium chloride concentration (5-30 mmol/L $CaCl_2$) and tan δ and G' values (i.e., as calcium concentrations increased, tan δ and G' appeared to decrease). The same authors also noticed the formation of thicker strands and more aggregated clusters at higher $CaCl_2$ concentrations (> 10 mM $CaCl_2$), which lead to a collapse of the gel network and floc formation. Furthermore, it was also observed that gels in the same study appeared looser and crumblier in appearance and appeared to have inconsistent pore sizes in comparison to samples with lower $CaCl_2$ concentrations. This can also be seen in the current study in Fig. 4.3A where samples with increasing concentration of $CaCl_2$ appear much rougher due to larger aggregates being produced.

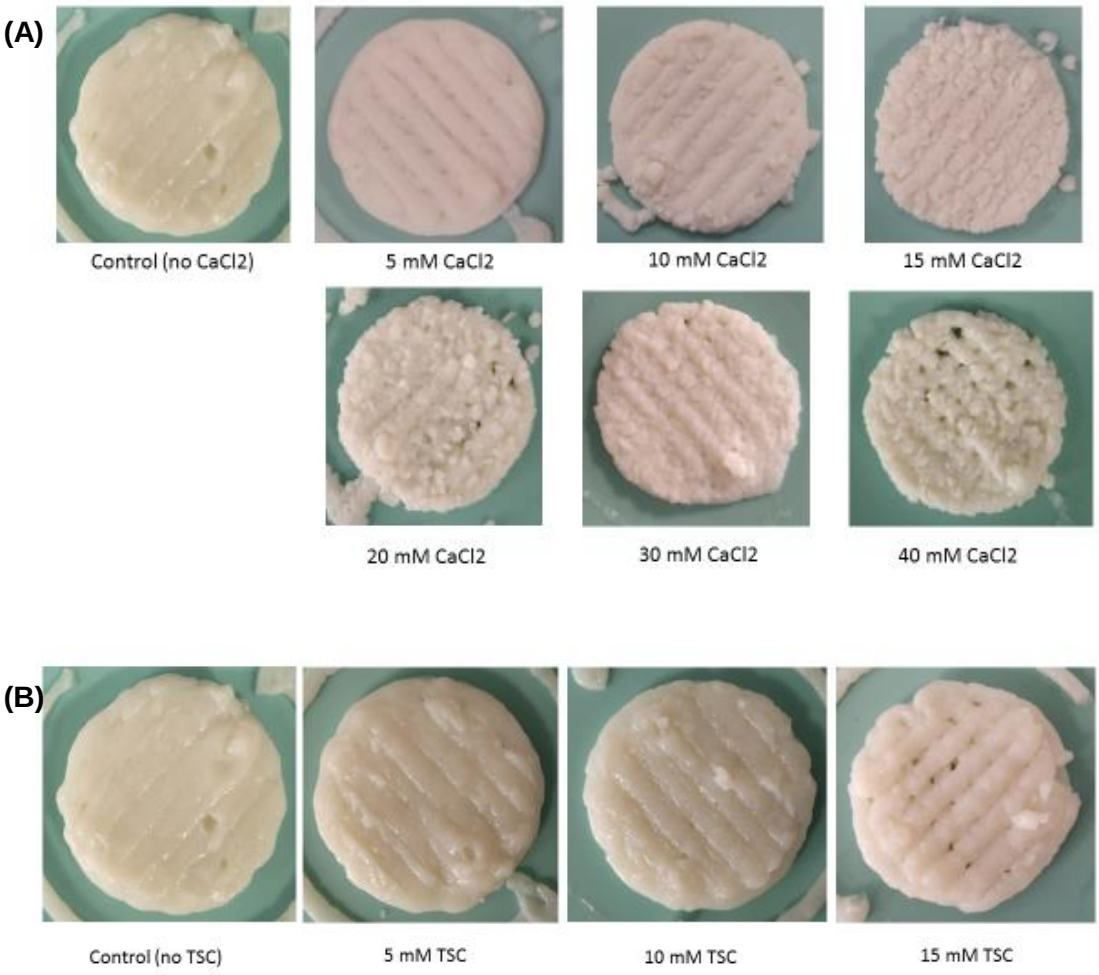

Fig. 4.3A and B. Effect of adding a range of (A) $CaCl_2$ or (B) TSC concentrations to 30% w/v MCC (3D-printed into disks (*d* = 40 mm) before rheological analysis).

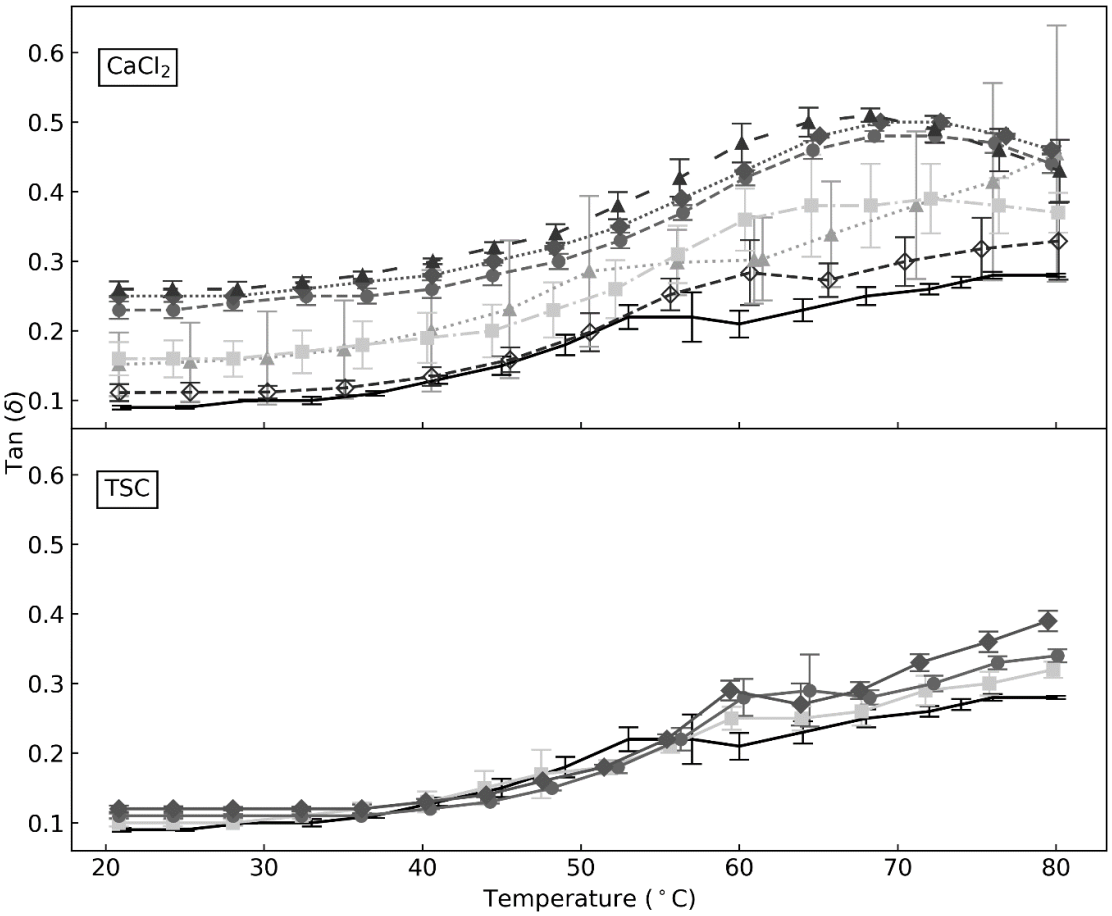

Fig. 4.4. Comparison of melting profiles of 3D-printed MCC samples. (Note: Control and TSC samples (unbroken lines) and $CaCl_2$ samples (broken lines)). Control (——), 1 mM $CaCl_2$ (--◊--), 2.5 mM $CaCl_2$ (-- ▲ --), 5 mM $CaCl_2$ (-- ■ --), 10 mM $CaCl_2$ (--●--), 15 mM $CaCl_2$ (--♦--), 20 mM $CaCl_2$ (-- ▲ --), 5 mM TSC (—■—), 10 mM TSC (—●—) and 15 mM TSC (—♦—).

There was a direct and positive relationship between TSC concentration and tan δ values, where samples with lower concentrations of TSC (i.e., 5 mM) were more resistant to melting than samples with higher TSC concentrations (i.e., 15 mM TSC). Ozcan-Yilsay *et al*. (2007) reported an increase in G' with low levels of TSC addition up to 10-20 mM TSC in yoghurt samples; however, concentrations above 20 mM TSC appeared to lead to a reduction in yoghurt stiffness. The authors contributed this phenomenon to a complete disruption of the casein micelles and dissolving of CCP crosslinks, leading to a weaker yoghurt gel. According to de Kort *et al.* (2011), an increase in viscosity in concentrated micellar casein suspensions with added TSC is associated with a swelling of casein micelles due to a reduction in calcium-ion activity.

The calcium-sequestering agent acts by chelating calcium from the aqueous phase along with a proportion of insoluble calcium and CCP, which in turn forms a soluble calcium-citrate complex (Holt, 1985; Kaliappan and Lucey, 2011; Lucey and Horne, 2009).

4.3.4 Factors affecting turbidity and protein solubility of melted 3D-printed MCC structures

To measure the rate at which the 3D-printed MCC melts in a liquid medium, spectrophotometry was used as outlined in Section 4.2.2.5 to record turbidity values during solubilisation at two temperatures (ambient (21°C) and 37°C). To support the interpretation of these data and to quantify the protein solubilised over time, the same aliquots of sample taken over set time intervals during dissolution were tested using BCA protein assays. Both methods (spectrophotometry and BCA protein assay) generate data to better understand the relationship between calcium supplementation, dissolution temperature and printed geometry, and the rate of dissolution and solubility of 3D-printed MCC. Only samples containing 1 and 5 mM $CaCl_2$ were chosen to compare the effect of lower and higher concentrations of calcium addition to a control sample without any calcium added; samples with > 5 mM $CaCl_2$ were quite brittle and unsuitable for printing larger and more complex structures like lattices. Samples containing TSC were also excluded from the primary comparison trial as it was found in preliminary trials that these samples exhibited very slow or incomplete dissolution., which could be contributed to the increased levels of hydration and dissociation of the casein micelle caused by the chelation of calcium, where solutions are already hydrated to a limited capacity and, therefore, require longer periods of time to dissolve and disintegrate (McCarthy *et al.*, 2017).

At both ambient temperature (Fig. 4.5) and 37°C (Fig. 4.7) there was a difference between the dissolution rate of the two printed structures. Samples printed into hemispheres had lower turbidity values over the recorded time period in comparison to printed lattice samples which would indicate a slower dissolution rate than samples printed into a lattice structure. Levels of protein solubility from the BCA analysis also supported this result. There was an apparent difference between the concentration of protein released during dissolution between the two printed geometries, where hemisphere-shaped samples (Fig. 4.6A and 4.8A) released lower concentrations of

protein in comparison to the lattice-shaped samples (Fig. 4.6B and 4.8B). This suggests that that the more exposed surface area and lower volume of the lattice facilitates more rapid dissolution than a printed structure with minimal surface area and larger volume, as in the case of a hemisphere shape (Table 4.1). Goyanes *et al*. (2015) noted, for 3D-printed pharmaceutical drugs with varying structural geometries, that those with higher surface area to volume ratio had faster dissolution rates versus those with a lower ratio. Others have also suggested that this ratio may be a suitable parameter in predicting drug-release kinetics in pharmaceutical applications (Reynolds *et al*., 2002).

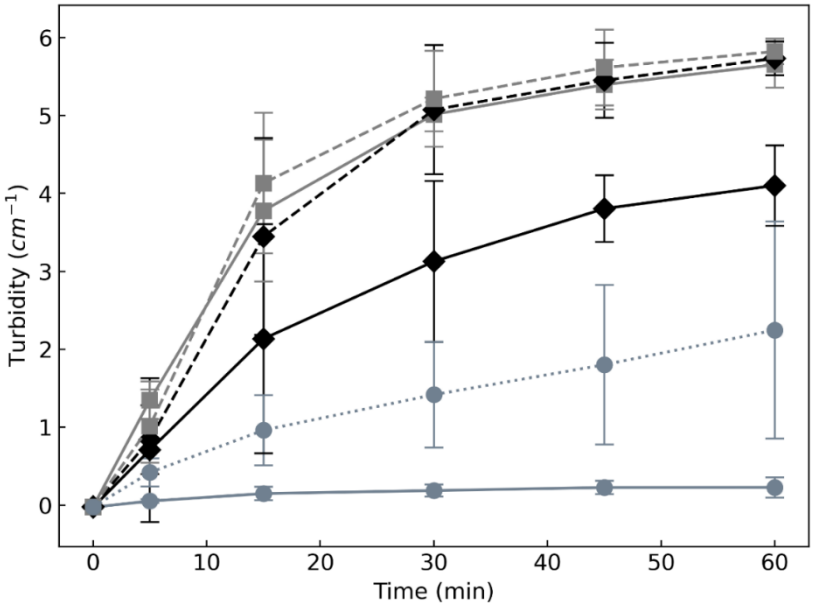

Fig. 4.5. Comparison of effect of calcium supplementation and printed geometry on melting rate as measured through turbidity at ambient temperature (HS = Hemisphere, LT = Lattice). (Note: HS samples (unbroken lines) and LT samples (broken lines). Control (HS) (—♦—), 1 mM $CaCl_2$ (HS) (—■—), 5 mM $CaCl_2$ (HS) (—●—), Control (LT) (--♦--), 1 mM $CaCl_2$ (LT) (--■--) and 5 mM $CaCl_2$ (LT) (--●--).

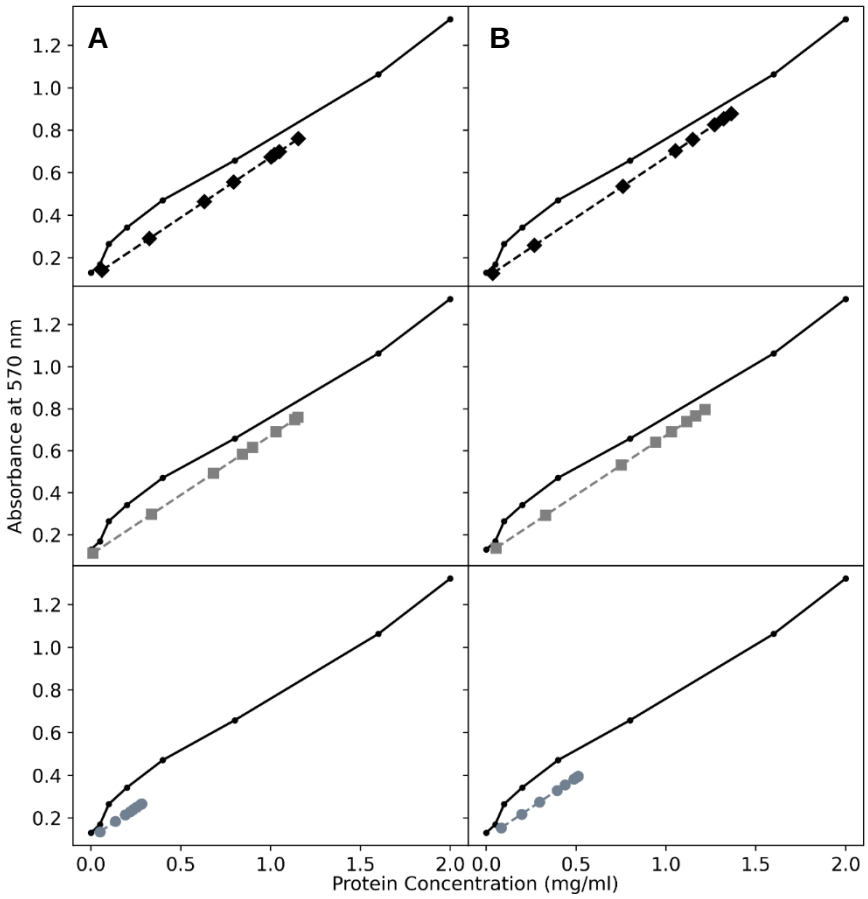

Fig. 4.6. Comparison of effect of calcium supplementation and printed geometry at ambient temperature (21°C) on protein solubility over time as measured using BCA analysis ((A) Hemisphere and (B) Lattice) (Note: samples were diluted 1 in 4 in order to fit the (1 % BSA) standard curve (—●—)). Control (--♦--), 1 mM $CaCl_2$ (--■--), 5 mM $CaCl_2$ (--●--).

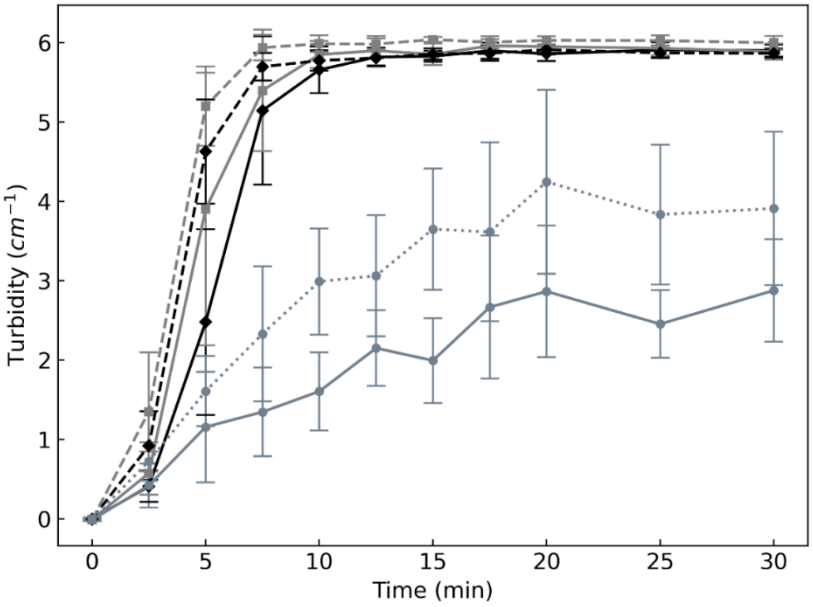

Fig. 4.7. Comparison of effect of calcium supplementation and printed geometry on melting rate as measured through turbidity at 37°C (HS = Hemisphere, LT = Lattice). (Note: HS samples (unbroken lines) and LT samples (broken lines). Control (HS) (—♦—), 1 mM $CaCl_2$ (HS) (—■—), 5 mM $CaCl_2$ (HS) (—●—), Control (LT) (--♦--), 1 mM $CaCl_2$ (LT) (--■--) and 5 mM $CaCl_2$ (LT) (--●--).

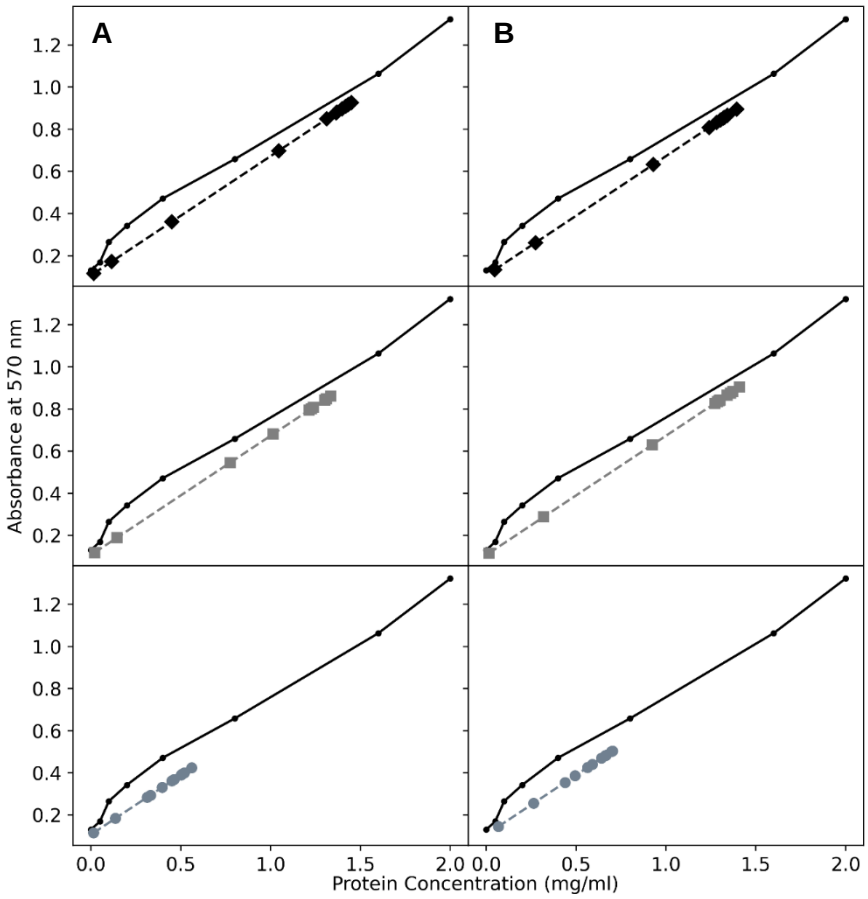

Fig. 4.8. Comparison of effect of calcium supplementation and printed geometry at 37°C on protein solubility over time as measured using BCA analysis ((A) Hemisphere and (B) Lattice) (Note: samples were diluted 1 in 4 in order to fit the (1 % BSA) standard curve (—●—)). Control (--♦--), 1 mM $CaCl_2$ (--■--), 5 mM $CaCl_2$ (--●--)

It was also noted that the suspensions with 5 mM added $CaCl_2$, 3D-printed in both geometries and subjected to both temperature conditions (ambient and 37°C), were found to be the least turbid of the three samples. According to studies involving casein-based high-protein content powders, such as micellar casein concentrate, a high activity of calcium ions can contribute to enhanced charge screening between casein micelles (Havea, 2006). This can result in the development of poorly dispersible aggregates or insoluble material due to hydrophobic interactions (Schuck *et al.*, 2002), which may have been responsible for the lack of turbidity in higher calcium-containing samples.

As might be expected, all 3D-printed samples in both geometries exhibited faster dissolution rates at higher dissolution temperatures of 37°C than at ambient temperature conditions. However, it was also observed that the difference in the rate of dissolution of samples containing 5 mM $CaCl_2$ was much more distinct than the control or samples containing a lower calcium concentration. It was seen that, at the final sampling point (after 30 minutes at 37°C), the turbidity value was almost four times that of the turbidity value of the same sampling interval at ambient temperature. Differences between the dissolution of the control and 1 mM $CaCl_2$ samples at 37°C (Fig. 4.7) appeared to be less distinct and contrasting than dissolution at ambient temperature (Fig. 4.5). After ten minutes of dissolution at the higher temperature, turbidity vales for control samples and those with lower calcium concentration are indistinguishable and appear to plateau and are assumed to be fully melted; whereas, at ambient temperature, the turbidity values for these samples plateaued at approximately 40-45 minutes.

4.3.5 Potential applications of 3D-printed MCC structures

Micellar casein concentrate has been described as having a relatively neutral flavour profile, with flavour notes characterised as 'cardboard', 'tortilla', 'astringent' and 'cooked/milky' (Smith *et al*., 2016). Due to a lack of strong competing flavours or colour, MCC could potentially be used a neutral base in which natural or synthetic colours and flavours could be added to create a personalised 3D-printed meal or snack. In conjunction with the ability to control dissolution and solubility with calcium addition and geometry, as shown in previous sections, the eating experience of these printed structures could also be modified in theory according to the desired texture and application. For example, it has been demonstrated that MCC dissolves faster at 37°C, or body temperature (i.e., to mimic temperature within the mouth), when printed in a lattice shape in comparison to a hemisphere, meaning porous structures could attribute to a melt-in-the-mouth sensation which may be preferable depending on the intended application. In the interest of demonstrating the potential capabilities of 3D-printed MCC, a selection of different designs (Fig. 4.9A-C) and an example of a fillable product (Fig. 4.9D) were 3D-printed.

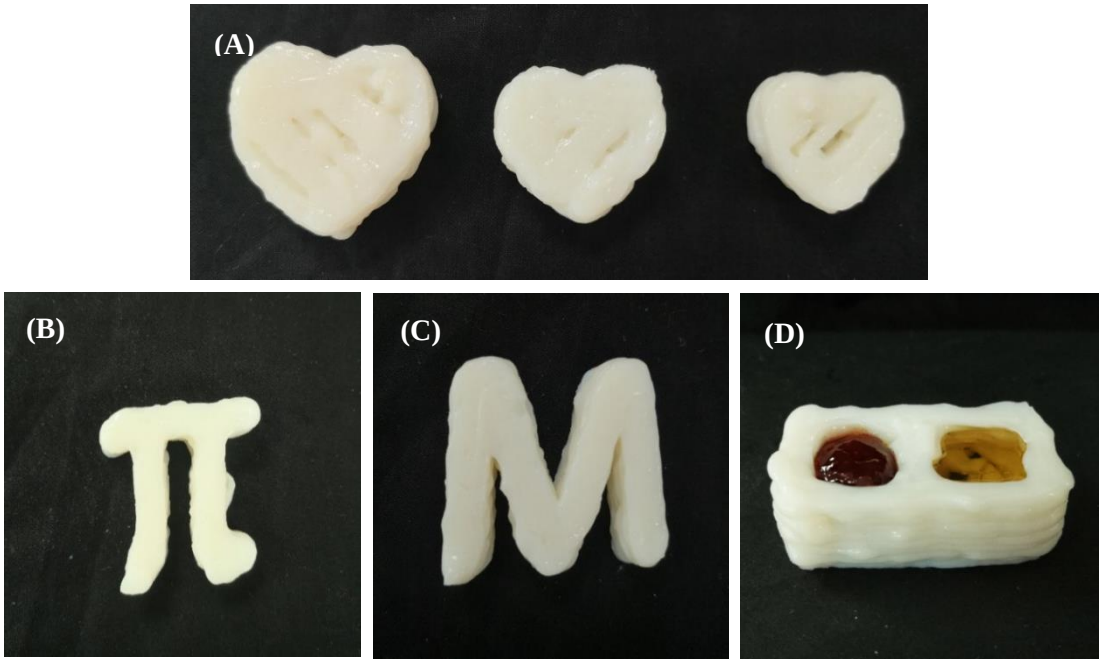

Fig. 4.9. Examples of various printable structures using MCC. (A) – (C) 3D-printed MCC shapes, symbol and letter; (D) fillable structure contains strawberry jam and honey in separate compartments to demonstrate that separate fillings could be utilised simultaneously in the same product.

The designs shown in Figures 4.9A-C were chosen to display the range of designs which could be produced in a creative way. Figures 9B and 9C show that, even though these structures are small in size (approx. dimensions - l = 40 mm, w = 40 mm, h = 10 mm), MCC can yield clear and detailed prints. In Figure 4.9D, a rectangular cuboid (l = 50 mm, w = 25 mm, h = 10 mm) with two hollowed out cuboids (l = 19 mm, w = 19 mm, h = 8 mm) surrounded by four-millimetre-thick walls was 3D-printed using 30 % w/v MCC. The base of the cuboid was 3D-printed 2-mm thick in a solid line-infill to ensure no leakage occurred when the hollowed-out sections were filled with honey and strawberry jam respectively. Though this measure was taken to reduce the likelihood of leakage occurring, there is a possibility that the 3D-printed MCC would absorb the moisture of the filling within and soften over time, leading to a potential collapse in the printed structure. Therefore, this must be taken into account, either by printing for immediate consumption, choosing low moisture content fillings, or adding higher levels of calcium, which was shown previously to delay the rate of dissolution.

It must be mentioned that the filling of this MCC structure was carried out manually by hand simply by using a spoon to drop each filling into the hollowed out sections;

however, studies completed by others have succeeded in incorporating the filling step during printing through the use of a dual-headed 3D food printer (Liu *et al.*, 2018b). This was achieved by assigning each print head to print a different section of the model (i.e., either perimeter or infill) respectively. In the same study, it was also found that, by varying infill percentage, the texture of the structure could be modified; where with increasing infill percentage (from 40-100%) the product would become harder and more adhesive. This would be an interesting concept to explore further with a dairy-based ingredient such as highly concentrated MCC suspensions, given the structural capabilities product which has been successfully demonstrated in this paper.

4.4 Conclusions

Depending on the printed geometry and added calcium chelator/supplementation of an MCC suspension, products can be created to suit the required application. A product with a high surface area: volume ratio and/or low(er) calcium may be suitable for use in fast dissolution applications, such as a high-protein nutritional beverage or snack for on-the-go where convenience is required. On the other hand, a 3D-printed product with low surface area: volume ratio and/or a higher calcium content may be more suitable for slow-release applications. Although only two geometries were used for the purpose of comparison for this study, 3D food printing provides an added advantage in the ability to create diverse and innovative structures that can be personalised to match users' preferences in terms of shape, size and degree of porosity. Calcium was shown to significantly affect textural attributes in 3D-printed MCC samples, which could also be useful in designing a product with customisable textures.

Overall, highly concentrated MCC systems can be used as a suitable base-material for 3D printing snack-sized dairy products. It has been proposed by others that 3D food printing could potentially be employed as a means of providing snacks and/or meals on demand with the option of customisable nutrient, flavour and colour profiles to suit the consumer (Caulier *et al.*, 2020; Lipson and Kurman, 2013). MCC could perhaps be suitable as a neutral tasting structural base and be complimented with added nutrients, flavours, and colours to suit consumers' preferences and needs and this could be explored in future studies.

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Supplementary material

Table S4.1: Experimental variables included in correlation matrix (Fig. S4.1) and their associated code for reference (For control, 1 and 5 mM $CaCl_2$ samples).

	Attribute	*Code*	*Units*
Calcium	CaCl2 added	A	g
TPA Indicators	Hardness	B1	N
	Adhesiveness	B2	Nm-1
	Springiness	B3	N/A
	Cohesiveness	B4	N/A
	Gumminess	B5	N/A
	Chewiness	B6	N/A
	Resilience	B7	N/A
Half sphere ambient	Turbidity (@5 min)	C1	cm-1
	Turbidity (@30 min)	C2	cm-1
	Solubility (@5 min)	C3	mg/ml protein
	Solubility (@30 min)	C4	mg/ml protein
Half sphere 37C	Turbidity (@5 min)	D1	cm-1
	Turbidity (@30 min)	D2	cm-1
	Solubility (@5 min)	D3	mg/ml protein
	Solubility (@30 min)	D4	mg/ml protein
Lattice ambient	Turbidity (@5 min)	E1	cm-1
	Turbidity (@30 min)	E2	cm-1
	Solubility (@5 min)	E3	mg/ml protein
	Solubility (@30 min)	E4	mg/ml protein
Lattice 37C	Turbidity (@5 min)	F1	cm-1
	Turbidity (@30 min)	F2	cm-1
	Solubility (@5 min)	F3	mg/ml protein
	Solubility (@30 min)	F4	mg/ml protein

Code	A	B1	B2	B3	B4	B5	B6	B7	C1	C2	C3	C4	D1	D2	D3	D4	E1	E2	E3	E4	F1	F2	F3	F4
A	1.0																							
B1	1.0	1.0																						
B2	0.8	0.8	1.0																					
B3	-1.0	-1.0	-0.8	1.0																				
B4	-0.9	-0.9	-0.4	0.9	1.0																			
B5	0.9	0.9	1.0	-0.9	-0.7	1.0																		
B6	-1.0	-1.0	-0.7	1.0	0.9	-0.9	1.0																	
B7	0.4	0.4	0.8	-0.3	0.1	0.7	-0.2	1.0																
C1	-0.8	-0.8	-1.0	0.7	0.4	-0.9	0.6	-0.9	1.0															
C2	-0.8	-0.8	-1.0	0.8	0.5	-1.0	0.7	-0.8	1.0	1.0														
C3	-1.0	-1.0	-0.9	0.9	0.7	-1.0	0.9	-0.6	0.9	0.9	1.0													
C4	-1.0	-1.0	-0.9	0.9	0.7	-1.0	0.9	-0.6	0.9	0.9	1.0	1.0												
D1	-0.7	-0.8	-1.0	0.7	0.3	-0.9	0.6	-0.9	1.0	1.0	0.9	0.9	1.0											
D2	-1.0	-1.0	-0.9	1.0	0.8	-1.0	0.9	-0.5	0.9	0.9	1.0	1.0	0.9	1.0										
D3	-0.6	-0.6	-0.9	0.5	0.1	-0.8	0.4	-1.0	1.0	0.9	0.8	0.8	1.0	0.7	1.0									
D4	-1.0	-1.0	-0.8	1.0	0.9	-0.9	1.0	-0.3	0.7	0.8	1.0	1.0	0.7	1.0	0.6	1.0								
E1	-0.9	-0.9	-1.0	0.8	0.6	-1.0	0.8	-0.8	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0							
E2	-1.0	-1.0	-0.9	1.0	0.8	-1.0	0.9	-0.6	0.9	0.9	1.0	1.0	0.9	1.0	0.7	1.0	1.0	1.0						
E3	-0.8	-0.8	-1.0	0.7	0.4	-1.0	0.7	-0.9	1.0	1.0	0.9	0.9	1.0	0.9	1.0	0.8	1.0	0.9	1.0					
E4	-1.0	-1.0	-0.8	1.0	0.9	-0.9	1.0	-0.4	0.8	0.8	1.0	1.0	0.8	1.0	0.6	1.0	0.9	1.0	0.8	1.0				
F1	-0.9	-0.9	-1.0	0.9	0.7	-1.0	0.9	-0.7	0.9	1.0	1.0	1.0	0.9	1.0	0.8	0.9	1.0	1.0	0.9	1.0	1.0			
F2	-1.0	-1.0	-0.9	0.9	0.7	-1.0	0.9	-0.6	0.9	0.9	1.0	1.0	0.9	1.0	0.8	1.0	1.0	1.0	0.9	1.0	1.0	1.0		
F3	-1.0	-1.0	-0.9	1.0	0.8	-1.0	0.9	-0.5	0.9	0.9	1.0	1.0	0.8	1.0	0.7	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	
F4	-1.0	-1.0	-0.9	1.0	0.8	-1.0	0.9	-0.5	0.9	0.9	1.0	1.0	0.8	1.0	0.7	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0

Green	Statistically significant (P <0.05) positive correlation
Red	Statistically significant (P <0.05) negative correlation
o	Open circle indicates strong negative correlation
•	Closed circle indicates strong positive correlation

Fig. S4.1: Correlation matrix between variables listed in Table S4.1 for control, 1 and 5 mM $CaCl_2$ samples. Values highlighted in green show positive correlations between variables which are statistically significant (P <0.05), whereas, values highlighted in red show negative correlations which are statistically significant (P<0.05). Open circle symbols indicate a strong negative correlation and closed circle symbols indicate a strong positive correlation. Symbols which are partially open/closed indicate weak or no correlation.

CHAPTER 5

Irish Consumer Acceptance of 3D Food Printing Technologies – a Qualitative Study

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Declaration

This chapter was written by the author MMR and reviewed by their co-authors. MMR co-designed the study, recruited participants, carried out the focus groups and analysed the resulting transcripts. This chapter was intended to be an exploratory piece which would direct the focus of the quantitative work included in the subsequent chapter (Chapter 6). The results of this chapter will be published as part of an international collaborative effort with colleagues in VTT (Research Centre of Finland) who carried out focus groups in both Belgium and Finland.

5.1 Introduction

Three-dimensional (3D) food printing involves the mechanical layering of food materials in such a way that a 3D-structure is built (Le-Bail *et al*., 2020). Structural designs can be created and developed using Computer Aided Design (CAD) software, and details such as layer height and infill percentage (i.e., density of the structure) and design can be adjusted accordingly in slicing software settings (Kikinis *et al*., 2014; Mantihal *et al*., 2019). Some foods which have been involved in 3D food printing research include processed cheese (Le Tohic *et al*., 2018), chocolate (Lanaro *et al*., 2017; Mantihal *et al*., 2019), meat (Dick *et al*., 2019), fruit and vegetable snacks (Derossi *et al*., 2018; Severini *et al*., 2018b), and dough (Liu *et al*., 2019; Yang *et al*., 2018), as well as more novel ingredient categories such as insect protein-based cereal snacks (Severini *et al*., 2018a).

It is important to understand the general consumer perceptions of 3D food printing and how they see this novel technology being applied through early engagement with potential consumers in order to reduce the risk of failure when initially introduced onto the marketplace. As 3D food printing is considered a prosumer-driven technology (i.e., where consumers are seen as simultaneously both the producer and consumer of the end product), consumers are involved from the very beginning of the design process, making it even more meaningful that the consumer's attitude of the technology is taken into consideration (Bravi *et al*., 2017). Through the use of 3D food printers, consumers can potentially choose the design, flavour, texture and nutrition values of their 3D-printed snack or meal according to their individual preferences and health status making it an extremely customisable and personalised experience (Bravi *et al*., 2017; Dankar *et al*., 2018; Sun *et al*., 2015). Therefore, with the degree of involvement the consumer is likely to experience with 3D-printed foods, it is of great importance that in-depth analysis is conducted into the attitudes of consumers on novel food technologies when considering the placement of the technology (Brunner *et al*., 2018).

There are many factors which have shown to influence consumer acceptance of novel foods/technologies, such as: cost, attitudes towards nature and technology, novel food technology neophobia, sustainability, culture, tradition and personal values and degree of risk-benefit (Cox and Evans, 2008; Giordano *et al*., 2018; Greehy *et al*., 2013;

Henchion *et al*., 2019; House., 2016; Lang., 2020; Tan *et al*., 2015). According to Greehy *et al*. (2013), consumers' may employ a heuristic approach when considering the use of certain food technologies, which is based on their own personal characteristics, values and belief systems. Consumer responses and acceptance can be as a result of consumer value orientation (i.e., a top-down process) irrespective of the benefits offered or based on attitudes formed based on knowledge known of the topic, for example the general attitudes and beliefs consumes may have on naturalness and technology. It has been demonstrated by Sütterlin and Siegrist, (2014), that certain ingredients or food processes carry a significant symbolistic weight in terms of degree of perceived naturalness. This may occur even to the extent where consumers place more value on products or processes that are perceived to be more natural, even though the benefits are comparable (Siegrist *et al*., 2016).

However, consumers may also employ a bottom-up process of information analysis, whereby consumers construct meaning by focusing on the information given in real-time, and not by resorting to previous or existing knowledge and experience. For example, consumers may perform risk-benefit assessments in the context of health and safety of the technology in order to draw a conclusion (Bredahl, 2001; Grunert *et al*., 2003). It has been reported by others (Lupton and Turner, 2016; Tran, 2016) that safety is a cause for concern for consumers when contemplating the use of 3D food printers, especially in the case of potential for cross-contamination and safety of the ingredients being used. In Lupton and Turner's, (2016) qualitative study (n = 30), a number of consumers stated that they would need evidence of the appropriate safety standard for consumption of food before consenting to try 3D-printed foods. It might be suggested that bottom-up decision-making is occurring, whereby consumers view the technology as high-risk based on the information provided to them (i.e., lack of evidence of safety), and therefore, are less willing to try 3D-printed foods.

Understanding which is the dominant mechanism (both top-down/bottom-up influences can operate concurrently) of attitude formation is significant in this context, as incremental innovations fall within normal acceptable social/value boundaries of how to behave, engage in practices and interact with others and our environment, while novel technologies tend to overextend the boundary for what is perceived as normal, appropriate, acceptable or ethical to consumers. Intuitive responses established

through top-down formations of attitude based on an individual's values and deep-seated beliefs, can often result in outright rejection of certain concepts. This may be due to the influence of the top-down process contributing to risk-benefit assessment, and consequently having a direct impact on technology evaluations and, as a result, general attitudes towards novel technologies are more likely to impact the risk-benefit assessment of 3D food printing (Bredahl, 2001).

Individuals may also compare technologies with others which are more familiar and recognisable in order to form a more confident risk-benefit analysis of the new technology and make decisions based on this analysis (Burri, 2009). The normative model of perceived risk has identified that a variety of factors influence consumers' risk-benefit reasoning for trying a new product, one of which is perceived control (Cardello, 2003). Novel food technologies tend to be perceived as spontaneous, unknown and involuntary when first introduced to consumers on the marketplace. Where the benefits of such technologies cannot be verified by the individual, due to a lack of personal interaction and experience of the technology, it becomes difficult to generate a level of trust in novel technologies (Ronteltap *et al*., 2007).

At present, there are a limited number of studies available exploring consumer acceptance of 3D-printed foods specifically, presumably due to the niche applications of the technology currently and the lack of availability of the technology to end-consumers (Brunner *et al.,* 2018). However, from these publications, there appears to be vastly mixed perceptions from the general public on the topic of 3D food printers. Lupton and Turner, (2016) found that consumers had an 'emotional response' when introduced to the idea of 3D food printing, where there were equal measures of fascination at the infinite potential of such a technology, but also, a certain amount of reluctance in believing that a machine could produce an edible and potentially wholesome meal. In other bodies of work, such as Caulier *et al*., (2020), where the degree of acceptance of 3D-printed snack bars was evaluated amongst Dutch military soldiers, they found that the 3D food printer's ability to customise meals and snacks specifically to the individual was the most valued attribute overall. However, it appears that consumer attitudes towards foods produced by novel food technologies is quite complex, where it was noted that, though the benchmark control snack bar was ultimately preferred over the 3D-printed snack bars, the overall liking of the 3D-

printed snacks increased over the duration of the trial with repeated consumption. The influence of exposure to unfamiliar foods or foods which have low initial acceptance has been consistently shown to be positively correlated with maintained or increased ratings of overall liking (Jønsson *et al*., 2019; Luckow *et al*., 2006; Stein *et al*., 2003), suggesting that overcoming the initial barriers to first purchase or consumption of the novel food may lead to eventual acceptance of that food.

In another study, carried out by Brunner *et al*., (2018), it was observed that novel food technology neophobia played a primary role in the negative attitudes formed towards 3D food printers. In this study, despite a description of the technology being provided to participants, novel food technology neophobia remained at the same level prior to the study. Brunner *et al*., (2018) also mentions that first impressions of the technology are vitally important in determining the overarching perception of the 3D printer. The name '3D food printer', unfortunately in most cases, allows the consumer to more quickly associate the technology with traditional ink and paper printers, more so than an association with edible and nutritious snacks and meals. It has been reported that the provision of information is an essential component in the formation of consumer attitudes and, therefore, consumer heuristics in judgement and decision-making (Jaeger *et al*., 2015; Tonkin *et al*., 2016), therefore, in this case, it is critical that the benefits and values of the technology (i.e., customisation, creativity, personalised nutrition etc.) are clearly evident to the customer on first contact to avoid giving rise to any potentially misconstrued perceptions (Brunner *et al*., 2018; Lupton and Turner, 2016).

In recent years, consumers' general knowledge of food production has declined due to increasingly complex and contradictory information which is available on food manufacture to the lay person, which can result in consumer uncertainty about food risks (Tonkin *et al*., 2016). Perceived uncertainty has also been shown to impact evaluations, with a lack of trust influencing the stability of attitudes and can lead to cautious behavioural responses. Existing knowledge (Moreau *et al*., 2001) suggests that consumers are open to accepting technologies which are continuous innovations (i.e., develop technologies gradually which have been previously known to be accepted by consumers). On the other hand, discontinuous or disruptive innovations (i.e., technologies which are radically different to technologies which are pre-existing,

therefore creating a new market and value network), such as 3D food printing, are generally accepted only when consumer expertise is accompanied by supplementary knowledge, enabling a knowledgeable consumer to understand and appreciate the discontinuous innovation.

There are many factors which influences the degree of consumer acceptance of novel food technologies, as explored briefly in this introduction. Studies involving perceived consumer acceptance are important in establishing a deeper understanding of the initial opinions and concerns with 3D food printing technology (Lupton and Turner, 2016). With such data and information, one can develop and pursue improved and more accepted 3D food printer designs and ingredient formulations with consumer attitudes in mind.

The present research is intended to be exploratory in nature, where an investigation was carried out into Irish consumer attitudes on 3D food printing and its associated technologies through the medium of guided discussions using focus groups. In terms of the industrial relevance of this study, opinions, suggestions and concerns discussed during these discourses are intended to be used in the interpretation of how different 3D food printing concepts may be adapted to better suit potential consumers.

The core research questions which were intended to be explored include, (1) What are the perceived potential barriers to acceptance of 3D food printing? (2) What are the evaluation processes behind consumers' attitude formation towards this novel food technology? And (3) Which elements of each 3D food printing application are preferred by Irish consumers and why, but also to whom is each application more suitably aimed at?

In the following sections, the methodological approach is outlined, followed by the research findings. Drawing on these results, a discussion will take place alluding to the responses to the research questions. Finally, the paper concludes with a brief note on the study limitations. This study was part of a broader complimentary research project done in collaboration with VTT Technical Research Centre of Finland, where qualitative data was gathered from three European countries (Ireland, Finland and Belgium). For the purpose of this chapter, the results of the Irish study will solely be discussed.

5.2 Methodology

The primary objective of this research was to explore the cognitive and affective responses of individuals to 3D food printing technology. In order to gain a better understanding of the evaluation processes and potential barriers to consumer acceptance of this novel technology, qualitative focus groups were employed to gather valuable insights from a sample of the Irish population.

5.2.1 Focus groups – a qualitative research method

Focus groups have been described as the most widely used qualitative research tool in applied social science according to Ayala and Elder, (2011). Focus groups can also be a useful tool in studies of attitude formation in consumers, where individuals are motivated to form new attitudes due to interactions with others in the group and external information provided during communication (Nielsen *et al*., 2009).

The classic focus group involves the use of a moderator whose role is to strategically guide the discussion through a number of pre-determined and carefully developed open-ended questions with minimal intervention (Krueger; 1997; Litosseliti, 2003). Focus groups with a large number of participants (i.e., 10 individuals or more) generally allow for effective brainstorming of ideas and suggestions to occur due to an increased diversity of responses. However, focus groups with smaller numbers of participants, similar to the current study, allows for the exploration of more complex, controversial and emotional topics which generally require more detailed responses (Litosseliti, 2003). A maximum of eight participants per focus group session was chosen to be the most practical group size in this study, both in terms of group manageability for the moderator and also allowing adequate time for each participant to express themselves within the two-hour duration of each session.

Due to the novel element of 3D food printing technologies as a discussion topic and exploratory nature of this study, focus groups were deemed the most appropriate qualitative research method to capture the initial responses and attitudes of participants towards 3D-printed foods. In comparison to other commonly used qualitative research methods (e.g., one-on-one interviews), focus groups are considered to be a more natural and encouraging environment for individuals to express and discuss their

opinions on a given topic in their own words (Ayala and Alder, 2011; Jervis and Drake, 2014). Focus groups allow individuals to build on other participants' comments and suggestions resulting in a broader perspective that may not have been reached in a survey or interview setting (Jervis and Drake, 2014; Marrelli, 2008).

5.2.2 Recruitment and sample

Concerning the overall sample size, 24 individuals were recruited in total and divided into three focus groups containing eight participants. Individuals (n = 24) from the university community were actively recruited by e-mail or face-to-face interaction at least one week prior to the selected date for the focus group session, based on clearly pre-defined exclusion and inclusion criteria presented in a screening questionnaire (See Fig. S5.1 in supplementary material). Applicable participants who were included in the sampling frame were chosen due to their active involvement in decisions surrounding food purchase, on the grounds that they would be able to share current food practices from their own households and potentially express their views on where or how 3D food printing technologies may be leveraged to support their food behaviours (Zepeda *et al.*, 2006). In terms of exclusion and inclusion criteria, participants were not recruited if they: worked or studied in the fields of food production, home appliance production or consumer/market research; or had participated in any focus group studies during the past six months, so as to reflect the attitudes of the average consumer's knowledge of food technology and prevent the recruitment of 'professional participants' who may anticipate the answer perceived to be 'right' to the researcher.

A number of studies have shown that consumer innovativeness is a consistent predictor of consumer willingness to try new products within a specific product category (Goldsmith, 2001; Goldsmith and Hofacker., 1999). Domain Specific Innovativeness (DSI) refers to the adoption of new items within a category of products, where in this particular study the specific domain of interest is 'food'. In the interest of diversity and comparison between consumer responses gathered, individuals who expressed interested in participating were obliged to completed an adapted Domain-Specific (DSI) scale (See table included in Fig S5.1 in supplementary material) (originally developed by Goldsmith and Hofacker (1991)) as part of the screening questionnaire at the recruitment stage. The adapted DSI scale was composed of a six-

item questionnaire using a five-point Likert scale (where 1 = low levels of innovation, 5 = high levels of innovation), where mean participant scores were calculated in order to determine their level of food domain innovation. According to Rogers', (2010) bell-shaped curve for his 'Diffusion of Innovation' theory, the first two categories of product adopters, 'innovators' and 'early adopters', are responsible for approximately 16% of the total consumer population. A mean DSI score of approximately 75% of the total score achievable (i.e., a value of 5) was deemed the most rational cut-off point in terms of eligibility for food domain innovator status. In other words, participants who scored highly (mean score, 3.8 or higher) were considered to be food domain innovators, whereas, participants who scored lower (mean score, 3.7 or lower) were considered to be 'regular' consumers.

In order to gain insight into the views and attitudes of both food domain innovators and 'regular' consumers, three food domain innovators and five 'regular' consumers were pre-allocated in each focus group session. To ensure that the views gathered from each focus group session were diverse, participants were assigned specific focus group sessions to allow for gender and age balance. It has been noted in previous studies that older and/or female participants tend to perceive higher risks and lesser benefits of novel food technologies than younger and/or male participants (Cardello, 2003; Costa-Font and Mosssialos, 2007). Therefore, a maximum distribution of 60% total female participants per group was allocated.

An overview of participants' profiles (n = 24) is presented in Table 5.1, which includes each participants' gender, age and DSI score. Participants were assigned a pseudonym to preserve anonymity and confidentiality of the individual part-taking in the study.

Ethics committee approval was sought and obtained from the University's Social Research Ethics Committee (SREC) from the university in June 2018.

Table 5.1. Participant profile of each focus group session, including pseudonyms used, gender, age and DSI score of each individual

Focus Group Session #	Pseudonym	Gender	Age	DSI Score
1	John	Male	27	4.2
1	Emma	Female	58	4
1	Ciara	Female	31	3.8
1	Jane	Female	63	3.7
1	Tom	Male	66	3.5
1	Aisling	Female	25	3.2
1	Beth	Female	42	3
1	Robert	Male	37	2
2	Áine	Female	26	4.3
2	Grace	Female	22	4.2
2	Aoife	Female	49	4
2	Ben	Male	30	3.7
2	Caitlin	Female	33	3.7
2	Seamus	Male	37	3.7
2	Liam	Male	46	3.5
2	Brigid	Female	29	3.3
3	Sophie	Female	47	4
3	Ruth	Female	54	3.8
3	Clodagh	Female	37	3.8
3	Dan	Male	21	3.7
3	Michael	Male	30	3.7
3	Cathleen	Female	54	3.2
3	Catriona	Female	37	2.8
3	Adam	Male	26	2.7

5.2.3 Discussion topic guide and focus group procedure

The objective of this research was to develop an understanding of Irish consumers' attitudes and evaluations of 3D food printing, which was achieved through the use of focus groups. To achieve this, a semi-structured focus group interview guide (For full guide – see Fig. S5.2 in supplementary material) was developed consisting of three distinct parts (summarised in Table 5.2) in order to obtain a broad range of responses from various perspectives through group interaction.

Table 5.2. Layout of the focus group sessions

Section #	Section Title	Description
1	3D Food Printing	Gauge consumer reaction before and after written 3D food printing description
2	Exploration of New Creative Ideas for 3D Food Printing	Encourage participants to consider the usage of 3D food printers and generate creative solutions for hypothetical individuals
3	Evaluation of 3D Food Printing Concepts	Discuss feasibility of 3 concepts related to 3D food printing o ***Concept 1*** – Digital Gastronomy; o ***Concept 2*** – Personalised Snacks at Fitness Centres; o ***Concept 3*** – 3D Food Printing Vending Machines in Public Spaces;

On first sitting down with participants, the moderator introduced themselves and the assistant moderator and explained what their roles would be during the entire process to create clear boundaries throughout the discussion. The group was also notified of the presence and position of the tape-recorder (i.e., recording device), and reassured complete confidentiality throughout the entirety of the focus group session, and in subsequent analysis and any publications. Each participant was then encouraged to introduce themselves to break the ice and become more familiar with each other.

Following this, a warm-up exercise consisting of a brief discussion and sharing session on the topic of the future of food (e.g., "What do you think food will look like in ten years time?) was conducted, both to encourage the group to engage in conversation and to allow the moderator to gauge the personalities in the room (i.e., shy/unengaged participants or dominant participants).

Once the group was engaged and acclimatised to the focus group setting, the topic of 3D food printing was broached. Participants were asked what their initial reaction to the idea of 3D food printing, whether they had heard of the technology before and in what context, but also what emotions were evoked on hearing the words 3D food printing. Following a brief discussion of their initial reactions, the assistant moderator distributed a brief written description of 3D food printing and how it is used (See S5.3 supplementary material). After allowing the group to read the description for a few moments, they were asked to describe their perception of 3D food printing and potential ways they might utilise the technology based on their knowledge from reading the text. The objective of this exercise was to determine the participants' baseline spontaneous reactions towards 3D food printing both before and after reading the basic description of the novel technology provided. Any indication of the primary barriers to acceptance of 3D food printing are anticipated to be alluded to during this particular exercise.

Following their introduction to the topic of 3D food printing, participants were requested to split into three pre-meditated subgroups, where two groups of 'regular' consumers were created, leaving one single group remaining which consisted exclusively of food domain innovators. Working together, the three groups were asked to brainstorm creative solutions involving 3D food printers to solve the lifestyle issues or conundrums these hypothetical individuals are experiencing. The primary objective of this group work was for participants to express their attitudes towards various possible applications of the technology, but also to gauge the variability in responses that may be created in this 'case-controlled' comparison dynamic between innovators and 'regular' consumers (Ayala and Elder, 2011). This portion of the focus group also facilitated the expression of both cognitive and affective attitudes and beliefs by gauging the capability of the technology and evaluate the extent to which having access to the printer might be advantageous in their lives.

Whilst individuals were encouraged in this part of the discussions to develop solutions without much prior situational knowledge of the technology, in the remaining portion of the focus group sessions participants are introduced to three theoretical 3D food printing concepts; (1) 3D food printers serving food at restaurants, (2) 3D food printers at fitness centres, and (3) 3D food printers in public spaces (e.g., train station). Here, participants were encouraged to express their opinions on whether each concept was viable or not in their opinion, and whether it would be a concept which with they would interact and use on a regular basis themselves. Inviting participants to list their likes and dislikes surrounding each concept allowed us to generate an idea of key characteristics of 3D food printing that were more/less acceptable to potential consumers/users of the technology.

Once the session was competed, an incentive (a voucher with a monetary value of €50) was presented to each participant who partook in the two-hour long study as a token of appreciation for their time. Participants were informed of this incentive at the recruitment stage.

From the detailed qualitative data collected surrounding the attitudes, concerns and innovative solutions on 3D food printing, key themes were systematically identified using thematic analysis derived from the methods outlined by Braun and Clarke (2006). Thematic analysis involves identifying, coding, analysing and reporting themes within the dataset and interpreting these emerging themes in a systematic manner. By highlighting reoccurring patterns and themes (i.e., certain words or opinions that would be repeated by participants) throughout the focus group transcripts, individual codes could be assigned in a way that condensed the data into robust categories which captured the essence of this research. Following this analytic approach facilitated a thorough exploration and deep understanding of participants' emerging evaluative stances on 3D food printing.

5.3 Findings

Five primary themes were identified during the initial phase of thematic analysis. The first theme relates to the difficulties participants experienced with comprehending the terminology used in describing 3D food printing technology, and the strategies employed by participants to create meaning. The second theme observed relates to

individuals' attitude towards naturalness and how 3D food printing may challenge the participant's perception of 'food'. The third theme deals with the impact of 3D food printing technology on the social and personal aspect of the participants' food experiences. The fourth theme, 'Perceived relevance of 3D food printing technology in society' concerns the effect of relevance to the participant and their acceptance of the technology. The final theme relates to the sense of control and autonomy which 3D food printing is perceived to provide to consumers, and how this impacts on participants' evaluation of the technology.

5.3.1 Theme 1: It's all in the name

This theme deals with the difficulties encountered during the focus groups with acknowledging and understanding 3D food printing as a novel technology. As mentioned previously in Section 5.2.3, a written description (See S5.3 in supplementary material) of the technology was presented to each individual, and were asked to share their opinion and initial reactions before and after reading this material. From the responses gathered, it was clear that most participants were unsure of the technology in a variety of respects, mainly in terms of operation, the descriptive language used, and the safety of the printing process. This theme also addresses some of the methods used by participants to better understand the technology.

5.3.1.1 Uncertainty and the need for information

On introduction of the topic of 3D food printing during the focus group, participants were asked whether they had any prior knowledge of 3D printing technologies before partaking in this study. Surprisingly, the majority of participants admitted to never hearing about the technology, yet some examples of 3D printing applications in the medical industry were mentioned by one or two individuals. Those with some prior awareness of 3D printing acknowledged that they had limited knowledge of its operation and benefits of use. Only one participant, Grace (22), a university student who lives at her parental home, was aware that the printer could be used to produce personalised confectionary: *"There's a place, is it Switzerland? Where they have it for kids, you can print your own sweets, with your own name, drawings and your picture on them".*

After reading the written description of 3D food printing, understandably, participants tended to react with caution to this novel food technology due to the scientific focus of the technology and an inability to visualise how this would deliver food. Participants suggested that the purpose and operation of 3D food printing through the title alone would not be self-evident on first hearing of the technology. There was unanimous agreement across the board that the name of the process be changed to something which would be more inviting to the everyday consumer. Clodagh (37), a counselor who house-shares with other professionals, suggested: *"They need to change the name of it. Make it more attractive (…) like "Fun-shapes! [laughs]"*. Other participants in this particular focus group session agreed with this sentiment, suggesting that leveraging the novelty aspect of the technology and reframing it from a serious science to something that is fun and playful would be more conducive to it being accepted. However, it must also be noted while this would be an interesting take in a marketing sense, the positioning of the technology in this space could result in this technology becoming a fad and quickly losing any traction in the marketplace.

Several individuals expressed reservations specifically with the term 'printing' due to an association with *"Cardboard"*, *"Paper"* and *"Processed"*. These associations, which stem from existing networks of meaning, appear to act as a key barrier to acceptance. Others believed that the words typically used to describe the novel technology, such as 'Fabrication', 'Interface', 'Template', 'Digital recipes' and 'Cartridges', were not indicative of food preparation, and therefore, were unattractive. Áine (26), a student nurse who lives with her spouse, mentioned that the description increased her apprehension rather than quelling it: *"The description, it feels more artificial than I thought when I heard it first. Cartridges with pastes (…)".* These associations made 3D-printed foods appear unappealing and unpalatable; thus, attitudes were beginning to form based on intuition and affected responses to the name of the technology.

Since the majority of participants had little to no prior experience of the technology, it was naturally challenging for them to make a confident evaluation and visualise how they would use the equipment themselves. Emma (58), an administrator who lives alone, found it *"hard to imagine"*, and many others communicated perplexity over how the 3D printer converted a food ingredient into a fabricated shape or structure.

"So, is it like you put a paste in and it comes out like nice food? Or is it like, you put a food in and it comes out like a paste?" (Aisling, 26, PhD student)

"Does it harden? Or is it always soft?" (Brigid, 29, research assistant).

A visual example of the technology in operation, such as an advertisement or instructional video, was seen as essential to enhancing acceptance. Seamus (37), an executive assistant living in a home-share, illustrated this with this observation: *"Yeah, I think it would be [long lasting] if you understand more of what it is and what it's about, and how does this come to be"*.

5.3.1.2 The "unknown" and sense-making strategies

The majority of individuals expressed reservations on first hearing of 3D food printing; Ciara (37), a business developer who lives at her family home, specifically mentioned a "*fear of the unknown*" when invited to share what feelings were evoked when the topic of 3D food printing was introduced. Participants seemed uneasy at the thought of using 3D food printing technology, mostly due to a lack of familiarity and understanding of the safety and specific function of the technology, thereby leading them to perceive 3D food printing as a potential risk. As 3D food printing might not be readily comparable or relatable to other kitchen equipment in the average home, this lack of reference to the familiar can hinder the acceptance to novel technologies and increase consumer risk perception. Lack of exposure to discussion surrounding the topic of 3D food printing can generate a sense of apprehension towards the technology. Hence, participants voiced a fear of the unknown when first introduced to the notion of 3D food printing, which Beth (42), a work placement manager who lives with her spouse and two young children described as follows: *"It [3D food printing] would be impressive, but there would be a concern of what it actually is"*.

However, some participants appeared to employ a heuristic approach (i.e., sense-making strategy) by drawing comparisons between science fiction and 3D food printing. This shortcut evaluation process (often referred to as system 1 information processing by Kahneman (2011), whereby individuals activate the decision-making system which is 'fast', comprising of various heuristics and biases that immediately perceive events. On the other hand, system 2, which is 'slow' and allows individuals to observe and evaluate the situation) enables participants to generate their own

interpretation of the technology by making inferences and connecting to ideas they are already familiar with, such as cinematic experiences. *"It kind of reminds me of the food machine on the Star Trek Enterprise, like something is going to appear from nothing"* (Brigid, 29), *"What brings to my mind when I hear it is Spock from Star Trek"* (Tom, 66, a retiree living with his spouse), *"It's like Willy Wonka"* (Seamus, 37).

Further along in discussions, when participants were more familiar with the idea of what the 3D food printing technology after some deliberation on the topic, some mentions of similarities between 3D printer and public coffee machines were suggested, where the personalisation aspect of the 3D food printer appeared to evoke a likeness with a machine which could provide the consumer with a range of personalised coffee product options (i.e., type of milk, coffee volume, type of sugar etc.). This example was given by Dan (21), a university student living at his family home: *"They have these [a vending machine] for coffee in Italy… you personalise it yourself…blends, sugar, coffee volume and milk…".* Acknowledging these similarities with familiar, trusted and more commonly-used technologies gave the impression that through the use of heuristics, and depending on the positive or negative associations made, can prove to be beneficial in terms of aiding consumer acceptance.

In considering where 3D food printing may be applied in everyday life scenarios, the connections with existing concepts and products were clear, where breakfast or protein bars, smoothies and nostalgic confectionary were regularly suggested, despite the capacity of 3D food printers to create entire meals and snacks with novel shapes and textures. This suggests the need to tie this technology to what is known, understood and familiar. Consumers appeared more open to the technology when they felt that 3D food printing was another way to create familiar food that form part of the everyday diet for some., Sophie (47), an administrator who lives with her parents, spoke to this with her observation: *"(…) we're not a million miles away from this (…) like the protein powders and bars and all of that. We're not too far away from this".*

5.3.1.3 Concerns over safety

Concerns about the technology manifested at two levels. At an abstract level, there was a sense of uncertainty associated with the application while at the more functional

level, safety concerns dominated. Participants were especially apprehensive about the safety and quality of ingredients used to create the food, as depicted by Beth (42), who shared her apprehensions with the technology: "*What's being used to make cheese flavour or milk flavour? What would be a serious concern of mine is what chemicals are being used to create [it]"*.

Certain participants also expressed concerns over the maintenance of appropriate equipment hygiene standards, such as cleanliness and hygiene levels of a 3D printing food-vending machine. Apprehension over potential risk of cross-contamination and food-borne pathogens was apparent when no description of how the machine would be maintained was mentioned. Brigid (29), and Beth (42) voiced their concerns as: *"How would you clean it and maintain it?"* and *"You wouldn't know what would be inside it".* Consumers perceived the technology as a potential risk to their health and wellbeing due to the lack of information and guarantee of safety, which therefore impeded their acceptance.

Many of the participants wanted a strong visual guarantee of safety, such as "*steam*" that they could see from the machine on completion of the printed meal. The possibility of a "*franchise*" being employed to maintain the machines was also mentioned. Some participants looked to trusted regulatory bodies and experts to address their concerns with the novel technology. Ciara (37) posed the question: *"Are they FDA-approved?"*. This idea of increased control over the safety standards of the 3D printer appealed to consumers, with most being willing to try the technology if there was an assurance of safety. Most individuals have a strong level of trust in regulators who govern potential technological risks; this level of trust can permeate through to consumer's perceived trust in novel technologies, if certified as safe by trusted-regulatory bodies or expert.

5.3.2 Theme 2: Natural offers good food

Consumers tend to be more accepting of food ingredients and products which they recognise as 'food', which may have been conditioned through social, cultural, provincial and ancestral circumstances. There are many complex factors at work to define what consumers believe are 'foods' and 'non-foods'. Participants associated the word 'paste' with things that are synthetic and non-food-like, due to the artificial

nature of the word. Consumers mentioned the term "*real food*" a number of times during the sessions when discussing home-cooked meals involving meats and vegetables. 'Real food' to participants appeared to represent foods which resemble the original form of the product, whereas a 'paste' is non-descript and has the potential to take on any form. This unfamiliarity and dissociation from what the consumer considers as 'natural' was apparently disconcerting to the participants.

Sophie (47) expressed this sentiment during her consideration of what the definition of food is to her: *"But would it be food though? (...) Would it be just a spoonful of glucose powder (...)?"*. Beth (42) also had her own definition of what the word 'food' meant to her, or more precisely, what wasn't considered as food: *"People are already doing this by taking a lot of protein and shakes and powders, which is very much chemical and not food-like"*. As these responses demonstrate, there appears to be a pre-formed and individual interpretation to what is considered unnatural, both in the context of pre-existing foods and 3D-printed foods, suggesting that top-down decision making is occurring, especially when very little knowledge and experience of the technology is available to the participants to enable bottom-up decision-making processes.

There was also a certain wariness of the exact contents of the cartridges, where participants branded the food paste as "*Chemical*", "*Artificial*" and "*Processed*", based on the description provided at the beginning of the focus group, though there was no mention of how the pastes were made in the description itself. "*I'd say to make it suitable to be processed like that there would have to be chemicals used*" (Sophie, 47). It appears that the degree of transformation of raw ingredients into ingredient mixtures impacts on the evaluation and acceptance of the technology. It could be suggested that participants did not consider the end product as having the same characteristics as the original form or ingredient, thus instilling a certain level of distrust. "*Ultra-processed*" was a term cited by Liam (46), a university lecturer living with his spouse, whereby he disputed that 3D food printing is processing (i.e., shaping) an already processed food substance (i.e., blended paste or purée): *"(...) like the cartridges with the paste-like foods in it, (...) they must be processed before you put them into a 3D printer, so you're kind of processing them again"*. This continuum of processing placed 3D food printing on the highly processed end of the scale, due to the apparent extreme degree

of transformation from the original form of the food. Some equated the number of steps in manufacture to the perceived 'healthiness' or overall 'naturalness' of the product, suggesting that products with little to no processing were judged as healthier and more nourishing than those that have multiple manufacturing steps.

The absence of additives or chemical processes was important to participants in constructing their view on the technology and the products produced from 3D food printers. Chemical processes were generally believed to be more 'unnatural' than physical processes, and participants tended to be dubious of 3D food printing if they believed the process has been manipulated in any way chemically.

Those who viewed the process as primarily physical (i.e., blending ingredients into a paste or purée), and not as a chemical reaction, were more accepting of the technology. Robert (37), a university lecturer living with his young family, illustrated this with a focus on natural ingredients in preparing the final food: *"It mentions that the mixes to be printed can be prepared using a wide range of conventional ingredients, so you can actually buy vegetables and put them inside there to prepare a food"*. This observation seemed to appeal to him and others in the group, when mentioned; however, the extent of agreeability to this concept appeared to be overshadowed by the use of the technology itself and doubts remained nonetheless.

During the course of the focus group sessions, it was clear that a majority tended to have a deep-seated affinity with natural, non-processed food, with some holding stronger views on the consequences of additional process as interference of the natural order led to an extreme view of the future. This view was demonstrated by Sophie (47), who believed that the rise of this type of technology would lead to *"chemical industries becoming bigger",* and directly impact on other industries (i.e., "*agriculture and fisheries*"), or even eradicating them altogether. Some other participants agreed with this statement and were also inclined to defend industries (e.g., agricultural) in Ireland that have been historically and culturally ingrained in society as dependable and honourable occupations. Participants pictured novel and unfamiliar technologies such as 3D food printing as the antagonist of Irish food tradition, whereby it is seen as a potential threat, or replacement of these respected and familiar traditional industries, which was not conducive to acceptance of the technology.

Contrarily, and most interestingly, during the group breakout sessions (See discussion topic guide and focus group procedure - Section 5.2.3), it was observed that many of the solutions generated to solve these hypothetical individual's food dilemmas included words such as, *"healthy"* and *"nutritious"*, which appears to contest the participants' general attitudes towards 3D-printed foods. However, it must be noted that during the course of this portion of the session, it was mentioned to the group that there was no limitation to be applied to their imagination, and not to be constrained by what they thought was possible to achieve with the technology when brainstorming ideas. Therefore, it may be implied that if their initial perceived concerns were eliminated or overcome, it may be possible to convince them that 3D food printing could live up to these expectations of producing healthy, convenient and nutritious foods.

5.3.3 Theme 3: Impact on current food practices and rituals

It was evident that the meanings associated with food varied across participants from some connecting most to the utilitarian/functional benefits with others being more concerned with psychological and social benefits. 3D food printing was envisioned as posing a threat to the social and psychological values some place on their current food practices and rituals, whereby this novel technology would replace the pleasurable ritual of food preparation and cooking and possibly change the deep-seated meaning of home-cooked meals. Ben (30) and Liam (46) both strongly articulated this sentiment against the use of 3D food printers, fearing that the technology would replace meal preparation: *"I enjoy cooking, so it would take this away from me (…) I'm not against having it available, but if I had it I wouldn't use it (…) "It's not just about eating food, it's about preparing food, there's a tactile element to cooking dinner (…) it would disrupt certain lifestyle factors"*. Cathleen (54), an administrator who lives with her spouse and children, also expressed her dismay at the thought of removing the social element from her cooking by using the 3D food printer: *"(…) it takes away all the surrounding to food (…) if it's at home and you're with your family (…) there's a ritual around how you prep and you get your dinner ready (…) to me it just seems to exclude all of that".* The importance of food preparation rituals was seen to be deeply important to these participants, but also to other participants who

delighted in cooked for others in their home, as it was seen as an expression of identity and caring for others through the labour of meal preparation.

When the topic of 3D food printers being used in professional kitchens was alluded to, Beth (42) linked the futuristic and novel aspect of 3D food printing to the British professional chef Heston Blumenthal, who is renowned for incorporating novel technologies and non-traditional cooking methods into his kitchen. Though this association was drawn, Beth communicated her thoughts with slight scepticism, believing that the involvement of so much technology is somewhat conflicting with artesian dishes: *"(...) you think of Heston Blumenthal (...) he makes something look like one thing and taste like something else. There are chefs using a lot of technology with their food".* Although there are certain benefits for chefs to incorporate 3D-printed elements into their dishes, such as performing repetitive tasks, results from this study suggest that the role of professional chefs would be redundant if 3D printers were integrated into their cooking process. Brigid (29), expressed her worry that 3D food printers would remove the unique skill and talent involved in creating extravagant fine dining dishes: *"I actually don't get the point of it. Like if you got food that's supposed to be from a star chef, and you're customising it that much, what's the point of having a star chef to make the recipe? I just don't get it."* Consumers tended to place little value on the novelty value of chefs utilising 3D food printers in the decoration of their meals, emphasising the significance of the unique and creative talent of the chef over the equipment used. John (27), jokingly, suggested that professional chefs would require a change in occupation title, subsequently coining the term *"food programmer"*.

Many participants also believed that 3D printers used in restaurants to print gourmet and artisan meals was a contradiction in the context of a value proposition. Artisan is synonymous with handcrafted and a high level of skill, whereas a 3D printer is deemed to be very much removed from this, and inadequate in comparison. Ciara (31), voiced her stance on the matter: *"I'm trying to figure out how you're going to equate gastronomical food to a 3D printer, it kind of sounds like a contradiction of sorts".* Some participants were concerned that the use of a 3D printer in the home or in a restaurant setting could possibly remove some of the pleasures of eating, such as sensory elements of dining. Ben (30), a university lecturer who house-shares with

other professionals, shared his concerns with eating 3D-printed food products: *"It might take away from the pleasure of eating. Like the cutting and chewing…"*. It appeared that Ben's main concern, and that other participants who nodded in agreement to this statement, with eating 3D-printed foods would be the lack of multidimensional textures within a dish comprised of 3D-printed pastes. Ben's definition of meals which were pleasurable to him were dishes with varying textures which would take him on a culinary and sensory adventure, one which would be perceived to be absent in 3D-printed foods due to the assumed one-dimensional texture (i.e., only consisting of soft, spoonable pastes).

The meaning of foods for certain individuals is also one of personal identity and social connections, which can greatly impact on openness to a disruptive technology such as 3D food printing. Emma (58) expressed her concerns on the social impact the technology may have: *"you would miss the dining experience of eating out, meeting other people…I wouldn't like it to replace the social aspect involved with eating…"*. Though the way we eat is always changing to suit the flow of our current lifestyle, and it appears that the social aspect of dining together, whether it is the sharing of a quick takeaway meal or a full 3-course meal at home or at a restaurant, it has always been considered to be meaningful and a unifying custom to most individuals.

Context was also an important gauge of acceptance, where in the case of 3D food printers in the home or restaurant setting was found to be less than favourable; contrastingly, the notional idea of a 3D printer located at a fitness centre where precise nutrition and sensory aspects (i.e., flavour, texture, shape etc.) could be selected according to consumers' calorie expenditure seemed to be a much more agreeable concept in comparison. Consumer attitude changed when the role of the printer was altered from usurping a pleasurable experience of food preparation for some to a role of providing a purpose that is naturally stripped of any social elements, unlike a restaurant or home setting. Sophie (47) mentioned that applications involving 3D food printers in a vending machine at the gym rather than a fine dining application is more suitable for this very reason: *"(…) I find this more acceptable as it's targeting calorie and portion control (…) I can at least see that this is very functional. Whereas the gastronomy idea should be more of a pleasurable experience" "(…) with this there's a functional need being met".* In the same vein, the technology was seen as having

some relevance by viewing it as a means of fueling oneself rather than a means of enjoyment and pleasure.

It was unclear as to whether the participants in this particular focus group session were regular gym-goers or not due to a lack of indication of personal experience of attending the gym; however, it must be noted that participants who reject a technology due to incompatibilities with their own lifestyle may suggest its uses for others with an alternative and more relevant lifestyle, despite not personally knowing the stance of these potentially better-suited individuals on this technology.

5.3.4 Theme 4: Perceived relevance of the technology

Participants were influenced in their opinions on 3D food printing by what they perceived as benefits of using the technology, either for themselves or others in society. Interestingly, most suggestions from consumers demonstrated how 3D food printing could be used to benefit mainly other segments of the population (i.e., hospitals, students, homeless etc.), even more than how 3D food printing may be used by consumers themselves in their everyday lives. Certain participants also described futuristic scenarios where 3D-printed food products would be designated to less affluent individuals of society, whereas what they described as "real food" or non-printed foods would be more suited to richer individuals who could afford whole foods. This theme deals with how consumers perceive 3D food printing in society and how their placement of the technology in civilisation affects their acceptance.

5.3.4.1 Perceived relevance to individuals and benefit to 'others'

Consumers tended to employ two pathways of thought when constructing their evaluations of 3D food printing: individual benefit or obstruction and benefits to 'others'. Individuals who saw the technology as personally beneficial (e.g., reducing food waste, portion control and encourage healthy eating etc.) were most accepting of this technology, whereas participants who visualised 3D food printing as a hindrance to their goals were not as accepting (e.g., slow printing speed, expense and excessive use of technology/lack of human interaction). Those that identified the technology as beneficial to other segments of the population were generally neutral in their acceptance, inclined neither to consider purchasing nor trying 3D food printing services nor to completely rule out the use of the technology in totality.

Evaluation of the technology varied amongst individuals, where some considered 3D food printing with an open and impartial view, others had a closed stance and a deep focus on personal relevance. Those that did not identify ways that 3D food printing technology might be of benefit to society also judged that it offered little benefit to them directly in their personal everyday lives. Liam (46) felt that 3D food printing didn't add value to his food experience, voicing that: *"with a 3D printer you're basically getting a prawn that has been turned into a paste that's been printed to turn it back into a prawn-shaped prawn paste. Which to me…I just don't get it".* 3D food printing was seen to some as a hindrance and counterproductive in terms of food preparation practices, and, for these individuals, the 3D food printer's ability to create complex textures and shapes did not align with their values or priorities in food preparation, thereby making the technology irrelevant to their everyday needs. In contrast, consumers such as Caitlin (33), a research scientist who house-shares with other professionals, identified certain attributes of the 3D food printer (e.g., automation of repeated culinary tasks) which she valued as useful and uniquely important to her as an individual who dislikes the hassle of cooking for large groups: *"If you're having a dinner party, and you're having themed food, you could use it to garnish your plates or something. I think that would be quite cool".* Nonetheless, acceptance of 3D food printing was dependent on the perceived relevance of the technology to the individual and their individual relationship with food and associated values.

Most consumers who participated in the focus group sessions identified various segments of the population who might benefit from 3D food printing technology, such as students, the elderly and hospital patients. The 3D food printer's ability to create appealing shapes from soft, paste-like foods was identified by participants as an advantage to individuals with dysphagia (i.e., swallowing difficulties) or elderly persons with chewing issues. Aoife (49), an administrator who lives with her spouse, viewed 3D food printing as a potential solution for her elderly father: *"I could see this being really useful for older people (…) I have a family member who, due to an illness, was constantly losing weight and had to get his food from these Fortisips, which are disgusting (…) But I could see this being very helpful for someone like that (…) Creating something that they would actually eat, rather than trying to force them to*

drink their calories from a carton". If 3D food printers provided individuals with effective solutions which affected them directly, such as enhancing family comfort or health, they tended to have a more open attitude to the technology than those who did not view the technology as relevant to their food lives and admitted that the technology was below the threshold of their needs. Ciara (31), illustrated this in her initial evaluation of the technology: *"I don't think I'd come to the point where I'm that time-poor that I would need to use something like that".*

Similarly, Tom (66), mentioned his wife multiple times during the focus group session; she is an allergy sufferer with coeliac disease. He was indifferent to 3D food printing on first learning about the technology: *"(...) it wouldn't be a thing for me at present"*; however, when Tom recognised the benefits it might have in enhancing his wife's food experience when it comes to safe and stress-free eating, he was more open to the technology: *"(...) those couple of lines [about omitting allergens] caught my eye. You know, my wife is coeliac and the trouble we have in restaurants (...) So I could kind of see there it could be handy there".* Adam (26), a master's student who house-shares, demonstrated similar views on the subject: *"I think it's beneficial (...) people with mobility issues, or aren't able to cook (...) or allergic to nuts, they know exactly what's going in and it's concentrated in that area (...) It enhances their living conditions".*

Other participants identified communities in which 3D food printing could provide effective service, such as the defence forces; Dan (21), suggests: *"The defence force would use it. Especially the navy, because they're away for so long (...) so they might as well have better quality food" (...) like if it's a portion per person, and you've a whole fleet of people to feed"*. Ruth (54), an administrator who lives alone, communicated a similar point of view: *"It's all about convenience and nutrition and what they can get in from a fuel point of view with little effort"*. Liam (46) also mentioned that students would be an ideal target market for 3D food printing services in providing cheap, mass-produced meals to universities: *"Depending on the cost of it, for example it could be used in a university (...) You could give them a gourmet meal if it's cheap enough to produce it (...) It could open up fine dining to a whole new market who wouldn't be able to afford it necessarily"*. Although consumers were unsure of the use of 3D food printing technologies personally in their daily lives, they

believed that the technology should be available nonetheless through the identification of benefits to certain groups of people, in other words while they did not see personal benefit/value in the technology they did not reject its introduction to the market on moral or ethical grounds i.e., as citizens they were open to the introduction of the technology.

5.3.4.2 Associations between food technologies and social status

A noticeable pattern emerged from the results overall, where consumers suggested that 3D printers would be primarily used by communities and individuals of socio-economically disadvantaged backgrounds, such as prisoners. John (27), a university lecturer who lives with his partner, mentioned that 3D food printers could provide a cheaper solution in feeding prison inmates with mass produced meals: *"(…) cost is an issue (…) I imagine this would be cheaper, because it's quite possible not real ingredients (…) you want to print something that tastes like beef but isn't beef (…) not the appearance, but the nutrition of it, but you want it really cheaply"*. Similarly, Seamus (37) suggested that 3D food printers could be a more practical resolution to feeding the homeless: *"(…) like places that feed the homeless (…) could be practical in terms of preparation and things like that".* Consumers tended to associate the use of 3D food printers with services which prioritise basic nutrition over the appearance and aesthetics of food.

Interestingly, Beth (42), suggested that there would be a *"two-tiered system"* if this technology became available, where there would be two options: *"(…) the wealthy will be able to afford fresh food and the poor will have to go for paste-like food"*. Beth continued to make comparisons with the *"paste-like foods"* used in the 3D food printer to the highly calorific paste, Plumpy'nut, used in developing countries as sources of emergency nutrition. Clodagh (37) shared a similar view, where 3D food printers could provide hunger relief to disaster areas or areas of over-population with food shortages: *"(…) if the paste isn't too heavy (…) for high population and poverty it might be easy to export"*. Participants were more likely to view 3D food printing as an inferior technology if it is commonly associated with underprivileged societal groups, thereby eliminating the desire to use the technology themselves. The suggestion that individuals from disadvantaged socio-economic backgrounds will be the primary users of 3D food printing technology, whilst more affluent individuals can

afford *"real food",* is intriguing. Participants did not hold 3D food printers in high regard for its ability to create both appetising and nutritious meals for middle or wealthy societal classes. This is due to a lack of affiliation of the word 'paste' with food ingredients used in more upscale associate meals, which Beth (42) alluded to: *"richer people will have a steak and potatoes; it'll be expensive to use real ingredients"*.

5.3.5 Theme 5: Consumer Autonomy

3D food printing could potentially allow consumers to exercise precise control over the nutritional content, flavours and texture of their meal. According to the data gathered, participants generally felt more accepting of a technology if they perceived that they have more control over it. Participants identified directly with the potential for personalisation which can be incorporated into 3D food printing technology, where meals and snacks can be created exclusively for an individual's nutritional requirements. Participants were more welcome to the concept of 3D food printing when they considered the technology as a means of enhancing their level of autonomy and control over well-being and health.

Targeted benefits of a functional nature were seen to be most acceptable to consumers, which was illustrated by the concept introduced of 3D food printers located at fitness centres. Control over impulsive consumption was found to be welcome where control was found to be lacking (i.e., diet, portion size, nutrition etc.), which Ciara (37) described, with her own personal experience: *"I know I come out of the gym sometimes and you're absolutely starving and your impulse is to go for something sugary and it's not good for you, whereas this might give you the necessary nutrients".* Most participants approved of the concept given as part of the study, where data collected (i.e., calories burned, distance travelled, heart rate etc.) from wearable devices (such as smart watches, Fitbit etc.) would be translated into customised and personalised 3D-printed snacks. Indeed, greater acceptance was evident when the technology was perceived as providing a necessary function that enriches their lives in some form which is meaningful (e.g., facilitating effortless healthier food ingredient swaps, and controlling portion sizes to achieve a healthy weight). As Sophie (47) aptly described: *"There's a functional need being met"*.

Some participants also considered 3D food printing as a potential solution for food allergy suffers, such as coeliac and lactose-intolerant individuals. Precise and consist control over the ingredients used in meals and snacks was an appealing factor for consumers with food allergies, where it was felt that 3D food printers could help regain control over their dietary struggles. It was suggested that a separate allergen-free 3D food printer could be employed in kitchens for hypersensitive diners to eat out bereft of fear of cross-contamination with allergens. Participants were found to be more accepting when 3D food printing could be presented as a solution in solving issues unique to them, compared to resolving a universal or more global issue. Tom (66) spoke of his wife who struggles with dining out as a coeliac: *"My wife would have a lot of allergies (...) it ruins the evening out to be honest (...) If you could all eat the same meal happily, but it wouldn't have the allergen in their food"*.

Participants also identified with other advantages of 3D food printing, such as the consistency of meals, in terms of creating familiar delicacies from their home-country to travellers and tourists when travelling abroad. Participant evaluation of 3D food printing seemed to move in favour of the technology which could restore a certain level of control when presented with familiar options, thus establishing confidence that safe, recognisable and comforting meals are printed. Beth (42) voiced her opinion on this: *"A lot of people like to know that they will be able to eat food that's safe and that's nutritious. You go to some countries and you're not sure how it's being cooked. Something like this might guarantee that you know that this is safe"*.

5.4 Discussion and conclusion

During the course of this study, there was a focus on the processes involved in consumer evaluations of 3D food printing as discussions progressed. Acceptance of the novel technology appeared gradual and evolutionary, as individuals were exposed to opinions and insights both for and against 3D food printing technology. As seen with other studies related to consumer acceptance and other novel food technologies (Brunner *et al*., 2018; Jaeger *et al*., 2015), it was determined that one of the primary barrier involved in impeding consumer acceptance was the terminology used in describing 3D food printing, and perhaps even the name of the technology itself. According to Brunner *et al*. (2018), to be accepted by consumers, novel technologies

such as 3D food printing should be described using lay terminology that is appropriate for consumer comprehension. It is evident that the provision of information is an important factor to the initial acceptance of the consumer; however, the language used when describing the technology must be carefully considered in order to appeal to the consumer. Clear terminology must be used which is familiar to the consumer (i.e., avoiding scientific jargon which would perhaps aid in removing the initial association of the technology with the use of "artificial" and "chemical" ingredients). However, this can often be a challenge in itself in the case of novel technologies, where it may be difficult to associate with more commonly used everyday kitchen technologies in households. The title of the technology must also be amended to a name more suitable and inviting to the consumer, since it is evident that there are many pre-existing associations with non-food related items when the subject of "printing" is introduced, which acts as a barrier to consumer acceptance of the technology. Therefore, it is of great importance that clear and accurate scientific information is provided to consumers in the most direct and unambiguous way possible in order to improve on consumer knowledge and thereby supporting more informed decisions and food choices (Tonkin *et al*., 2016).

There was an additional difficulty amongst participants in initially identifying similarities between 3D food printers and kitchen appliances found in the average home. As Olsen *et al*. (2010) highlighted, consumers tend to find it easier to accept technologies that are familiar to them than those that are unfamiliar, such as 3D food printing. As discussions progressed, some individuals noted certain comparisons to everyday technologies, for example the level of personalisation offered by certain self-service coffee machines, where consumers can choose the type of milk, strength of coffee and flavours of syrup. Those who were able to identify a reference to a familiar technology by that was also present in the 3D food printing concepts (i.e., personalised nutrition) were perceived to be more accepting of the technology. In order to maximise the level of consumer acceptance of unfamiliar technologies such as 3D food printing, marketing strategies must focus on the clear benefits of the technology and also endorsements from recognised organisations (i.e., scientific, governmental etc.). Consumer trust in authoritative figures with well-established food sector credentials who have a level of control and risk regulation, can be significant in forming

evaluations of perceived risk of novel technologies such as 3D food printing (Frewer *el al*., 1997).

However, potential adopters of the technology who may still have had reservations, suggested a trialling period could be useful, where 3D food printers would be freely accessible in order to instil confidence and lower the perception of high risk in the novel technology before investing time and energy in 3D food printing.

Other more reserved attitudes towards 3D food printing were mainly expressed by consumers who perceived 3D food printing as 'unhealthy' or 'unnatural', which was principally linked to certain descriptors, such as 'paste', used in the written description provided (See S2 in Supplementary Material). Food manufacturing processes involving multiple technological steps are typically portrayed amongst trusted healthcare professionals, food activists and gastronomists as unhealthy, less nutritious, and chemically/physically-altered, and therefore viewed with suspicion by consumers (Bredahl, 2001; Tenbült *et al*., 2005). According to Hartman *et al.* (2018), perceived 'naturalness' is viewed as a vitally important variable for the acceptance of foods and their associated technologies. Consumers tended to associate 'naturalness' with positive and desirable food product attributes and will therefore reject food products that are not perceived as this highly abstract construct of 'naturalness'. Consumers can be easily and understandably overwhelmed by the sheer volume of the often sensationalised and conflicting messages received from the media concerning what is considered '(un)healthy' (Hogbin and Hess, 1999). This disorientation of information was apparent when participants were required to contribute ideas for the use of the 3D printer in hypothetical situations. Solutions suggested were generally related to 'healthy' snacks and creative desserts, which, compared to initial responses and reactions (i.e., 'chemicals', 'non-food', 'GMO' etc.), was unexpected.

Alongside concerns over degrees of processing and risk to health, individuals were of the belief that 3D-printed food products should be labelled as such on the packaging, to provide complete transparency to the consumer. If this information is not freely offered to consumers, there may be a sense of deception and falsehood, which may result in a reduction of trust in that brand or product. Food labelling can be seen as a symbol of perceived risk in terms of consumer evaluation of the product (Tonkin *et*

al., 2016). Therefore, it is of vital importance that the authenticity is explicitly guaranteed through the labelling of 3D-printed foods (Tran, 2016).

Consumers also remarked that use of 3D food printers in public spaces, such as the example at the fitness centre, was more acceptable than that of 3D food printers located in restaurants or in the home place. Therefore, 3D food printers were more acceptable to participants when the technological application was solely reserved in providing consumers with physical nutrition, but do not prioritise the hedonic aspect of the printed product.

In contrast, 3D food printers in a social dining application were seen as a threat to deeply ingrained cultural and social traditions of food preparation and mealtimes, thereby reducing the level of acceptance of the novel technology, as many participants mentioned how 3D food printing wouldn't be compatible with their relationship with cooking and food.

Towards the end of discussions, it was evident that the provision of information and sharing of ideas and solutions using 3D food printing, was important in developing participant acceptance. Some participants shared that they felt more open-minded about 3D food printing now that they had explored the topic. This sentiment was also shared and mirrored in other 3D food printing studies addressing consumer acceptance of the technology (Caulier *et al*., 2020; Brunner *et al*., 2018)

Although 3D food printing is a novel and reasonably underdeveloped technology at present, there is substantial potential for growth within the area. As many consumers agreed, whether in favour of 3D food printing or not, current technology is ever-evolving and improving. With that said, it may be a possibility that, in a decade's time, 3D food printing may gradually be integrated, and accepted as a common sight in dining services or ultimately as household appliances.

5.5 Limitations

One of the most prominent limitations of this study is the number of focus groups completed. The reasoning behind this decision was based on the sample size available and time and budget constraints of the project. However, it was also determined that a certain saturation point was reached as the study progressed in terms of the number of

novel viewpoints emerging from later focus groups. Therefore, it could be argued that there may be a limit to the range of diversity in consumer responses.

During the decision-making process of recruiting strategy, it was also decided that the selection of participants would originate solely from the university community to ensure that the upper limit of participant numbers was met. It might be argued that a lack of diversity in participant backgrounds could lead to similar mind-sets due to an aligning factor shared with the sample set (i.e., attending or employed by the same institute), however, due to resource constraints, recruitment was limited to the most accessible sample of the population available.

Another detail that was realised post-focus groups during data analysis, was that the participants were not exclusively of Irish origin. There were 1-2 individuals who mentioned their home country during the course of the focus group sessions, and how opinions would differ from their culture compared to Irish attitudes and culture. This was an oversight that should have been addressed in the screening phase, with an additional question screening applicants who do not have Irish citizenship. However, it was concluded after reviewing the focus group transcripts that the small number of non-Irish participants had not disrupted or skewed the overall results due to the minor impact of their comments in their respective focus groups.

It must also be noted that one of the potential areas of initial interest during the planning stage of this study was between possible divergences in responses and attitudes between food domain innovators and 'regular' consumers. In order to facilitate a clear comparison between the originality and creativity of the two groups, during the group brainstorming portion of the study (see section 5.2.3 for more information), food domain innovators were purposefully grouped together creating a homogenous grouping. Using this method allows for a greater focus to be held on the factors which are most appealing to the consumer and therefore, aid in acceptability (Ayala and Elder, 2011)). However, it was observed that the level of innovative ideas and suggestions created between innovators and regular consumers appeared to be negligible. Despite deliberately alternating the three hypothetical individual cases to ensure the equal distribution of cases per innovator group, there was little variation in diversity and creativity between either group. Though this finding in itself is interesting, it does not contribute to the overall findings of this study.

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Compliance with Ethical Standards

Ethical approval

The study received ethics approval from the Social Research Ethics Committee in University College Cork; participants were reimbursed with vouchers with a monetary value of €50 for their time.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Supplementary material

Fig. S5.1 – Recruitment screening questionnaire

SCREENING QUESTIONNAIRE

The blue text is not to be shown to the respondent; it is information for the recruiters only

Q1	Are you, or any member of your family or close friends, working or have worked in, any of the following occupations?		
	Market research or consumer research		CLOSE
	Development or manufacture of food products		CLOSE
	Retail, wholesale development or manufacture of kitchen appliances		CLOSE
	Refused to answer		CLOSE
	NO / Don't know		
EXCLUDE ANY RESPONDENT WHO WORKS OR WHOSE FAMILY OR CLOSE FRIENDS WORK IN ANY OF THE ABOVE EXCLUDED PROFESSIONS / FIELDS			

Q2	Have you attended a market research focus group discussion or depth interview within last 6 months?		
	YES	1	CLOSE
	NO	0	

Q3	Gender		
	MALE	1	
	FEMALE	2	
MAX 60 % WOMEN			

Q4	Age		
	Write In ……		
ALL PARTICIPANTS SHOULD BE 24 - 65 YEARS OLD			

Q5	Who in your household is responsible for the household grocery shopping and cooking?		
	Mainly myself	1	CONTINUE
	Me and someone else in the household jointly	2	CONTINUE
	Mainly someone else	3	CLOSE
ALL RESPONDENTS MUST BE SOLELY OR JOINTLY RESPONSIBLE FOR THE HOUSEHOLD GROCERY SHOPPING			

Q6. Please indicate how much you agree or disagree with each of the statements below

	(presentation order to be mixed)	Strongly disagree	disagree	Neutral	agree	Strongly agree
1	In general, I am among the first in my circle of friends to buy a new food product when it appears	1	2	3	4	5
2	If I heard that a new food product was available in the store, I would be interested enough to buy it	1	2	3	4	5
3 REV	Compared to my friends, I own few kitchen appliances	1	2	3	4	5
4	I am aware of new food product/brands before other people do	1	2	3	4	5
5	I will buy a new food product, even if I haven't tried it yet	1	2	3	4	5
6 REV	In general, I am the last in my circle of friends to be aware of food novelties appearing in the markets	1	2	3	4	5

The score will be calculated as follows: First reverse the scores for items 3 and 6. Then compute a mean score.
The respondent will be considered as "innovator" if the mean score is ≥ 3,8

EACH OF THE FOUR GROUPS SHOULD INCLUDE 2 - 3 "innovators".

Q7. Could you please describe the most exciting new/different foods, you have bought or tried within about 6 months

_____

_____

_____

_____

_____

_____

_____

S5.2 – Focus group guide

Introduction (5 minutes)

Welcome and thank you for attending. We appreciate you for giving up your time to participate today, your contribution and insights are very important.

Introduction: My name is Megan Ross and my colleague Séan Tanner will make some notes for the duration of this focus group session.
Duration: Approximately 110 minutes in total.
Project: This interview is linked to a Finnish research project coordinated by VTT, Finland and carried out in Ireland by UCC. The project is funded by VTT, Finish Government through local innovation agency Tekes and some Finnish companies.
Topic: The focus of this research is on **food consumption** and **new ways of creating this new food.**

(MODERATOR **DOES NOT MENTION 3D FOOD PRINTING HERE**. IF SOMEONE ASKS TO SPECIFY THE TOPIC OF THE STUDY, TELL THEM THAT IT WILL BE REVEALED AT THE END OF THE DISCUSSION TO AVOID BIAS IMPACT ON THE RESULTS)

This is not an interview but a discussion. To gain a rich description of the study topic, it is important to gather different insights and opinions as widely as possible from each participant. Therefore, we encourage you to share your opinions freely, no matter how diverse it might be. **There are no right or wrong opinions.**

My role is only to guide your discussion so that we will focus on the topic and keep to the schedule.

Confidentiality, tape recording and photos: Before we begin I would like to assure that everything you say here today from the group discussion will be tape-recorded to ensure nothing vital is forgotten or missed. Should any quotes or excerpts from the discussion be used at a later stage in any reports or publications, they will be fully anonymous. The data gathered from today will be stored securely on a password protected computer and on completion of the project; they will be retained for a further ten years and then destroyed. You are also free to withdraw from the study within two weeks of the focus group session.

Please talk one at a time so that it will be possible to analyse the results from the tape. We would also like to take some photos during the focus group. The photos are taken for documentation purposes only and will not be published anywhere. **Does anyone have any objections?**

Short introduction of participants: To start off please tell us your first name and something about yourself

Part 1: Future of Food and Food Consumption (10 minutes) – *warming up the participants*

(MODERATOR: IT IS NOT NECESSARY TO GO THROUGH THIS PART ENTIRELY IN CASE THE DISCUSSION IS EXPECTED TO EXCEED THE PLANNED TEN MINUTES)

Now, let's talk about the future of food consumption. What do you think food will be like ten years from now?

Will it be different and how?

- IF YES → What causes the differences?
- IF NO → Why do you think there will not be any changes?

What kind of needs or demands will be there be in relation to food, if any?

- IF NEEDS APPEAR → What causes these needs?
- IF NO NEEDS APPEAR → Why?

What do you expect from the food in the future?

- What is important? Why?
- Do you have some concerns when you think about food and future? Why?
- Do you have some hopes for the future in terms of food and food consumption?

Part 2: 3D Food Printing (20 minutes) – *1) Participants' spontaneous reactions to the topic, 2) Beliefs, perceptions, fears and 3) Potential way of using*

(MODERATOR: REMEMBER THE IMPORTANCE OF **WHY** QUESTIONS THROUGHOUT THIS PART OF THE SESSION)

Now I would like to talk about **3D food printing.**

1. *Spontaneous reactions*
   - What comes to mind for you when 3D food printing is mentioned? Do you know what it means? Does it evoke any emotions?
   - Have you heard about it before? Where?

(MODERATOR: GIVE THE GENERAL (WRITTEN) DESCRIPTION OF 3D FOOD PRINTING TO PARTICIPANTS AND GIVE THEM 2-3 MINUTES TIME TO GET FAMILIAR WITH IT)

2. *Perception, beliefs and fears*
   - What comes to mind for you based on the description of 3D food printing?
   - What are the positives of this approach in creating meals and snacks?
   - Do you have any concerns related to it? Why?
   - Does the idea of 3D Food Printing make any sense?

3. *Potential ways of using*
   - Would you use a 3D food printer? Why/why not?
   - How would you use it?
   - Where would you use it?
   - Could someone else be interested in 3D food printing? Why? How and under which circumstances would they use it?

Part 3: Exploration of New Creative Ideas for 3D Food Printing (40 minutes – 25 minute group work and 15 minutes presentation) – *brainstorming in small groups to create new ideas for 3D food printing*

Next we will do brainstorming in small groups to create ideas about how 3D food printing could be used by different consumers.

(MODERATOR: DIVIDE THE PARTICIPANTS IN SMALL GROUPS IN THE FOLLOWING MANNER: INNOVATORS IN ONE GROUP AND "NORMAL CONSUMERS" IN TWO GROUPS)

GROUPS BASED ON THEIR USER PROFILES (It doesn't matter which user profile the innovators are working with):

- Vitality and energy/ DEBORAH
- Healthiness/ JOHN
- Creativity/MICHAEL

There is a theoretical user-profile of a fictional person for each sub-group. Your task is to come up with some ideas on how 3D food printing could be used to create food solutions that help this person.

You should describe the following characters of the solution you create:

- What are the key features of the solution? What is needed to fulfil the person's goal?
- What is the end product like?
- Where is the product produced and how is it delivered?
- What is the price?
- How should it be promoted?

Utilise the circle in the work-sheets to create your idea. You can write your ideas directly to the circle or use post-its.

You do not need whether the solution could be technologically feasible or not. Do not hold back! Be as creative as possible!

You have 25 minutes time. After that, each sub-group will present their solution.

Part 4: Evaluation of 3D Food Printing Concepts (30 minutes) – *1) Participants' perceptions of the concepts and 2) Further Development of ideas*

Now I would like to hear your opinion about some 3D food printing concepts.

(MODERATOR: PROVIDE THE "DIGITAL GASTRONOMY" DESCRIPTION TO PARTICIPANTS AND GIVE THEM 2-3 MINUTES TO GET FAMILIAR WITH IT)

Now you have had some time to get familiar with the concept, what do you think food and food consumption will be like ten years from now?

- In general, what do you think about the idea? How does it make you feel? Is it interesting or boring?
- What benefits do you associate with the concept?
- What downsides do you associate with the concept?
- Do you have some concerns related to the concept?
- Would you add something to the concept or take anything away?
- Could the idea presented to you in the concept be applied in some other context? In which and for what reason?
- Would you be interested in using Digital Gastronomy as described in the concept?
- Who else could be interested in Digital Gastronomy? What would be the reasons for your interest?
- In your opinion, would Digital Gastronomy be a momentary fad or would it last?
- Could the idea be applied in some other context than fine dining?

(MODERATOR: PROVIDE THE "PERSONALISED SNACKS AT FITNESS CENTRES" DESCRIPTION TO PARTICIPANTS AND GIVE THEM 2-3 MINUTES TO GET FAMILIAR)

Now that you have had some time to get familiar with the concept…

- In general, what do you think about the idea? How does it make you feel? Is it interesting or boring?
- What benefits do you associate with the concept?
- What downsides do you associate with the concept?
- Do you have some concerns related to the concept?
- Would you add something to the concept or take something away?
- Could the idea be presented in the concept be applied in some other concept? In which and for what reason?
- Would you be interested in the solution?
- Who else would be interested in the solution? What would be the reasons for interest?
- In your opinion, would this solution be a momentary fad, or would it last?

(MODERATOR: PROVIDE THE "3D FOOD PRINTING VENDING MACHINES IN PUBLIC SPACES" DESCRIPTION TO PARTICIPANTS AND GIVE THEM 2-3 MINUTES TO GET FAMILIAR)

Now that you have had some time to get familiar with the concept…

- In general, what do you think about the idea? How does it make you feel? Is it interesting or boring?
- What benefits do you associate with the concept?
- What downsides do you associate with the concept?
- Do you have some concerns related to the concept?
- Would you add something to the concept or take something away?
- Could the idea be presented in the concept be applied in some other concept? In which and for what reason?
- Would you be interested in the solution?
- Who else would be interested in the solution? What would be the reasons for interest?
- In your opinion, would this solution be a momentary fad, or would it last?

Part 5: Ending (5 minutes) – *End Survey, Thanks and Incentives*

Thank you! We have now reached the end of the focus group. I would like to ask you to fill out the end survey and sign the participant list before leaving. Once you have returned the end surveys and signed the participant list, I will give you the incentives. I can also respond to any of your questions concerning the study.

(MODERATOR: DISTRIBUTE THE END SURVEY TO PARTICIPANTS AND CIRCULATE THE PARTICIPANT LIST)

S5.3 - Description of 3D Food Printing

A 3D Food printer is a piece of kitchen equipment which in appearance resembles a microwave oven that can be loaded with cartridges filled with a wide range of paste-like foods and turn these into personalized snacks or meals. Creative personalization is possible in terms of a wide range of flavours, textures and look of food, and also nutritional contents. The mixes to be printed can be prepared using a wide range of conventional ingredients (e.g., milk, flour, cheese etc.), and texture is a key product attribute. The printed food will look and can then be consumed similar to other everyday snacks and meals.

CHAPTER 6

Overcoming barriers to consumer acceptance of 3D-printed foods in the food service sector

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Overcoming barriers to consumer acceptance of 3D-printed foods in the food service sector

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Declaration

This chapter was written by the author MMR and reviewed by their co-authors. MMR co-designed the study, constructed the survey and helped analyse the resulting data. AMC performed the CFA and structural modelling of the study. A version of this chapter has been accepted for publication as: Ross, M.M., Collins, A.M., McCarthy, M.B., and Kelly, A.L. (2022) Overcoming barriers to consumer acceptance of 3D-printed foods in the food service sector, *Food Quality and Preference*, **100**, 104615.

Abstract

The purpose of this study is to investigate the potential uptake and inhibitors of 3D-printed food applications in the food service market to provide market salient evidence to inform business investments. An online survey was designed and distributed to an adult Irish population and was completed by 1,045 participants. The collected data was analysed using Structural Equation Modelling to test a hypothesised model of willingness to try 3D-printed food applications. Results showed that perceived personal relevance of the technology is a strong positive determinant of willingness to try (Standardised $\beta = 0.614^{***}$). Novel Food Technology Neophobia (NFTN) represents a barrier to willingness to try 3D-printed food applications as evident from its significant negative direct effect (Standardised $\beta = -0.167^{***}$). NFTN is also found to have a depressing indirect effect when mediated through perceived personal relevance (Standardised $\beta = -0.202^{***}$), while the importance consumers attach to naturalness is yet another barrier (Standardised $\beta = 0.053^{*}$). Overall, considering its total effect, NFTN (Standardised $\beta = -0.369^{***}$) presents the greatest barrier to willingness to try 3D-printed foods.

The role of trust in science by directly diminishing the effects of NFTN (Standardised $\beta = -0.445^{***}$) and the importance of naturalness also emerges (Standardised $\beta = -0.137^{***}$). Consequently, this work has identified some of the major obstacles facing the technology in the forms of NFTN and the importance of naturalness but has pointed to possible resolutions in building continued support and trust in science, and a focus on designing and delivering both customisable consumer-focused food products and accompanying marketing strategies that communicate and emphasise the personal benefits that this novel food technology affords.

6.1 Introduction

The purpose of this paper is to investigate the potential for 3D printing technology in Ireland and the potential constraints and inhibitors to market acceptance and uptake of the technology. To address this question, the paper seeks to reveal the factors that either promote or inhibit consumers' acceptance of the technology in the foodservice market. Foodservice, or out-of-home dining, may be defined as "all food consumed and prepared out of home" (Bord Bia, 2019). The decision to focus on this market is due to the fact that it is a substantial market and has a high penetration and exposure rates among consumers given its spatial distribution and its attractiveness given today's consumer lifestyles. In 2019, pre-Covid 19, the market was worth in the region of €6.33B in terms of consumer expenditure. The foodservice sector in the Republic of Ireland is highly competitive with over 27,000 outlets distributed across a variety of business types, but faces considerable cost pressures due to a shortage of skilled staff and high labour content (Bord Bia, 2019). Given the conflicting force of the need to differentiate yet facing high operational costs, 3D printing offers enormous potential to operators within the sector. More importantly, given consumers' wide exposure to the foodservice sector, it provides an entrée for this novel technology and an opportunity for consumers to experience it for the first time, building a sense of familiarity with 3D printing and opening up further opportunities in the consumer market, the holy grail for commercialisation.

Benefits of 3D printing in the food environment present exciting possibilities in terms of customization to meet specific dietary requirements, managing food waste (Fuller, 2021) or, more generally, to appeal to consumers' desire for new hedonic and self-expressive benefits through the physical construction and presentation of food itself (Burke *et al*., 2021). However, lack of familiarity with a novel food/ technology is often cited as a barrier to acceptance (Jung *et al*., 2022; Schäufele *et al*., 2019). This has led to the recommendation, in the case of novel foods, to establish them in the context of the familiar (Lombardi *et al*., 2019; Siegrist, *et al*., 2008a). Consequently, our interest is to identify the determinants of consumers' willingness to try 3D printing applications situated within a familiar context, such as the food service market, where the person is aware of the technology being applied to create the food, but is not

required to learn how to use it (Bos *et al*., 2018). Placing the technology in a familiar context enables the consumer to visualize the technology in their everyday lives in ways that offer benefits to them, such as, convenience, health and enjoyment. Additionally, understanding the determinants of willingness to try the 3D food printing applications set in this context provides a critical insight into responses to foods derived from this technology in more transparent supply chains (i.e., where the consumer knows that it is a 3D-printed food or contains a 3D-printed food component). Exposure to the technology through food service applications can also support broader uptake of application. This perspective aligns to the position that exposure is the main building block of familiarity, so it is reasonable to argue that food service has a potential role in driving consumer acceptance of the technology.

Consumer acceptance of new food technologies can be challenging due to its newness and unfamiliarity to end users. These concerns are likely to also exist with novel 3D food printing applications. 3D food printing is considered to be a relatively young and developing technology, having been in existence since the early 2000s, with the development of the first open-source 3D food printer called 'Fab@Home' (Malone and Lipson, 2007). Since then, the number of studies exploring the characteristics of 3D-printed foods has climbed steadily, where foods such as fruit and vegetables (Derossi *et al*., 2018; Severini *et al*., 2018b), pasta (Van der Linden, 2015), cheese (Le Tohic *et al*., 2018), meat (Dick *et al*., 2019), bread dough (Liu *et al*., 2019; Yang *et al*., 2018), chocolate (Hao *et al*., 2010; Mantihal *et al*., 2018), and more novel products such as insect-based cereals (Severini *et al*., 2018a) have been investigated.

3D food printing may be described as the layering of food material using a computerised process (Huang *et al*., 2013a). Using this method, a multitude of intricate and complex 3D objects can be printed, limited only by the physical properties of the food material (i.e., sufficient structural integrity for layering) and stability of the design (i.e., one with a stable equilibrium or low centre of gravity is better suited) (Liu *et al*., 2017). 3D food printing is thought to be a beneficial concept in helping to improve an individual's health status in terms of personalised nutrition. Using 3D printing technology, a user can potentially 3D-print a meal or snack tailored to their specific health needs, as opposed to a generalised portion which is mass manufactured to suit the average person (Caulier *et al*., 2020; Pérez *et al*., 2019; Sun *et al*., 2015).

Although there are many publications discussing the fundamental science behind 3D food printing technologies and printability of potential food materials (Le-Bail *et al*., 2020; Liu *et al*., 2021; Zhu *et al*., 2019), there are few available on consumer attitude and acceptance of the technology. This is an important knowledge gap and increases the risks of investment in this novel technology. Siegrist (2008) reported the vital importance of involving the consumer at the initial stages of product development in order to increase chances of success in the marketplace, as a multitude of factors, beyond stated benefits, can impact on consumer acceptance of novel food technologies and products which cannot be overlooked at the research and developmental phase.

Based on the few studies currently available on consumer acceptance of 3D food printing, the terminology used in association with the technology (e.g., "printer"), the degree of safety and palatability of the food produced by the printer, and the potentially impaired nutritional value of the food were some of the primary consumer concerns that negatively impacted acceptance (Brunner *et al*., 2018; Lupton and Turner, 2016). A common consensus across studies currently available involving consumer perceptions of 3D food printing shows that many participants were previously unaware of the technology and initially did not recognise any true value in using the technology for themselves (Brunner *et al*., 2018; Lupton and Turner, 2016). As argued by Caulier *et al*. (2020), an awareness of the perceived benefits of the technology did not have a significant effect on the consumer's acceptance or perception of 3D-printed foods; however, it was observed that repeated consumption (over a period of four weeks) was found to be much more compelling in positively impacting participant liking of the 3D-printed food product. Similar to other forms of innovations in food and food processing, factors such as neophobia (both of novel foods and novel food technologies) and degree of trust (in food regulatory bodies, scientists, government etc.) can also pose challenges to consumer acceptance and must be overcome in order to encourage consumers to be willing to purchase and/or try a product produced by novel food technologies in the first place (Lim *et al*., 2021; Losada-Lopez *et al*., 2021; Wezemael *et al*., 2010).

In keeping with the knowledge deficit perspective, much discussion has focused on whether or not the provision of risk benefit information to consumers can alleviate their concerns about novel food technologies and combat neophobic tendencies

(Bruhn, 2007; Hansen *et al*., 2003; Rollin *et al*., 2011). This 'knowledge deficit' perspective has received some criticism as it represents a relatively flawed and simplistic approach to explaining the rational for risk aversion in consumers when faced with food produced using novel food technologies (Runge *et al*., 2018). Rollin *et al*. (2011) argued that understanding and addressing consumer evaluations of, and responses to, novel technologies is a much more complex and multifaceted issue, involving an understanding of consumers' risk-benefit analysis, socio-demographic characteristics, and established public knowledge and information, including the level in trust in the source of information. As Hansen *et al*. (2003) stated "[…] where trust is lacking, merely 'stating the facts' is not a viable strategy in risk communication, no matter how sound the underlying science is".

The present study aims to address the current paucity of research into the determinants of 3D-printed foods acceptance. It will achieve this by identifying the predominant factors determining the willingness of Irish consumers to try 3D-printed foods in the food service context. The research is based on an online representative sample of 1,045 Irish consumers. It is anticipated that the research findings will help inform global ingredient suppliers, manufacturers, and food service operators when designing, developing and implementing the use of 3D food printing technology in such a way that 3D food printers become an enduring and practical technology rather than a fleeting novelty. As 3D food printing has been shown to be beneficial in valorising food waste streams (Jagadiswaran *et al*., 2021) and providing personalised nutrition to enhance the health of individuals (Caulier *et al*., 2020; Sun *et al*., 2018), it is of current relevance to promote sustainable food technologies to global consumers and those operating in the food industry in order to address these immediate challenges. Whether that is by utilising the full potential of food products to avoid unnecessary food waste, or producing tailor-made snacks and meals to satisfy each consumers' unique nutritional needs instead of a mass manufacture of a generic product which only satisfies a portion of the population.

The structure of the paper is as follows. The next section will develop a series of hypotheses to explain the relationships between constructs implicated in the willingness to try variable based on findings in earlier studies. Considered together, these hypotheses form our overall model of willingness to try (Fig. 6.1) and will help

reveal the mediating relationships that need to be understood in explaining the willingness to try 3D food printing applications. Following the hypotheses, we will progress to the methods applied and particular attention will be paid to our measurement model establishing convergent and discriminant validity using pre-validated items. Then, we test our model, before discussing the findings and implications for practitioners.

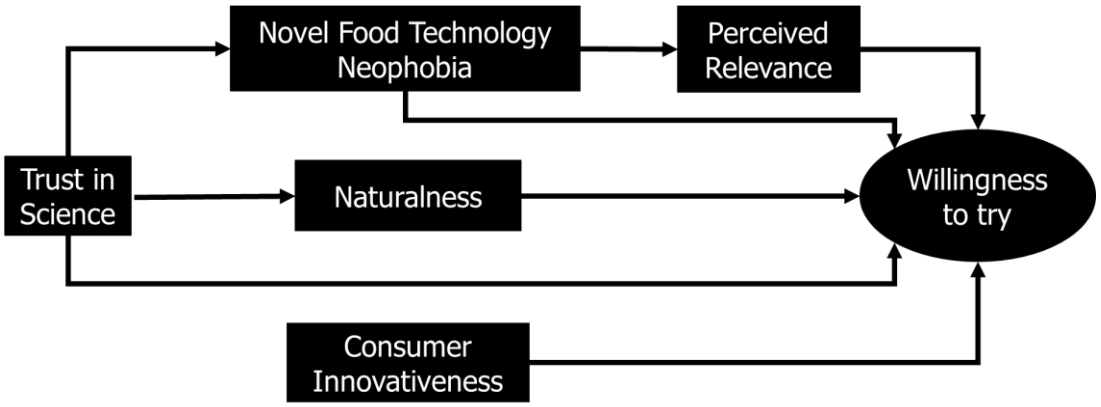

Fig. 6.1. A basic model of Irish consumers' willingness to try 3D food printing applications

6.2 Hypotheses

Based on the literature available on the factors effecting general consumer acceptance of novel food technologies, the following section identifies the key factors which would be expected to influence consumer acceptance of 3D-printed food applications. The majority of these factors have also been accounted for in the few studies involving consumer acceptance of 3D-printed foods, where novel food technology neophobia (NFTN), perceived relevance/benefit, and attitudes towards naturalness have been shown to be significant factors in determining consumer acceptance (Brunner *et al*., 2018; Hassel, 2018; Lupton and Turner, 2016). The current work will establish a more complete model of the intention to use 3D printing technology through the development of a set of hypotheses that will reveal both the direct and indirect (mediated) effects between our independent and dependent variables.

6.2.1 Consumers' perceived personal relevance of novel food technologies

Perceived relevance is defined as the extent of which a person thinks something will contribute positively to their life (Celsi *et al*., 1992; Davis *et al.,* 1989). According to Greehy *et al*. (2013), individuals evaluate and prioritise products produced by novel food technologies according to their personal orientations and values, and perceived relevance of benefits offered by that product. As each individual has a unique life path and set of circumstances, personal values and benefits associated with technologies relevant to them personally can vary immensely (Henchion *et al*., 2013).

Other studies have shown that, when an individual perceives a product to be of high personal relevance to them, they are more likely to form a positive attitude towards that product and are more likely to purchase (Dean *et al*., 2012; Kang *et al*., 2013). For example, individuals who are eco-conscious might perceive environmentally sustainable textiles as highly relevant to their own life and will therefore, feel a stronger desire to purchase in order to actively reinforce their interpretation of their projected self-image (Kang *et al*., 2013). In essence, individuals might be more willing to purchase items which they perceive as a way of enabling them to live the type of life for which they strive.

Therefore, it is proposed that:

H1: *There is a direct and positive relationship between the perceived personal relevance of the novel technology and consumers' willingness to try 3D-printed foods applications.*

6.2.2 Novel food technology neophobic behaviours affect willingness to try 3D food printing technologies

Novel innovative technologies are often rejected by consumers as the technology enters the marketplace due to negative and suspicious feelings (Chen *et al*., 2013). This can be due to a phenomenon known as 'Novel Food Technology Neophobia' (NFTN), which is a multidimensional concept comprised of perceived needs, choices, risks and media, as embedded in the scale developed by Cox and Evans (2008) to measure consumer acceptance of foods produced using novel technologies.

NFTN varies across different contexts. For example, Siegrist *et al*. (2008b) reported that consumer acceptance can fluctuate based on the technological application, where consumers were seen to be more accepting of nanotechnology being used in food packaging rather than in the food itself. Vidigal *et al*. (2015) also observed a difference in levels of consumer neophobia of different manufacturing processes of foods where Brazilian consumers were more accepting of processes which were seen as familiar and safe (e.g., traditional processing, pasteurisation); on the other hand, consumers were less accepting of these same products if they were produced using non-traditional and novel processes such as nanotechnology and Genetic Modification (GM), due to an increased level of perceived risk and negative perception of these technologies.

In the few studies of consumer attitudes towards 3D-printed foods studies available to date, it has been found that foods produced by 3D food printers are looked upon with suspicion by consumers, where fears generally derive from the uncertainty of the manufacture and nutritional quality of the printed foods (Brunner *et al*., 2018; Lupton and Turner, 2016). Hartmann and Siegrist (2017) suggested that consumers may regard foods which are 3D-printed as riskier to consume when compared to foods produced using gene technology, as the food as well as the technology can be seen as novel, meaning there is additional uncertainty and unease to comprehend. Similarly, in a quantitative study carried out by Brunner *et al*. (2018), it was observed that, despite the provision of targeted communication to their participants (n = 260) relating to the application of the technology, food technology neophobia persisted and even increased in some cases.

Therefore, it is proposed that:

H2: *There is a direct and negative relationship between novel food technology neophobia and the consumer's willingness to try 3D-printed food applications.*

6.2.3 Consumer relationship with naturalness

In recent years, consumer demand for foods which are perceived as natural has been increasing (Staub *et al*., 2020). However, the term naturalness is not well-defined and tends to vary depending on context and type of product (Schiano *et al*., 2021). Some have suggested that the degree of processing which food products are subjected to can be considered a measurement of naturalness, where foods which most resemble their

native form are more natural than those which have been processed (Rozin, 2006). Whereas, others have proposed that physical changes (i.e., cutting, mashing etc.) are more natural than chemical changes (i.e., GM, additives) (Evans *et al*., 2010). According to Román *et al*. (2017), when food products are labelled as being natural, they are perceived to be better tasting, more nutritious and less harmful to the environment. In response to this, those involved in the manufacture and marketing of food have come to realise the considerable value which consumers place on perceived naturalness and have therefore made an increased effort to accentuate this particular aspect of their product over other characteristics on packaging labels and in marketing campaigns, further fueling the narrative that natural is superior (Siegrist and Hartmann, 2020).

Studies involving consumer perception of 3D-printed foods have reported that there is a general sense of apprehension surrounding the levels of processing 3D printing would add to already processed (i.e., cooked, chopped, blended etc.) food purées (Brunner *et al*., 2018; Lupton and Turner, 2016). There is a general consensus that the consumers' perceived level of processing subjected to foods is inversely proportional to that of perceived naturalness (Etale and Siegrist, 2021; Evans *et al*., 2010a; Rozin, 2005; Rozin *et al*., 2012). This sentiment was also evident in works by Rozin (2005) and Rozin *et al*. (2012), where consumers were of the belief that where a food product had more steps involved in its manufacturing process, it was less 'natural'. Therefore, despite the possibility of 3D-printing ingredients which should be perceived as natural, such as fruits and vegetables (Derossi *et al.*, 2018), consumers for whom naturalness is important may perceive 3D-printed foods as ultra-processed and unnatural due to the number of processing steps (e.g., blending of mixtures and sometimes addition of additives such as texturisers to create suitable textures for printing).

Therefore, it is proposed that:

H3: *There is a direct and negative relationship between the importance of naturalness and consumers' willingness to try 3D-printed food applications.*

6.2.4 Consumer innovativeness and adoption behaviour

Consumer innovativeness has been described by Steenkamp *et al.* (1999) as a consumer's predisposition towards the purchase of new or different products or brands, in favour of repeat purchasing of common or familiar products purchased previously. There is evidence (Rogers, 2010) that the degree of consumer acceptance of 'newness' can be a predictor of the probability of consumers adopting novel technologies, and the level of consumer innovativeness has been shown to have a direct and positive effect on innovation adoption behaviour (Arts *et al*., 2011; Wang *et al*., 2008). Clearly, 3D food printing can be described as a novel, innovative and disruptive food technology (Berman, 2012; Kietzmann *et al*., 2015), which can be attractive to innovators and early adopters who want to be the first in their social group to explore the newest creative culinary technology. Therefore, it is proposed that:

H4: *There is a direct and positive relationship between consumer innovativeness and consumer willingness to try 3D-printed food applications.*

6.2.5 Consumers' level of trust in science impacts their willingness to try

Consumer trust in the industry, media, scientists and the government has been shown to play a decisive role in opinion and attitude formation towards acceptance of novel food technologies (Cvetkovich and Lofstedt, 1999; Søndergaard *et al*., 2005). It has been recognised by Roosen *et al*. (2015) that consumers with a high degree of trust generally exhibit lesser efforts of self-protection behaviour (i.e., are less risk-adverse) and are more willing to pay for novel foods than those with a lower degree of trust. Moreover, Bord and O'Conner (1990) observed that consumers who show trust in the food industry, government regulatory agencies or science have been more accepting and receptive of foods manufactured by novel food technologies.

Therefore, it is proposed that:

H5: *There is a direct and positive relationship between consumers' level of trust in science and their willingness to try 3D-printed food applications.*

6.2.6 Relationship between trust in science and the importance of naturalness to consumers

According to Siegrist and Hartmann (2020), consumers often use a combination of cues and heuristics, such as perceived naturalness and or trust, to evaluate and form judgements of novel foods and foods produced using novel methods. Science has brought many advances beyond nature in terms of processes which have enabled the variety and availability of safe foods that characterise contemporary society (Chávez-Dulanto *et al*., 2021). In doing so, trust in science has acted as a substitute for naturalness when making food-related decisions. However, a recent rise in consumers' perception of the importance of naturalness can be seen to coincide with a reduced level of trust in science, which has been amplified by high profile cases of food related dilemmas such as GM foods (Maghari and Ardekani, 2011) and E-numbers (Haen, 2013), and may have led to consumers preferring to form their opinions and judgements based on naturalness, rather than trust.

The emergence of more recent science-led modern food technologies, such as *in-vitro* meat production, can be seen as "extending human control" over what is considered natural and resonate in a negative light for consumers (Rozin, 2005). Despite many of the companies involved in *in-vitro* meat or GM foods production stating these technologies to be more environmentally friendly and to support sustainable food supplies (Aleph Farms, 2021; Phillips, 2008), consumers who have a 'natural preference' may view these technologies as destructive to what is natural and, therefore, dismiss those involved in the food chain, such as scientists, who may be associated with unnatural or ultra-processed food manufacture (Rozin *et al*., 2004). In addition to this, such as in the case of food irradiation or genetic modification of food, consumers may perceive new food technologies to have unknown consequences which cannot be understood or prevented without long-term testing and observation (Frewer *et al*., 2011). To counteract this fear of human intervention, consumers have developed more of an affinity towards natural food products which are perceived to be lower risk (Frewer *et al*., 2011; Rozin *et al*., 2004). For example, Siegrist and Sütterlin (2017) reported that, despite being informed of a greater risk of developing colon cancer with the consumption of traditional meat, consumers will often choose traditional meat

products over cultured meat (i.e., *in-vitro* meat) products due to issues of trust in the information given.

Conversely, Bearth *et al*. (2014) suggested that, when trust in food regulators and risk communicators are high, consumers don't equate natural with being unequivocally safe. In the same study, it was observed that consumers who perceived more benefits in the use of artificial additives in foods were reported as being more trusting in regulators and had less preference for natural additives. Due to a higher level of trust in food regulators, consumers are able to remove or lessen the doubt that there are sufficient processes in place to ensure the safety of their food, thereby reducing the reliance on the natural heuristic (Bearth *et al*., 2014).

Therefore, with these factors in mind, it is proposed:

H6: *There is a direct and negative relationship between the level of trust in science and the perceived importance of naturalness.*

Given H3, this leads to the proposition;

H7: *There is an indirect and negative relationship between trust in science and consumer willingness to try 3D-printed food applications, mediated through the importance of naturalness.*

6.2.7 Trust versus mistrust: Impact of technology neophobia on willingness to try

Cox and Evans (2008), conceptualise NFTN as comprised of risk, lack of benefit and quality or health concerns. Trust is often a substitute for knowledge in the process and safety of food manufacturing (Siegrist and Cvetkovich, 2000) and, therefore, the degree of trust in scientists and other actors within the food chain to do their job to the highest standard is an important factor influencing the level of perceived risk and quality concerns (Siegrist, 2000) and, as a consequence, their level of NFTN. Studies have established that scepticism in formal institutions involving the food industry are linked to higher incidences of novel food technology neophobia (Dolgopolova *et al*., 2015, Huang *et al*., 2019, Siegrist *et al*., 2008a). This is also supported by findings that people who exhibited higher trust in institutions also tended to associate new technologies with increased benefits and less risks (Siegrist *et al.*, 2008b).

Furthermore, without the combination of perceived benefit and trust in the actors behind the manufacture of that food product, consumers are less likely to be willing to purchase (Frewer *et al*., 2003).

Based on the argument above, the following hypotheses are proposed:

H8: *There is a direct and negative relationship between the level of trust in science and consumers' level of novel food technology neophobia.*

Drawing upon H2 it is proposed:

H9: *There is an indirect and positive relationship between trust in science and consumer willingness to try 3D-printed food applications, mediated through novel food technology neophobia.*

6.2.8 Barriers to willingness to try: Novel Food Technology Neophobia and perceived personal relevance

Certain studies have shown that the purchase of novel food products by consumers can be perceived as a way of building social status (Barrena and Sánchez, 2013; Perrea *et al*., 2017; Rogers, 2010), which is a key element of the consumer value model (Papista and Krystallis, 2012) and perceived benefit that enhances perceived personal relevance. Thus, while novelty has been linked directly to personal relevance, it is to be expected that higher levels of NFTN based on perceptions of risks or the view that the technology is unnecessary or perhaps raises quality concerns (Cox and Evans, 2008) would be anticipated to have a negative relationship on perceived personal relevance. NFTN can lead to dissonance or incongruence in terms of association with the consumer's lifestyle and what they perceive as useful or relevant (Giordano *et al*., 2018).

Others involved in similar areas of study have also suggested that, where consumers believe there to be a lack of necessity for new food technologies, they also perceive novel food technologies as high risk with low/no clear benefit and are less likely to be willing to try the product in the first place (Napier *et al*., 2004). Therefore, we argue that, without personal relevance, consumers will not be willing to try 3D-printed food applications.

With these points in mind, the authors would propose the following hypotheses:

H10: *There is a direct and negative relationship between novel food technology neophobia and the consumer's level of perceived personal relevance of 3D food printing applications.*

H11: *There is an indirect and negative relationship between novel food technology neophobia and consumers' willingness to try 3D-printed food applications mediated through perceived personal relevance.*

6.5 Methods

6.3.1 Data collection and sample

In total, 1045 individuals who identify as Irish and are long-term residents in the Republic of Ireland were recruited to participate in completing the online survey (See S6.1 in supplementary material for complete survey) during November/December 2020. The survey was delivered online via the Qualtrics platform (QualtricsXM, Dublin, Ireland) to their consumer panel. Quality control protocols were in place to ensure the integrity of the data (e.g., avoidance of over-surveying participants and professional survey takers). Three screening questions were included in a preempt mini questionnaire before the primary survey in order to screen out individuals who failed to meet the inclusion criteria. Participants who are currently working/studying in or have had previous experience in the fields of food production, home appliances, production or consumer or market research were omitted from the recruitment process in order to exclusively gather data from the average lay citizen. If a participant selected a country of residence other than Ireland, they were also excluded from completing the survey. Survey participants who were mainly or partially responsible for the purchasing of food for their household were included in the survey in order to involve individuals who are more likely to be in regular interactions and decision-making surrounding food purchase. The age bracket of the sample cohort recruited ranged from 24 to 65 years of age; 49.8 % of the total sample were male (n = 520) and 50.25 % were female (n = 525). The overall sample was representative of adults in Ireland with respect to age and gender distribution when compared to Census 2016 (Central Statistics Office, Ireland, 2016). Details of the recruited sample characteristics can be

found in Table 6.1. Ethical approval was sought and granted in October 2020 by the Social Research Ethics Committee at University College Cork.

Table 6.1. Demographic profile of respondents

	Number	Percentage (%)
Total Sample	1045	-
Gender		
Female	520	49.8
Male	525	50.2
Subtotal	1045	100
Age (Years)		
24-34	263	25.2
35-44	263	25.2
45-54	256	24.5
55-65	263	25.2
Subtotal	1045	100
Education		
Primary	3	0.3
Secondary	344	34.1
Bachelors	453	44.9
Masters	189	18.8
Doctoral	19	1.9
Subtotal	1008	100
Employment		
Full time	531	52
Part-Time	172	17
Unemployed	111	11
Student	23	2
Retired	65	6
Self-employed	63	6
Unable to work	48	5
Subtotal	1013	100
Marital status		
Single	236	23
Married or living with partner	738	71
Widowed	11	1
Separated/divorced	60	6
Subtotal	1045	100

6.3.2 Measurement Model

To test the series of hypotheses established above, measures for the focal constructs were identified and selected by drawing on pre-existing and validated scales and items. Novel Food Technology Neophobia (NFTN) drew upon the work of Cox and Evans (2008), the importance of naturalness drew on multiple sources (Hansen *et al*., 2018; Squires *et al*., 2001, Tobler *et al*., 2001). Trust in science drew on the work of Bak *et al*. (2001), indicators for perceived personal relevance were sourced from Kang *et al*. (2013), while measures of consumer innovativeness were drawn from Goldsmith and Flynn (1992).

While the original NFTN scale (Cox and Evans, 2008) was originally constructed with four dimensions, one item was employed for information and media. As a single item cannot be tested for reliability or validity this dimension of NFTN was dropped from our conceptualisation. Tables 6.2 and S6.2 (see supplementary material), presents the specific indicators (items) used to measure each latent construct.

All latent variables in the model were conceptualised and measured as reflective variables. All independent variables were measured using a 1-7 point (strongly disagree- strongly agree) Likert scale. The dependent variable, willingness to try 3D-printed food applications (adapted from Bäcktröm *et al.* (2004)), was measured using a 1-7 point (extremely unlikely to use – extremely likely to use) Likert scale.

A maximum likelihood Confirmatory Factor Analysis (CFA) was carried out to test the measurement model using AMOS 25. As proposed by Podsakoff *et al*. (2003), a common methods latent factor was introduced to ensure that our measures were free of common methods bias.

Table 6.2: CFA, factor loadings and reliability for dependent and independent variables

Constructs	*p*-Value	Standard Loading
Willingness to try various 3D food printing applications (α = 0.84; CR = 0.81; source: adapted from Bäckström *et al.*, 2004)		
Snacks freshly 3D-printed on demand from a vending machine in public spaces (e.g., cinema, shopping centres, airports, train stations etc.)	[a]	0.811
3D food printer in the gym/fitness centre	0.001	0.793
3D-printed meal at a restaurant	0.001	0.701
Consumer Innovativeness (α = 0.75; CR = 0.70; source: adapted from Goldsmith and Flynn, 1992)		
In general, I am the last in my circle of friends to know the latest food brand names and new food products	[a]	0.661
In general, I am among the last in my circle of friends to buy a new food product when it appears in the shops	0.001	0.802
Trust in Science (α = 0.80; CR = 0.72; source: adapted from Bak, 2001)		
Most scientists want to work on things that will make life better for the average person	[a]	0.601
Science and technology are making our lives easier and more comfortable	0.001	0.667
Science and technology are helping to improve our health	0.001	0.759

Constructs	*p*-Value	Standard Loading
Importance of Naturalness (α = 0.82; CR = 0.76; source: adapted from Hansen *et al*., adapted from 2018; Squires *et al*., 2001; adapted from Tobler *et al*., 2001)		
When I purchase foods, I make sure that these are natural	[a]	0.775
I seek to choose food products which are minimally processed	0.001	0.642
I avoid foods that contain artificial preservatives	0.001	0.732
Perceived Relevance (α = 0.94; CR = 0.91; source: adapted from Kang *et al*., 2013)		
The purchase and/or use of 3D-printed food products would let others see me as I ideally would like them to see me	[a]	0.820
The purchase and use of 3D-printed food products could help me attain the type of life I strive for	0.001	0.843
I believe that I could make connections or associations between the purchase and use of 3D-printed products and other experiences and/or behaviours in my life	0.001	0.762
I think that the purchase and use of 3D-printed food products would be of personal importance to me	0.001	0.842
The purchase and use of 3D-printed food products would help me to express who I am	0.001	0.847
Novel Food Technology Neophobia (α = 0.87; CR = 0.95; source: Cox and Evans, 2008)		
Risk	[a]	0.952
Lack of benefits	0.001	0.925
Quality concerns	0.001	0.913

[a] set to 1 for estimation purposes

Consumer innovativeness drew on items used by Goldsmith and Flynn (1992). Initially, it contained six items; however, all but two items were deleted due to low factor loadings. Regarding Novel Food Technology Neophobia (NFTN), the model draws upon the conceptualisation of Cox and Evans (2008). The original NFTN scale identified 13 items which loaded into four factors, one as single item factor which we dropped as outlined earlier. During the initial stage of data analysis, NFTN was identified as a second order construct with three underlying sub-dimensions, risk, lack of benefits and quality concerns (Cox and Evans, 2008) (see supplementary material S6.2). This configuration was integrated into our measurement model.

The CFA for our measurement model yielded the following results; Chi-square (χ^2) = 678.5, df =256, p = .000, (χ^2)/df = 2.651, goodness of fit index (GFI) = 0.949, adjusted goodness of fit index (AGFI) = 0.936 comparative fit index (CFI) = 0.967, the Tucker-Lewis index (TLI) = 0.961, and the root mean square error of approximation (RMSEA) = 0.040, demonstrating good fit (Byrne, 2001). Individual items, standardised loadings, P-values and reliability values are provided in Table 6.2. Reliability is demonstrated through the use of both Cronbach's alpha scores and composite reliability (Table 6.2) with all constructs meeting the desired 0.70 threshold (Nunally, 1981).

All standardised factor loadings demonstrate statistical significance (P < .01) on their corresponding construct. With the exception of Trust in Science (AVE=0.47), the average variance extracted (AVE) for each construct (Table 6.3) meets the 0.50 threshold (Hair *et al*., 1995). To justify the retention our measure of trust in science we draw on the works of Acquila-Natale and Iglesias-Pradas (2020) and Huang *et al*. (2013b) who argue that AVE values higher than 0.4 are acceptable if composite reliability (Trust in Science CR= 0.72) is higher than 0.6 (Fornell and Larcker, 1981). Furthermore, Cheung and Wang (2017) suggested that the case for convergent validity is stronger when the standardized factor loadings of all items are not significantly less than 0.5. Thus convergent validity is supported for all our latent constructs.

Table 6.3: Convergent and Discriminant Validity

	Correlations in the Upper Diagonal, Variance Extracted in the Diagonal, and R^2 in the Lower Diagonal					
	Willingness to try	**Trust in Science**	**Perceived relevance**	**Naturalness**	**Consumer Innovativeness**	**Neophobia**
Willingness to try	**0.59**	0.24	0.71	-0.22	-0.02	-0.54
Trust in Science	0.06	**0.46**	0.18	-0.15	-0.03	-0.60
Perceived relevance	0.51	0.03	**0.68**	-0.15	-0.07	-0.47
Naturalness	0.05	0.02	0.02	**0.51**	-0.18	0.25
Consumer Innovativeness	0.00	0.00	0.00	0.03	**0.54**	-0.73
Neophobia	0.30	0.36	0.22	0.06	0.53	**0.87**

Discriminant validity is supported by the fact that the average variance extracted exceeds the squared correlation coefficient for each pair of latent factors (Table 6.3; Fornell and Larcker, 1981). Consequently, the measures of the constructs used in the model achieve satisfactory reliability, convergent and discriminant validity while accounting for common methods bias.

6.4 The Structural Model and Results

6.4.1 The structural Model

The structural model, including three covariates, age, gender and education, was tested and yielded the following fit results; Chi-square ($\chi 2$) = 1061.658, df =320, p = 0.000, ($\chi 2$)/df = 3.318, goodness of fit index (GFI) = 0.933, adjusted goodness of fit index (AGFI) = 0.915, the comparative fit index (CFI) = 0.943, the Tucker-Lewis index (TLI) = 0.933, and finally the Root Mean Square Error of approximation (RMSEA) = 0.047. All met accepted thresholds (Byrne, 2001), enabling us to proceed to test our hypotheses and interpret our results.

A descriptive account of the data is presented in Table 6.4. Consistent with the findings of others (Eurobarometer, 2005; Fell *et al*., 2009), it was observed that males were more likely to use novel food technologies (e.g., 3D food printing applications) than females, though it must be noted the overall scores for both genders were relatively low. In the age category, a negative relationship was seen where willingness to try declined as the age of consumers increased. In the employment category, students were more likely to be interested in trying 3D food printing applications than those who were retired or fully/partially employed. A Eurobarometer report (2005) also found that students and other young people were generally more likely to have positive perceptions of novel technologies, such as biotechnologies.

Table 6.4. Mean scale values (mean of items) for age, gender, education and occupation categories (Scale: 1, low; 7, high).

	Naturalness	Trust in Science	Novel Food Technology Neophobia	Perceived Relevance	Consumer Innovativeness	Willingness to try 3D food printing applications
Mean	5.2	5.4	4.7	3.2	4.1	3.6
Gender						
Female	5.3	5.3	4.8	3.0	4.2	3.4
Male	5.1	5.5	4.6	3.4	4.0	3.8
Age (Years)						
24-34	5.1	5.5	4.7	3.7	4.1	4.0
35-44	5.3	5.6	4.5	3.6	4.3	3.9
45-54	5.1	5.2	4.7	3.0	4.2	3.5
55-65	5.3	5.3	4.7	2.7	3.9	3.0
Education						
Primary	4.8	5.1	4.4	4.0	4.3	3.7
Secondary	4.9	5.2	4.7	3.0	4.0	3.4
Bachelors	5.3	5.5	4.6	3.3	4.2	3.7
Masters	5.5	5.7	4.6	3.4	4.1	3.7
Doctoral	5.4	5.8	4.8	4.0	3.7	4.2
Employment						
Full time	5.2	5.5	4.7	3.5	4.2	3.8
Part-Time	5.4	5.3	4.7	3.0	4.1	3.5
Unemployed	5.0	5.3	4.6	3.2	4.2	3.6
Student	5.1	5.3	4.5	3.7	4.1	4.3
Retired	5.3	5.5	4.8	2.5	3.7	2.7
Self-employed	5.2	5.2	4.8	2.9	4.1	3.1
Unable to work	4.7	5.4	4.6	2.6	3.6	3.2

6.4.2 Results

The model explains 57.0% of the variation in our dependent variable, willingness to try 3D-printed food products in out-of-home situations. Tables 6.5 and 6.6 present substantial support for our hypotheses, with the exception of H4 and H5. The standardized estimates for our direct effects are provided in Table 6.5. The first feature to note is that perceived personal relevance is the dominant factor that influences the willingness to try 3D-printed food applications in a foodservice context. Its standardized coefficient is substantial and significant (H1 β = 0.614***) and considerably larger than the direct effect of NFTN (H2 β = -0.167***) (Table 6.5). However, NFTN also has a significant negative effect on perceived personal relevance (H10 β = -0.329***). Thus, NFTN had both direct and indirect effects on willingness to try. To estimate this indirect effect, we followed the approach of Preacher and Hayes (2004) and Zhao *et al*. (2010) and applied bootstrapped tests (5000 samples). This approach revealed that the indirect effect of NFTN, mediated through perceived personal relevance, is negative and significant (β = -0.202***) (Table 6.7) while the total effect of NFTN on willingness to try is also found to be substantial (β = -0.369***).

On analysing the relationship between trust in science and willingness to try 3D-printed food applications, we find the direct effect to be insignificant (H5 β = 0.055 ns). AMOS 25 provides unstandardized (standardized unavailable) estimates for specified indirect paths where there is more than one mediator. Consequently, we were able to test the proposed indirect effect between trust in science and willingness to try, mediated through NFTN. Table 6.6 presents the unstandardized estimates of this indirect path and reveals an indirect and mediated effect between trust in science, via NFTN, to willingness to try that was positive and significant (H9 β = 0.137***). Consequently, trust in science, by reducing NFTN, increased willingness to try 3D-printed food applications.

Trust in science also has a direct and negative effect on the importance of naturalness (H6 β = -0.137***), while the importance of naturalness reduced willingness to try 3D-printed foods (H3 β = -0.053*) (Table 6.5). Returning to Table 6.6, the unstandardized estimates reveal the mediating effect of naturalness, where we find that the path was positive, but only significant at the 90% confidence level (H7 β = 0.014*)

Table 6.5. Bootstrapped standardised direct effects

Direct Effects	Standardised β	SE (Bootstrapped)	P
(H1) Perceived Relevance to Willingness to try	0.614	0.037	***
(H2) Novel Food Technology Neophobia to Willingness to try	-0.167	0.056	***
(H3) Naturalness to Willingness to try	-0.053	0.036	*
(H4) Consumer Innovativeness to Willingness to try	-0.022	0.042	NS
(H5) Trust in Science to Willingness to try	0.055	0.046	NS
(H6) Trust in Science to Naturalness	-0.137	0.065	**
(H8) Trust in Science to Novel Food Technology Neophobia	-0.445	0.072	***
(H10) Novel Food Technology Neophobia to Perceived Relevance	-0.329	0.040	***

****** 99%, ** 95%, * 90%***

Table 6.6. Unstandardized indirect effects

Indirect Effects (Bootstrapped sample = 5,000)	**Unstandardised β**	**SE (Bootstrapped)**	**P**
(H7) Trust in Science through Naturalness to Willingness to try	0.014	0.014	*
(H9) Trust in Science through Novel Food Technology Neophobia to Willingness to try	0.137	0.059	***
(H11) Novel Food Technology Neophobia through Perceived Relevance to Willingness to try	-0.337	0.047	***

****** 99%, ** 95%, * 90%***

Table 6.7. Breakdown of the Total Effects (Bootstrapped 5,000 samples)

	Direct Effect			**Indirect Effects**			**Total Effect**		
	Standardised β	**Bootstrapped SE**	**P**	**Standardised β**	**Bootstrapped SE**	**P**	**Standardised β**	**Bootstrapped SE**	**P**
Trust in Science to Willingness to try	0.055	0.046	NS	0.172	0.045	***	0.226	0.040	***
Novel Food Technology Neophobia to Willingness to try	-0.167	0.056	***	-0.202	0.025	***	-0.369	0.057	***

****** 99%, ** 95%, * 90%***

Considering the direct and both mediated effects of trust in science and willingness to try 3D-printed food applications, we can see (Table 6.7) the standardized total effect ($\beta = 0.226^{***}$) is positive and significant. Trust in science matters when it comes to the future demand for 3D-printed foods. However, there is no evidence to support a direct effect; instead, the effect of trust in science was operant through its effects on NFTN and the importance of naturalness.

The analysis proceeded to examine the relationship between our co-variates, age, gender and education, on willingness to try using the standardized total effect to factor in both direct and indirect effects operant through our other variables. The results shown in Table 6.8 indicate that both age and gender impact willingness to try. Older consumers are less willing try 3D food printing applications than younger consumers. Gender was also found to be related with males displaying a greater willingness to try 3D food printing applications than females.

Table 6.8. Total effects of gender, age and education on willingness to try

Co-variates	Standardised Total Effect	Standardised SE	Two-tailed Sig.
Gender	0.182	0.033	***
Age	-0.307	0.033	***
Education	-0.037	0.035	NS

***** 99%, ** 95%, * 90%***

6.5 Discussion and Conclusion

While many technologies involved the preparation and provision of food in the foodservice market have been silent and unobserved by the consumer, the use of 3D-printed food applications will reveal significant changes in the ways in which our foods are processed. Thus, the need to address our key findings on the impact of NFTN need to be considered. The evidence shows the direct negative effect of NFTN on willingness to use. In many respects this is what can be expected. However, by separating out indirect effects, the current analysis clearly shows the way NFTN undermines an individual's perception of relevance of the 3D food printing technology to them personally.

Marketing strategists need to be aware of this indirect effect in their endeavours to reach their target audiences by highlighting the personalisation factor of 3D food printing in order to promote the relevance of the technology. Introducing 3D food printing applications into the ubiquitous food service sector allows consumers the freedom and autonomy to try 3D-printed foods with relatively low risk (versus purchasing the technology for home use) which may help to negate initial neophobic inclinations towards 3D-printed foods. Others have also shown that contexts where consumers are perceived to be in control of their exposure to hazards are more accepted than those where exposure is not voluntary (Siegrist *et al*., 2018).

Although 3D food printing technologies are still relatively unknown among consumers (Brunner *et al*., 2018), it is very important to gain a deeper understanding of the thought processes and behaviours contributing to potential barriers to acceptance before attempting to extend the use of the technology into the mainstream marketplace. Findings from this study point to multiple, and sometimes interconnected, influences on consumer intention to use. In line with existing literature (e.g. Greehy *et al*., 2013, Kang *et al*., 2013) perceived relevance was identified as a significant determinant of willingness to try 3D-printed food applications. Thus, a clearer understanding of the consumer through the application of a needs-based segmentation approach in the design of food service products and implementation of marketing and communication strategies will be critical. This reaffirms the importance of holding a consumer-oriented mind-set in the design and development of new products/technologies. Enhancing consumer experience through

designing and communicating salient benefits is essential to offset any potential risks that may be perceived. Indeed, Henchion *et al*., (2019) suggests that off-setting risk is dependent on technology application, benefits offered and consumer segment needs. Applications of 3D food printing will be required to communicate the many benefits that the technology offers from the delivery of personalised nutrition snacks and meals where the technology can be used to help individuals reach their own unique nutrition goals in a convenient way (Burke-Shyne *et al*., 2020; Caulier *et al*., 2020) to its ability to support consumers' sustainability concerns through waste reduction.

Extending beyond immediate personal relevance, to be successful the technology will need to be compatible with existing and evolving values and social norms (Rogers, 2010). In socialising these technologies, marketers may tap into consumer-innovators' drive for stimulation, novelty-seeking and/or a need to express identity (Roehrich, 2004). With this in mind, it would be beneficial for potential 3D food printing applications within the food service sector to emphasize the customisability and personalisation features of the technology to appeal to the consumers' individuality so that they feel as though they can enjoy a unique user experience. For example, the technology facilitates the physical construction of foods in novel ways to appeal to the consumer. This presents operators in the food service market, for example restaurants, to construct unique food items themed around special occasions without incurring prohibitive costs. There are emerging examples where these initiatives are being implemented by chefs in high-end European restaurants who have 3D-printed meals of a theatrical nature with novel flavours and textural combinations to showcase the full capabilities of the technology (Ahmed, 2017; byFlow, 2018a; byFlow, 2018b; La Boscana restaurant, 2016).

It interesting to note that the hypothesised role of consumer innovativeness in determining willingness to try was not supported. This is contrary to much of the existing literature which suggests that consumer innovativeness has a positive and direct effect on willingness to try/adopt. In this study we used a domain level innovativeness measure that focused on food. However, given the nature of the application, participants may not have perceived the resultant foods as innovative (e.g., snacks), and thus, rather than food innovativeness being important, it is their perspective of the technology that was key. The analysis highlights the significant direct and indirect influence of NFTN on willingness

to try the technology. Equally while the hypothesised direct effect of trust in science on willingness to try was not supported the significance of trust in science lies in its diminishing effects on NFTN and naturalness. These indirect effects have interesting consequences at a higher level and point to the potential role for various stakeholders in improving the prospects and consumer openness to innovative applications of technology in food production.

At a more general level, the paper highlights how the negative effect of NFTN might be mitigated. The findings clearly show that trust in science has a substantial and significant depressing effect. While our complete model addresses the specific application of 3D printing, this particular finding may be generalised to other novel food technologies. Increasing confidence in scientists and science will have a positive impact in the consumer environment and the willingness to embrace new technologies. Thus our findings have broader implications for the nature and content of marketing communications by industry and even policy makers. This will require a more long-term approach and a renewed focus on how food science is portrayed in consumer markets.

6.6 Limitations and future work

3D food printers are considered a novel and disruptive technology (Lupton and Turner, 2016). With that said, we are in the midst of experiencing a massive growth of technological advancements at an unprecedented rate and, consequently, the evolution of 3D food printer technologies can be predicted to continually change and adapt according to future trends and demands. Therefore, the results of this study are contingent on the fact that 3D food printers are not currently at the time of writing considered a mainstream technology or commonly used by consumers. However, we must be mindful that, in the near future, the factors affecting Irish consumer acceptance of 3D food printers may change if the technology becomes more readily available and commonplace in consumers' lives, and this must be considered when approaching future studies.

As exclusively Irish consumers were included in this study, this could be considered a limitation as cultural and societal norms tend to differ in other European countries and elsewhere in the world, therefore, attitudes towards the use of 3D food printing

technologies might differ depending on the consumer's country of residence. It would an interesting investigation to compare the attitudes of other consumers worldwide and determine differences, if any, in the level of acceptance of 3D food printing applications.

Another aspect that could be explored further is to determine the relative importance of the mix of potential benefits that might be more or less salient among consumer segments. The technology offers so many opportunities through customisation in terms of functional, emotional and self-expressive benefits that determine those that are most likely to resonate in different consumer environments (out of home vs in-home) merits consideration. For example, it has been suggested by Sun *et al*. (2015) that 3D food printers could be utilised as a way of reducing food waste and increasing the use of sustainable food sources by disguising less appetising, but sustainable food ingredients such as algae and fungi, in appealing 3D-printed shapes in order to increase consumption of foods that would normally go to waste or alternatively to transform less attractive but healthier foods into forms that are more likely to be consumed by resistant consumer segments such as children.

In addition to this, there are reports of the potential use of 3D food printers as a means of portion control for individuals, where snacks and meals are 3D-printed in portion sizes unique to that individual based on biometric data supplied to the printer software (Lupton and Turner, 2016; Sun *et al*., 2018). Therefore, it would be of value to include items relating to sustainability and the environment in a future study, especially in more recent times when actions against climate change have become increasingly more urgent and compelling (Bouman *et al*., 2020; Mariam *et al*., 2020).

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Supplementary material

Fig. S6.1: Layout of online survey

Information Sheet

Thank you for considering participating in this research project. The purpose of this document is to explain to you what the work is about and what your participation would involve, so as to enable you to make an informed choice.

The purpose of this study is to determine the measures which affect the acceptance of Irish consumers to try 3D-printed foods. Should you choose to participate, you will be asked to complete a survey, which will include items on attitudes towards food, levels of trust in government and science and the consumer's relationship with food.

Participation in this study is completely voluntary. There is no obligation to participate, and should you choose to do so you can refuse to answer specific questions, or decide to withdraw from the study. All information you provide will be confidential and your anonymity will be protected throughout the study. IP addresses will not be collected at any point, meaning the data you provide cannot be traced back to you.

You maintain the right to withdraw from the study at any stage up to the point of data submission. At this point your data will be collated with that of other participants and can no longer be retracted.

The anonymous data will be stored on the University College Cork OneDrive system and subsequently on the UCC server, for a minimum of ten years. The information you provide may contribute to research publications and/or conference presentations, and also to my doctoral thesis.

We do not anticipate any negative outcomes from participating in this study. Should you experience distress arising from participating in the research, the contact details for support services provided below may be of assistance.
This study has obtained ethical approval from the UCC Social Research Ethics Committee.

If you have any queries about this research, you can contact me at megan.ross@student.ucc.ie or my research supervisor at m.mccarthy@ucc.ie.

If you agree to take part in this study, please check the 'yes' box below. If you have changed your mind about taking part in this study, please check the 'no' box.

□ Yes, I consent
□ No, I do not consent

Eating habits information and screening questions

Many thanks for consenting to partake in this survey. Before we begin, I would like to ask a brief set of questions with regards to some basic information and your day to day involvement with food practices in an ordinary day. Please answer them as accurately as you can.

What is your gender?

☐ Male

☐ Female

☐ Prefer not to say

☐ Other (please specify)

What is your age?

☐ 24-34

☐ 35-44

☐ 45-54

☐ 55-65

What is your country of residence?

☐ Ireland

☐ UK

☐ Europe

☐ Australia

☐ USA

☐ Canada

☐ Africa

☐ Latin America

☐ Mexico

☐ Asia

How much responsibility do you have for food shopping in your household?

□ Mainly

□ Jointly (i.e., myself and another person/other people)

□ None (i.e., someone else)

Where do you usually go to buy food? (you can choose more than one answer)

□ At farmer's markets

□ In the supermarket

□ In small shops (e.g., butchers, bakers, grocers etc.)

□ In organic/health food shops

□ Online

□ Other (please specify)

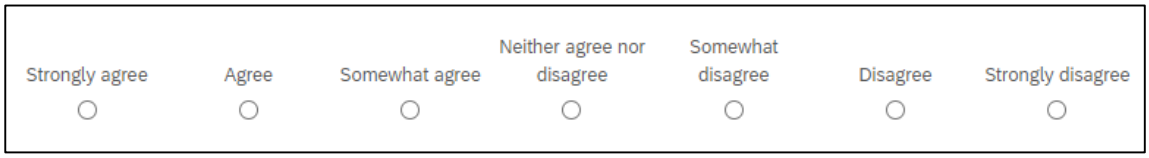
Do you/have you worked in areas linked to the food sector? (i.e., developer and/or manufacturer of foods or food appliances)

□ Yes

□ No

Survey Questions (Note: Question order was randomised)

The following set of questions involves expressing the degree that you agree or disagree with the following statements on a 7-point scale, where 1 = strongly agree and 7 = strongly disagree (example below).



Please read each question carefully and press continue to proceed to the first set of statements.

Consumer Innovativeness

To what extent do you (dis)/agree to the following statements?

Please rate from a scale range of (1 = strongly disagree) to (7 = strongly agree)

Q1. In general, I am among the last in my circle of friends to buy a new food product when it appears in the shops

Q2. If I heard that a new food product was available in a shop, I would be interested enough to buy it

Q3. Compared to my friends I have bought/tried few new food products

Q4. I will buy a new food product, even if I have not heard of it yet

Q5. In general, I am the last in my circle of friends to know the latest food brand names and new food products

Q6. I know the names of the latest food brands and new food products before other people do

Novel Food Technology Neophobia

To what extent do you (dis)/agree to the following statements?

Please rate from a scale range of (1 = strongly disagree) to (7 = strongly agree)

Q7. New food technologies are something I am uncertain about

Q8. New foods are not healthier than traditional foods

Q9. The benefits of new food technologies are often grossly overstated

Q10. There are plenty of new tasty foods around so we do not need to use food technologies to produce more

Q11. New food technologies decrease the natural quality of food

Q12. New food technologies are unlikely to have long term negative health effects

Q13. New food technologies give people more control over their food choices

Q14. New products using new food technologies can help people have a balanced diet

Q15. New food technologies may have long term negative environmental effects

Q16. It can be risky to switch to new food technologies too quickly

Q17. Society should not depend heavily on technologies to solve its food problems

Q18. There is no sense trying out high-tech food products because the ones I eat are already good enough

Q19. The media usually provides a balanced and unbiased view of new food technologies

Trust in science

To what extent do you (dis)/agree to the following statements?

Please rate from a scale range of (1 = strongly disagree) to (7 = strongly agree)

Q20. Science and technology are making our lives easier and more comfortable

Q21. Science and technology are helping to improve our health

Q23. Most scientists want to work on things that will make life better for the average person

The following part of the survey involves reading a text with the definition and applications of 3D food printing technology laid out for you. Please read the description carefully before answering the questions following the text.

The questions will be laid out as before where you will express the degree that you agree or disagree with these statements on a 7-point scale, where 1 = strongly agree and 7 = strongly disagree.

3D Food Printing Information Sheet

Please read the following description of 3D food printing technology carefully

- Three-dimensional (3D) food printers are essentially a piece of equipment which can build 3D structures/designs by extruding any flowable food materials from a nozzle onto a platform in a layer-by-layer fashion.
- Foods that can be used in the food printer include, but are not limted to: pasta, chocolate, cheese, butter and fruit and veg purées.
-

After reading the description, please answer the questions on the next page.

<u>Naturalness</u>

To what extent do you (dis)/agree to the following statements?

*Please rate from a scale range of (1 = strongly disagree) to (7 = strongly agree**)***

Q24. I seek to choose food products which are minimally processed

Q25. I avoid foods that contain artificial preservatives

Q26. When I purchase foods, I make sure that these are natural

Perceived relevance

To what extent do you (dis)/agree to the following statements?

Please rate from a scale range of (1 = strongly disagree) to (7 = strongly agree)

Q27. The purchase and/or use of 3D-printed food products would let others see me as I ideally would like them to see me

Q28. The purchase and use of 3D-printed food products could help me attain the type of life I strive for

Q29. I believe that I could make connections or associations between the purchase and use of 3D-printed products and other experiences and/or behaviours in my life

Q30. I think that the purchase and use of 3D-printed food products would be of personal importance to me

Q31. The purchase and use of 3D-printed food products would help me to express who I am

3D food printing applications questions

Thank you for your patience for the duration of this survey, we are almost at the end of the survey. The following questions involve concept applications of 3D food printing and where they might fit in in every day society. Could you take a moment, and choose how likely it would be that you would hypothetically use the following applications in your own life.

The questions will be laid out as before where you will express the degree of your likelihood in trying each application on a 7-point scale, where 1 = extremely likely and 7 = extremely unlikely.

Dependent Variable - willingness to try

Q32. Snacks freshly 3D printed on demand from a vending machine in public spaces (e.g., cinema, shopping centres, airports, train stations etc.)

Q33. 3D food printer in the gym/fitness centre

Q34. 3D-printed meal at a restaurant

Demographics questionnaire

You have now completed the survey. Before you go, there are a few basic questions we would like to ask you, if you could kindly spare 1-2 minutes to answer them, it would be much appreciated. Thank you.

Which race/ethnicity best describes you? (Please choose only one)

□ American Indian or Alaskan Native

□ Asian/Pacific Islander

□ Black or African American

□ Hispanic

□ White/Caucasian

□ Multiple ethnicity/other (Please specify)

What is the highest level of education you have received? (please choose only one)

□ Primary school

□ Secondary school

□ Undergraduate/bachelor's degree

□ Master's degree

□ Doctoral degree

□ Other (please specify)

Which of the following best describes your current occupation?

□ Employed (Full-time, more than and including 30 hours per week)

□ Employed (Part-time, less than and including 29 hours per week)

□ Unemployed (Currently looking for work)

□ Unemployed (Not currently looking for work)

□ Student

□ Retired

□ Self-employed

□ Unable to work

□ Other (please specify)

What is your chief earner in the household's yearly income (after tax)?

□ Less then €20,000

□ €20,000 - 40,000

□ €41,000 - 60,000

□ €61,000 – 80,000

□ €80,000 – 100,000

□ More than €100,000

Which of the following best describes your current marital status?

□ Married

□ Widowed

□ Divorced

□ Separated

□ In a domestic partnership or civil union

□ Single, but cohabitating with a significant other

□ Single, never married

□ Other (please specify)

How many people currently live in your household? (please enter a whole number in the text-box below)

Table S6.2: Items for the dimensions of Novel Food Technology Neophobia

Measures for the dimensions of Novel Food Technology Neophobia	
Risk	New food technologies are something I am uncertain about
	New food technologies may have long term negative environmental effects
	It can be risky to switch to new food technologies too quickly
Lack of Benefits	There are plenty of new tasty foods around so we do not need to use food technologies to produce more
	Society should not depend heavily on technologies to solve its food problems
	There is no sense trying out high-tech food products because the ones I eat are already good enough
Quality Concerns	New food products using new technologies are not healthier than foods created using traditional methods
	The benefits of new food technologies are often grossly overstated
	New food technologies decrease the natural quality of food

Chapter 7

General discussion and future perspectives

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7.1 Overview of key themes

As the work relating to this thesis is multidisciplinary in nature (See Fig. S7.1 in supplementary material for example of timeline demonstrating works of two of more disciplines concurrently at times), the following section is ordered by experimental chapter to combine and highlight the key themes concerning topics related to engineering, food science and consumer science collectively (Chapter 1, which precedes the experimental chapters, outlines the current knowledge in these areas surrounding 3D food printing, but also the opportunities and challenges which guided the focus of Chapters 2-6). Beginning with Chapter 2, the rationale behind certain decisions involving printer design is briefly discussed, followed by an overview of the relevance of identifying and controlling key printing parameters. Attention is also given to the importance of including consumer insight at early stages of printer design and application concept. The effect of physicochemical changes and printing parameters on the printability and functionality of dairy-based systems, such as processed cheese and concentrated casein-based formulations are also reviewed (Chapters 3 and 4). The relationship between understanding the effect of these physiochemical changes on texture and printability and how this could allow consumers more choice in sensory profiles is also discussed. To conclude, the relationship between the key barriers and incentives to consumer acceptance of 3D-printed food from Chapter 5 and 6 were explored, which will serve as the basis for recommendations to mitigate challenges to acceptance.

7.1.1 3D food printer design and consumer perceptions

In Chapter 2A, the primary aim was to develop a solid foundation of fundamental knowledge of 3D printers that could be built upon in subsequent chapters (Chapter 3 and Chapter 4). A thorough review of the types of 3D printers available helped identify the key characteristics of a printer suitable for the applications proposed in Chapter 3 and Chapter 4. Two traditional plastic printers with different mechanical characteristics were chosen to explore two different methods of feed delivery, syringe-extrusion and positive displacement (i.e., peristaltic pump). Identifying the rheological characteristics of the foods intended for printing (i.e., Newtonian/non-Newtonian/ideal plastic), as well as the

conditions necessary for printing (i.e., is temperature control required for sufficient flow? Which food-grade materials are suitable for construction for this particular application?) helped to guide the design for the modifications made to each printer. Approaching the initial stage of printer design in this manner allowed for a much better understanding of the function of each component featured on the printer (e.g., heater wires and stepper motors) and how they could be employed to print food rather than plastic.

As different elements of this thesis project were pursued concurrently in some cases (e.g., carrying out focus groups, making modifications to 3D printers and initial formulation development could occur within a few weeks of each other), it was possible to consider the consumer perspective when designing the printer. From a written description of the printer, focus group participants expressed concern about the operation of the printer and the "ultra-processed" appearance of the printed products. Therefore, it was important to take this observation into consideration and avoid adding too many features to the printer which might exaggerate the perception of complexity of the printer. Though this printer was designed purely as a prototype model for research purposes and not a commercial printer, it was useful to have this perspective from which to draw and help focus the key elements which would feature on the printers (e.g., neat, simple and safe mechanical design, temperature control to maintain food material consistency, and ease of cleaning and dismantling).

7.1.2 Fundamental printer operation and understanding

Following on from the initial design of the printers, the next step was to address the variables that have the potential to affect the overall quality of the print (Chapter 2B), such as print speed, material viscosity, printing temperature, nozzle diameter and distance to the print bed. This step was crucial to understand which elements of printability were important to monitor and control.

It was also important to note that only a subset of printing parameters was studied in Chapter 2, others have identified additional processing parameters such as extrusion rate, infill porosity, air pressure and flow volume of cooling air which have been suggested to be key processing parameters to measure with the aim of characterising printability (Derossi *et al*., 2018; Hao *et al*., 2010; Kern *et al*., 2018; Lanaro *et al*., 2017; Lille *et al*.,

2018). Understanding the relationship between these parameters is essential for researchers to fully explore what this technology has to offer in terms of novel products with innovative textures and designs and customisation potential. However, to draw from a particular theme from the consumer acceptance work outlined in Chapter 5, focus group participants expressed a preference for convenient and easy-to-use 3D food printing applications; therefore, for the technology to be practical for consumer use, it is important that an automated or A.I. (Artificial Intelligence) system be implemented in these applications which would control for these factors without the need for consumer intervention (e.g., printing processing parameters such as nozzle diameter and printing temperature automatically change based on a code or similar identification method that is read when the food cartridge is loaded into the printer).

Observations from Chapter 2B, such as maintaining a suitable printing height and selecting an appropriate print speed and nozzle diameter, were useful to consider prior to commencing work with different food systems. This was a key element in maintaining the reproducibility and uniformity of printed structures in subsequent chapters (Chapters 3 and 4).

7.1.3 *Exploring the effect of physio-chemical changes of 3D-printed cheese and the impact of these observations on consumer acceptance*

Dairy-based processed cheese products (PCPs) (i.e., products formulated using a blend of natural cheese, emulsifying salt, water and other optional ingredients) have been studied extensively in the conventional sense (i.e., blocks, sliced, shredded PCPs), and many factors, such as composition and processing parameters, have been identified that have an effect on the texture and functionality on traditional manufacture of PCPs (Černíková *et al*., 2017; Guinee, 2022; Lenze *et al*., 2019; Mozuraityte *et al*., 2019; Salek *et al*., 2019; Talbot-Walsh *et al*., 2018; Talbot-Walsh and Selomulya, 2021).

However, a gap was identified in the literature involving the effect of some of these factors, such as pH and the structural protein content of Cheddar, on the printability of processed cheese. Le Tohic *et al*. (2018) previously demonstrated the effect of printing on commercially manufactured processed cheese; however, the work of Le Tohic *et al*. (2018) primarily focused on the effect of printing parameters (such as extrusion rate and

heat treatment prior to printing) on the printability of a singular recipe, whereas the experimental work presented in Chapter 3 built upon this study and delved deeper into the effects of pH, structural protein content and printing temperature on the printability of a basic processed cheese recipe. Another consideration, which was not included in the work of Le Tohic *et al*. (2018), was the addition of temperature control to the printing process to maintain overall temperature of the processed cheese within the syringe barrel. This feature also allowed for the effect of different printing temperatures on the printability of processed cheese to be compared.

Processed cheese was identified as a suitable candidate for the 3D printing process as the heating and shearing steps typically used in PC manufacture are also features of 3D printing (i.e., extruding and shearing a heated processed cheese through a narrow orifice or nozzle). One of the challenges with working with a novel technology such as 3D food printing is selecting and developing the methodology for measurement of the key characteristics of the printing material. However, the use of the Starch Pasting Cell (SPC) attachment on the rheometer was an ideal tool for this application, allowing for small batches (30 g) of processed cheese recipes to be produced using the heating and shearing capabilities of the double-walled jacket and rotating impeller, respectively. Using the SPC, the rheological characteristics of each of the processed cheese recipes could be monitored and the effect of pH change or structural protein content could be compared. This method proved to be a beneficial pre-screening step before printing, as a viscosity profile could be constructed for each recipe and used to determine the optimum range of viscosities suitable for the printing process.

Another challenge was the process of defining which elements would be important in measuring printability of processed cheese, as the parameters defining printability tend to differ depending on the type of material measured and the focus of the study (e.g., flowability, accuracy, structural stability) (Wilms *et al*., 2021). The elements selected to describe printability were chosen primarily based on certain key features of 3D printing, such as accuracy and reproducibility. The use of an innovative tool such as imaging analysis (ImageJ software) allowed for the printed processed cheese grids in Chapter 3 to be objectively measured and be compared to the dimensions of the original CAD model to test the accuracy of each print. At the time of doing the study in Chapter 3 (2018),

imaging analysis was not a commonly used method of quantifying printability; a more typical and subjective method was to visually compare images of printed structures and allocate an ordinal scale for printable and un-printable materials (Lille *et al*., 2018). Correlating the data for each recipe for their viscosity profile, printing accuracy and texture allowed for a detailed description of how pH and structural protein content can affect these factors, which could be helpful to other researchers or those in industry when creating processed cheese recipes with customised textures and product applications. Creating products that can be personalised to suit consumer preferences could also be a way of increasing consumers' level of perceived relevance, which was shown to be a significant barrier to acceptance in the consumer science works outlined in Chapter 5 and 6.

7.1.4 Understanding the effect of calcium and structural geometry on functional characteristics (i.e., melting, dissolution and solubility) of printed MCC

Micellar Casein Concentrate (MCC) is a dairy-based ingredient which has been the subject of recent interest in the literature, primarily due to its unique thermoreversible characteristics, but also its water-binding and stabilising capabilities, which have been demonstrated in products such as processed meats, nutritional bars, desserts, soups and coffee creamers (Hammam *et al*., 2021; Lu *et al*., 2015). MCC solutions (18 % protein) can have a liquid, flowable state at temperatures above 22 °C and form a gel when cooled (< 22 °C) (Amelia and Barbano, 2013). This thermoreversible behaviour is an attribute which inspired the use of MCC in potential 3D food printing applications, where ideally the printable material will be free-flowing when heated during extrusion and form self-supporting layers once allowed to cool on the surface of the print bed.

MCC powders are also known to have poor rehydration properties, which makes them difficult to use in applications requiring fast rehydration. Some authors have proposed that manufacturing liquid MCC concentrates that can be melted can help overcome this problem (Amelia and Barbano, 2013; Lu *et al*., 2015); however, the rate at which these products melt has received little attention. In Chapter 4, the goal was to explore the potential 3D food printing to enhance the rate of dissolution of these liquid concentrates,

particularly in a consumer context (e.g., casein-based sports nutrition products). Solubility tests were also included to quantify the amount of protein released during dissolution, to further characterise the functionality of printed MCC, especially in the case of products that may be marketed as a good source of protein.

As the ability to create custom shapes and structures is a unique feature of 3D printing, the effect of different geometries (a hemisphere and a porous lattice structure) on dissolution rate and protein solubility was of particular interest in this study. Depending on consumer preference for a faster or slower melting product, they could choose or print the lattice structure or hemisphere respectively. To the authors' knowledge, there are no studies at present looking at the effect of structural geometry on melting or dissolution properties of dairy-based printed structures; however, this effect has been studied in pharmaceutical applications. For example, Ayyoubi *et al*. (2021) observed that composition of the extruded material had a more significant effect on dissolution rate than geometry. Another study reported that surface area and dissolution rate of 3D-printed tablets were directly correlated (i.e., increasing surface area led to an increase in dissolution rate) (Sadia *et al*., 2018) Controlling the rate of dissolution of drugs during digestion is an important factor to consider in pharmaceutical contexts, as certain active ingredients may require slow or fast release throughout the body depending on the application (e.g., fast pain-relief for temporary treatment versus slow-release pain-relief for more long-term treatment). Although the dissolution tests included in the present study were completed using water as the dissolution medium to determine roughly how 3D-printed MCC samples would melt at body temperature (37 °C), it would be interesting to complete an *in-vitro* study to accurately monitor the effect of geometry and composition (i.e., calcium chelation/supplementation) on dissolution in a model digestive system, similar to that carried out in the studies mentioned previously (Ayyoubi *et al*., 2021; Sadia *et al*., 2018). An *in-vitro* study could be a useful tool in the food industry, especially in sports and clinical food settings, whereby 3D printing geometry and ingredient composition could be used together to control energy and micronutrient release in the body.

The effect of calcium supplementation and chelation on dissolution rate and protein solubility was also investigated. Depending on the application, especially whether the

product will be eaten post-printing or dissolved in a carrier fluid (i.e., smoothie, milk, water etc.) and then consumed, the variables surrounding rate of dissolution (i.e., calcium addition or chelation, temperature of the carrier fluid and printed geometry) can be altered to suit the desired consumer experience. This particular application could be useful to incorporate into the hypothetical gym/fitness centre application, which was the preferred application to focus group participants in the work carried out in Chapter 5. Individuals were more accepting of 3D-printed food products which provided a convenient and a personalised service; therefore, providing consumers with a range of textures and customisable applications to choose from could be a pragmatic use of the technology. Caulier *et al*. (2020) also proposed that reaching the desired level of personalisation required by consumers in terms of customisable nutritional content and sensory attributes is a key way to enhance consumer acceptance.

7.1.5 Impact of consumer perceptions of 3D food printing on printer and product applications

The inclusion of studies involving consumer science (Chapter 5 and 6) in this thesis was important, if not vital, in order to develop a well-rounded perspective of 3D food printing as a whole. As 3D food printers are marketed as a prosumer technology (Bravi *et al*., 2017), it is especially meaningful to incorporate a consumer-focused element when considering the design and implementation of 3D food printers, as well as during the initial development of 3D-printed foods. Chapter 5 studied Irish consumers' initial impression of 3D food printing applications through the use of focus groups, which allowed for a number of themes, such as perceived personal relevance, attitudes towards naturalness and consumer autonomy, to be identified.

The dominant response across all groups was uncertainty, especially surrounding the function, operation and safety of 3D food printers. Consumers were also concerned with the terminology associated with 3D food printing, especially the term "3D food printing" itself. Therefore, it is essential to keep these concerns in mind during the initial stages of printer design and product formulation, but also during the promotion of the technology, to help to dispel apprehension towards 3D food printing applications. For example, in Chapter 2, using food-grade materials in the design for an easily removable printer syringe

and print bed matting was an essential design feature to ensure that food safety was a priority.

Focus group participants were also reluctant to entertain the idea of using a 3D food printer at home, and indicated that vending machine and out-of-home use applications were preferred. Food can have symbolic meanings for consumers beyond the nourishment it provides, especially when associated with meaningful experiences and rituals (Brown, 2011). Therefore, it is important to take consumer perceptions, values, beliefs and behaviors into account and introduce 3D food printing applications in a way that consumers will be most open to experiencing, rather than coercing the population into purchasing and using printers for at-home use. Focus group participants were also hesitant to use the technology for themselves, and were quick to identify applications for others (e.g., students/individuals with lower income, those with food allergies or medical conditions) rather than for their own use. It appears that consumers are more accepting of 3D food printing at a societal level, in comparison to technologies with higher perceived risk (e.g., irradiation and GM); however, a lack of perceived personal relevance and willingness to use 3D food printing technology appears to be where the challenges lie in this case. Identifying and promoting features of 3D food printing (such as reducing food waste, personalised nutrition and customised structures) which align with consumer values and priorities in life could be another key step in establishing personal motivation to purchase or try 3D-printed foods.

7.1.6 Identifying significant factors and their relationship to consumer willingness to try 3D-printed food applications

Based on themes identified from the focus group work outlined in Chapter 5, a survey was designed to generate data in order to determine the primary factors affecting Irish consumers' willingness to try 3D-printed foods. Identifying the most significant factors affecting the dependent variable (i.e., 'willingness to try 3D-printed foods), enabled more informed decisions to be made throughout the work of this thesis. For example, results showed that perceived personal relevance was among the most significant factor in determining consumer willingness to try 3D-printed foods and giving consumers more agency and personalised choices in their printing experience (e.g., customisable textures,

flavours, shapes, nutrition etc.) may enhance perceived relevance of the technology to their everyday food lives. The ability to modify these parameters have been demonstrated in Chapters 3 and 4, where, for example, texture can be modified based on pH changes or calcium concentration. Increasing the number of available sensory profile options (e.g., flavour and texture) of customisable 3D-printed snacks has been shown to led to enhanced consumer satisfaction, according to Caulier *et al*. (2020).

Overall, the relationship model developed in Chapter 6 could be a useful tool for stakeholders involved in the food service and food technology marketing space to use when considering the most significant barriers to consumer acceptance. Keeping in mind that establishing and enhancing consumers' trust in science is a key element to overcoming neophobic tendencies towards novel technologies and reducing reliance on what is natural, it is important that technologists and other actors within the food industry consider their consumer first. This is especially the case during the initial stages of printer design (Chapter 2) and development of formulations suitable for 3D food printing (Chapters 3 and 4). By ensuring that communication and messaging surrounding 3D food printing applications is clear, straightforward and inclusive, a successful introduction into the marketplace may be more achievable.

Exploring the barriers to acceptance of 3D-printed foods in a food service context allowed for a deeper dive into one possible avenue of 3D food printing applications, a perspective that has not been previously offered in the available literature. More often than not, a general view is taken on consumer acceptance of 3D food printing technologies (Brunner *et al*., 2018), which can be useful in gauging consumer perceptions at the surface level; however, exploring more specific contexts, such as food service or out-of-home applications, allows more focused insights into consumer perceptions to be revealed. Others have reported similar observations involving novel food technology neophobia (NFTN) when investigating consumer attitudes towards 3D food printing (Brunner *et al*., 2018; Caulier *et al.*, 2020); however, none have studied the relationships between other related variables such as perceived relevance, trust in science and NFTN in the specific context of 3D food printing. By narrowing the scope of our investigation to applications surrounding food service specifically, it could be argued that tangible solutions for barrier-to-acceptance are more achievable. Conversely, attempting to address a range of general

issues which include a number of diverse contexts (e.g., in-home, restaurants, automated applications, delivery/catering service, fast food, independent businesses such as bakeries and convenience stores etc.) could be counterintuitive in identifying pragmatic remedies to the challenges associated with consumer acceptance of novel technologies.

7.2 Applications of outcomes

Incorporating the results and knowledge gathered as a whole over the course of this PhD project will be useful for others involved in the 3D food printing or novel food technology spheres. Stakeholders interested in developing the technology further may use the information in this thesis to build on the fundamental understanding of printer operation, the printing parameters and characteristics of printing dairy-based materials, and also barriers affecting consumer acceptance of 3D food printers.

The methodology used throughout this body of work may also be useful for other researchers to draw from and build on when developing methods to quantitatively measure attributes relating to 3D-printed foods. For example, in Chapter 3 the use of image analysis techniques to create printing precision values in conjunction with viscosity profiles generated using a rheometer can be used to characterise the effect of changes in formulation in processed cheese. In Chapter 4, the use of spectrophotometric measurement methods to record dissolution and solubility rates could be useful for describing the relationship between certain variables like printed geometry and functional characteristics, e.g., dissolution or melting, of the printed product. In addition to this, there is little to no research available at present on the topic of quantitative measurement of factors relating to consumer acceptance of 3D-printed foods, especially research with a robust sample size such as that included in Chapter 6. The relationship model created in the quantitative work (Chapter 6) provides a solid framework with tangible information which can be used by those studying or working in consumer behaviour analysis in relation to acceptance of novel food technologies.

The work in this thesis also provides comprehensive information on the topic of 3D food printing which can be used either in its individual parts or as a whole in creating potential 3D-printed food products or in developing 3D food printer designs. The data generated

may be useful for other researchers, food professionals or those with an interest in 3D printing to create novel and innovative food structures by applying the practical information on physicochemical reactions and rheological characterisation of dairy-based formulations to potential future products.

7.3 Recommendations for future research

Additional temperature and humidity control in 3D printer design

One major priority following on from this work is creating a controlled environment to regulate external environmental factors, such as humidity and ambient temperature. A container could be constructed using clear Perspex or similar material which is moisture and heat resistant to encase each 3D food printer and allow users to view the printer clearly during operation. A digital humidity controller would also have to be installed to allow for a fixed humidity level to be maintained within the container whilst the 3D printer is running. A dedicated area at the side of the container would need two holes to be cut out in order to allow the USB cable and 3D printer power supply cable to feed through and be connected to the laptop and power supply which will be located outside the container for easy access. These holes will need to be made airtight to maintain the designed humidity and ambient temperature within the container. The construction of a unit which is temperature- and humidity-regulated would enable users to eliminate external factors that might affect the quality of the print. As temperature and humidity levels can fluctuate depending on the location of the printer, this type of approach would standardise the ambient conditions affecting the operation of the printer.

A further step in improving the functionality of the printer would be the addition of a cooling feature to the print bed. Depending on the material being printed, it can be beneficial to speed up the rate of solidification of cold-gelling materials (such as dairy-based ingredients like micellar casein concentrate; Lu *et al*., 2015) so as to reinforce the structural stability and strength of the material as it is being extruded in layers.

To develop a cooling system suitable for the 3D printers, one solution might be to install a Peltier plate under the print bed or even replace the print bed entirely with the Peltier plate to ensure direct contact with the base of the printed sample. Another solution may

be to add a water-cooled condenser underneath the print bed as means of drawing away excess heat from the print bed and, by extension, the printed sample. However, this solution would require much more space, as it would require an external volume of water set to the required temperature, whereas the Peltier plate would operate electronically and only require a power supply. Another consideration would be the proximity of water to the electrical circuits of the 3D printer, which may present an electrical hazard; therefore, this option may not be feasible without the proper precautions for electrical safety.

Incorporating new delivery system designs for 3D food printers

As mentioned in Chapter 2, the delivery system for the Delta printer (i.e., using peristaltic pumps) would require adjustment to be suitable for printing more viscous food materials. If the equipment was readily available, a more powerful peristaltic pump with a higher power output, or a gear system, would be beneficial in generating the required energy for sufficient flow through the silicone tubes. Another system that might be worth exploring that operates in a similar way to that of the peristaltic pump is a tube roller. Instead of the food travelling through silicone tubes to be extruded at the nozzle, a large piping bag, or similar flexible container with a nozzle attached, is used to hold the food material (Fig. 1). The bag is suspended upright in a support frame with the nozzle facing downward and a tube roller (i.e., an implement that is sometimes implemented to squeeze out the remaining toothpaste once the product is almost used up) is attached at the top of the bag and held in place on support rails at either side. As the 3D printer is printing, the tube roller, powered by stepper motors, gradually slides down the support rails and squeezes the material within the bag downwards simultaneously. The downside to this mechanism is the limit to the volume of food material which can be stored in the bag; however, the volume would more than likely be larger than a regular syringe used for 3D food printing (e.g., 20-ml syringe volume used in the Cartesian printer mentioned in Chapter 3 and 4).

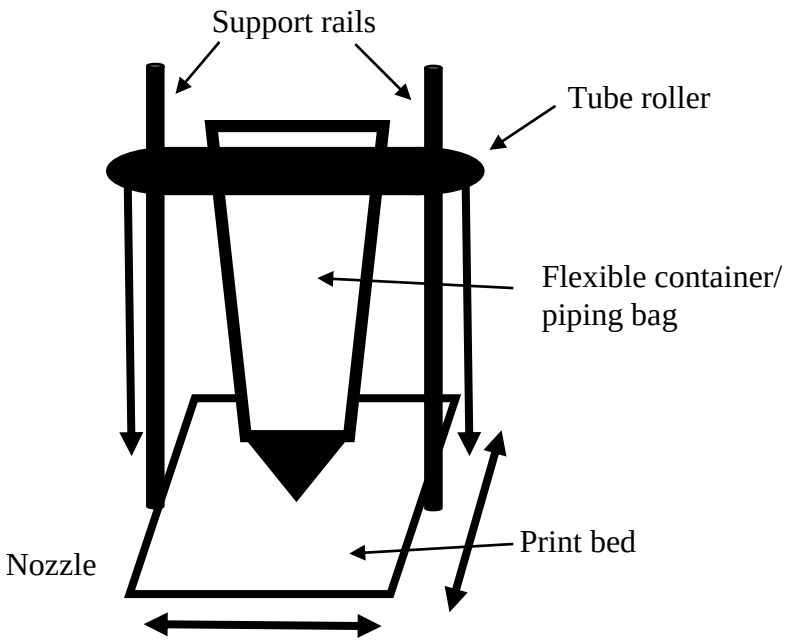

Fig. 7.1. Mock-up of possible alternative printing design involving a tube and roller system

Another research route that would be of interest is the use of 3D food printing to crosslink suitable food systems to form gels. Shah *et al*. (2020) utilised the crosslinking capabilities of sodium alginate and calcium lactate to 3D-print single-layer shapes while exploring the viability of the resultant hydrogel in supporting tissue-cell growth in bioprinting applications. The authors mentioned two methods of gelation whereby sodium alginate and calcium lactate are added together in a certain ratio to form a gel (which was the chosen method in this study), or sodium alginate could be extruded in a container filled with calcium lactate to facilitate initial crosslinking on the external surface area of the gel. Though a multi-layered structure was not demonstrated in this study, it would be useful if the deposition of cross-linking agents could be controlled and print a certain pattern that would result in a mixture of textures. For example, the crosslinking agents could form solid gel-like layers in the external areas of the desired shape, but have a liquid centre where cross-linking does not occur. This could be achieved by a multi-nozzle printer where there are two streams of sodium alginate and calcium lactate feeding into one nozzle. The latter stream could be connected and disconnected *via* a valve system depending on where cross-linking is desired during printing.

The use of a dual or multi-nozzle system could also be useful for other food systems in order to determine the effect on texture and flavour intensity, depending on the distribution of certain ingredients (e.g., salt, sugar etc.) within the layers and infill patterns (Fahmy *et al*., 2021). As mentioned in Chapter 4, Micellar Casein Concentrate (MCC) is a neutral-flavoured dairy-based ingredient and could be employed as a high-protein structure in 3D food printing. In a multi-nozzle system, flavours and colours could be added *via* a second nozzle at set intervals to create a customised printed product. This concept would be interesting to explore further, especially with the combined use of trained tasting panels and rheological analysis (i.e., Texture Profile Analysis (TPA) and melting behaviour). Using tasting panels could allow for a broader description of the product to be generated, which could be quantitatively supported with appropriate textural analysis measurements.

Implementing the use of tasting panels, focus groups with food professionals, and conjoint analysis to further examine consumer behaviour towards 3D-printed food applications

An element that was absent from the scope of this research, but would have been of interest to explore, was the implementation of trained tasting panels. The main reasoning for excluding this type of analysis was primarily due to the use of non-food-grade ingredients in the processed cheese and MCC recipes (i.e., hydrochloric acid, sodium hydroxide, calcium chloride) which would be unsafe for human consumption. However, if these recipes were reproduced using food-grade substitutes, then it would be useful to organise human tasting panels to determine the overall acceptability of these 3D-printed structures. Furthermore, it would be beneficial to introduce 3D-printed samples or a demonstration of the 3D food printer as part of focus group discussions, to allow participants to encounter a more realistic experience when discussing their initial impressions of 3D-printed foods. It can be difficult for participants to assess a product produced from a technology they have never seen in person before and providing physical samples or a demonstration of the printing process, which can be experienced using all five senses (i.e., sight, taste, touch, smell and hearing), may allow consumers to form a more rounded and candid evaluation.

It would also be useful to carry out a larger number of focus groups in addition to the groups mentioned in Chapter 5, and expand the recruitment area to a broader selection of the Irish community. It would also be worth delving deeper into the motivations behind consumers' perceptions of 3D food printing and perhaps doing a mixed-methodology study and incorporating the use of surveys as well as in-depth focus groups to provide quantitative data alongside more descriptive data to complement each other. It might also be of interest to do focus groups exclusively with food professionals, such as chefs, food manufacturers and caterers, to compare their impression of 3D-printed foods to that of non-food professionals and regular consumers. As some commercially-available 3D food printers at present are primarily directed at, and sold to, food professionals (e.g., byFlow, 2022), there may be a difference between the levels of perceived relevance of food professionals and those with non-food related careers.

Conjoint analysis is another useful consumer research technique that can provide insight into the specific factors affecting consumer choice behaviour (Bogue *et al*., 2009; Henchion *et al.,* 2019). For example, consumers could be presented with a number of hypothetical 3D-printed foods or applications which have a list of attributes attached to them (e.g., cost, texture, flavour etc.) and are required to rate them based on preference. By changing the level of a singular attribute (e.g., Context 1: low cost, crunchy texture, strawberry flavour; Context 2: low cost, smooth texture, strawberry flavour) each time using a pre-determined statistical experimental design, this would allow researchers to determine the optimum combination of factors in an objective manner (Cardello *et al*., 2007).

Additional methodologies to further characterise the printability of dairy-based food materials

On reviewing the methodology included in Chapter 3 and Chapter 4, it would be of interest to incorporate the use of other forms of analysis in order to further characterise these printed structures. For example, Computational Fluid Dynamics (CFD) would be useful in monitoring the pressure and flow of dairy-based food materials through the syringe nozzle during printing (Guo *et al*., 2020). CFD operates based on virtual simulations which are fed by external numerical data (i.e., density and viscosity of the

food material) and could be a beneficial screening step in characterising each formulation and quantifying their level of printability.

Another method of interest, which has only been used in one 3D food printing study involving cooking 3D-printed chicken using lasers (Blutlinger *et al*., 2021), is heat-mapping. Blutlinger *et al*. (2021) used Infrared (IR) cameras to map the surface temperature of the 3D-printed chicken, which gave an overview of the distribution of heat during cooking. This technology could be useful in other 3D food applications that do not require cooking (such as highly concentrated protein pastes) to track the cooling rate as the heated pastes are extruded onto the print bed. This could be especially interesting for ingredients with thermoreversible properties like MCC, where heat-mapping could be used in conjunction with rheological measurements to record gelation rates and the relationship with structural stability. Similarly, IR cameras could also be used in multi-layered structures to map the rate of cooling as each layer is printed and determine which factors (such as line width, infill pattern and infill percentage) affect cooling rate and how this might subsequently affect layer adhesion and texture.

Exploit the cold-gelation mechanism of Micellar Casein Concentrate for 3D printing

Others have exploited the cold-gelation mechanism that MCC offers for the purpose of 3D food printing, where an MCC and whey protein suspension was cooled using a double-walled cooling jacket connected to a water bath, resulting in a printable semi-solid gel (Daffner *et al.,* 2021). It would be interesting to explore this mechanism further and observe how the rate of cooling and printing temperature can affect texture and printability. Perhaps utilising the IR camera, in this instance, would be useful in identifying changes in temperature during printing, and whether external temperature control (i.e., using a cooled print bed or temperature-controlled atmosphere) is necessary to maintain the texture and printability of these printed structures (i.e., whether the preceding layer would be robust enough with sufficient layer adhesion to build upon).

A preliminary trial (not presented in this thesis) was conducted to determine the viability of 3D printing MCC (30 % w/v) at refrigeration temperatures (4 °C), where the 3D food printer was placed in a walk-in refrigeration unit. However, the operation of the printer

was inconsistent and struggled to print at lower temperatures and, therefore, the project was abandoned. The primary issue involved the exposure of the printer hardware to suboptimal functioning temperatures. To prevent this, a chamber could be constructed around the building space (i.e., print head and print bed) to create a controlled atmosphere surrounding the printer which could allow the temperature and humidity to be adjusted, and the operating hardware could be placed externally to this chamber. If this set-up was not feasible, perhaps the use of a cooling jacket as used by Daffner *et al*. (2021) would be a suitable alternative, with the addition of a cooled print bed (i.e., through another cooling jacket similar to a plate heat exchanger, or perhaps a Peltier plate system) to add a certain element of temperature control. This would allow the main points of contact between the printer and food material to maintain a cooler temperature, although the electrical components of the printer have the capacity to operate at ambient temperatures safely and without issue.

In addition to the work completed in Chapter 4 of this thesis, it would also be interesting to explore the rheological measurement of the cooling cycle of 3D-printed MCC in order to see the effect of calcium chelation and supplementation on the hardening or solidifying the printed MCC structure. As creating self-supporting layers is key to a successfully printed 3D structure, it would be important to understand the cooling mechanism and how this can be exploited to improve the solidification properties of thermoreversible materials such as MCC.

Recommendations for further exploration of micellar casein as a printing material

In Chapter 4, micellar casein concentrate was selected to be the printing material of choice due the unique thermoreversible properties the ingredient appears to offer. However, there are additional queries surrounding the internal dynamics of the casein micelle in this particular system that would be interesting to investigate. With this information, it might be possible to increase our understanding of the physiochemical factors affecting printing at an even deeper level. For instance, one such area for exploration would be the mechanistic impact of modifying the balance of salts (i.e., addition of calcium chloride/tri-sodium citrate) on the structural properties of the casein micelles in MCC. Others have

measured differences between calcium and phosphate concentration in the casein micelle and the serum phase in concentrated casein systems using High Pressure Liquid Chromatography (HPLC) (Reiter *et al*., 2022). It was reported that only a portion of added calcium (in the form of calcium chloride) is actually transported to the casein micelle in concentrated milk systems in comparison to natural casein concentrations in skim milk. This was said to be due to higher saturation of calcium present in the form of CCP in casein micelles in concentrated systems, such as MCC, than in less concentrated systems such as skim milk (Reiter *et al*. 2022).

It would also be interesting to investigate whether there are significant differences in printability or functionality of printed structures between the use of less casein dominant ingredients such as Milk Protein Concentrates (MPC). Others have studied the printing performance of MPC/WPI (Whey Protein Isolate) or MCC/WPI suspensions (Daffner *et al*. 2021; Liu *et al*., 2018), but a functional comparison between MCC and MPC in terms of printability has never been investigated. Another interesting study could be the addition of MCC to the processed cheese recipes mentioned in Chapter 3. As MCC is a casein dominant structural agent, it could be worthwhile adding it to recipes which were shown to have poor structure forming capabilities in order to improve the print quality.

It has also been suggested by others that differences in processing of MCC liquids prior to spray-drying have an effect on the functionality of 3D-printed MCC structures, which could be an important factor to consider when selecting raw materials for printing. Ferrer *et al*. (2011) and Liu *et al*. (2012) have observed a decrease in soluble calcium in the casein micelle when either evaporation or nano-filtration, respectively, as a means of concentration of liquid MCC prior to spray-drying. However, in the case of evaporation, this reduction in insoluble calcium can be reversed upon rehydration (Ferrer *et al*., 2011). Differences in types of filtration (i.e., diafiltration versus ultra-filtration) also appear to play a part in the degree of turbidity of the suspension (Ferrer *et al*., 2011).

Another factor to explore may be the effect of other calcium chelators such as sodium hexametaphosphate (SHMP) and sodium phytate (SP) on the functionality and printability of MCC. de Kort *et al*. (2011) reported that the addition of different calcium chelators to concentrated dairy systems can have a distinct effect on viscosity and turbidity, primarily

due to differences in calcium-binding abilities. It would be of interest to explore whether using an alternative calcium chelator, as opposed to TSC, would make a difference to the printability of MCC, especially as viscosity has been found to be an important element in determining printability (Chapter 3). With a deeper understanding of the potential factors affecting printability, this information could be used to formulate versatile and customisable products which perform optimally for the consumer in any 3D food printing application (i.e., at home or out-of-home use). This, in turn, could boost the perceived functionality of the technology and convince consumers of the value which 3D food printing can offer.

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Supplementary material

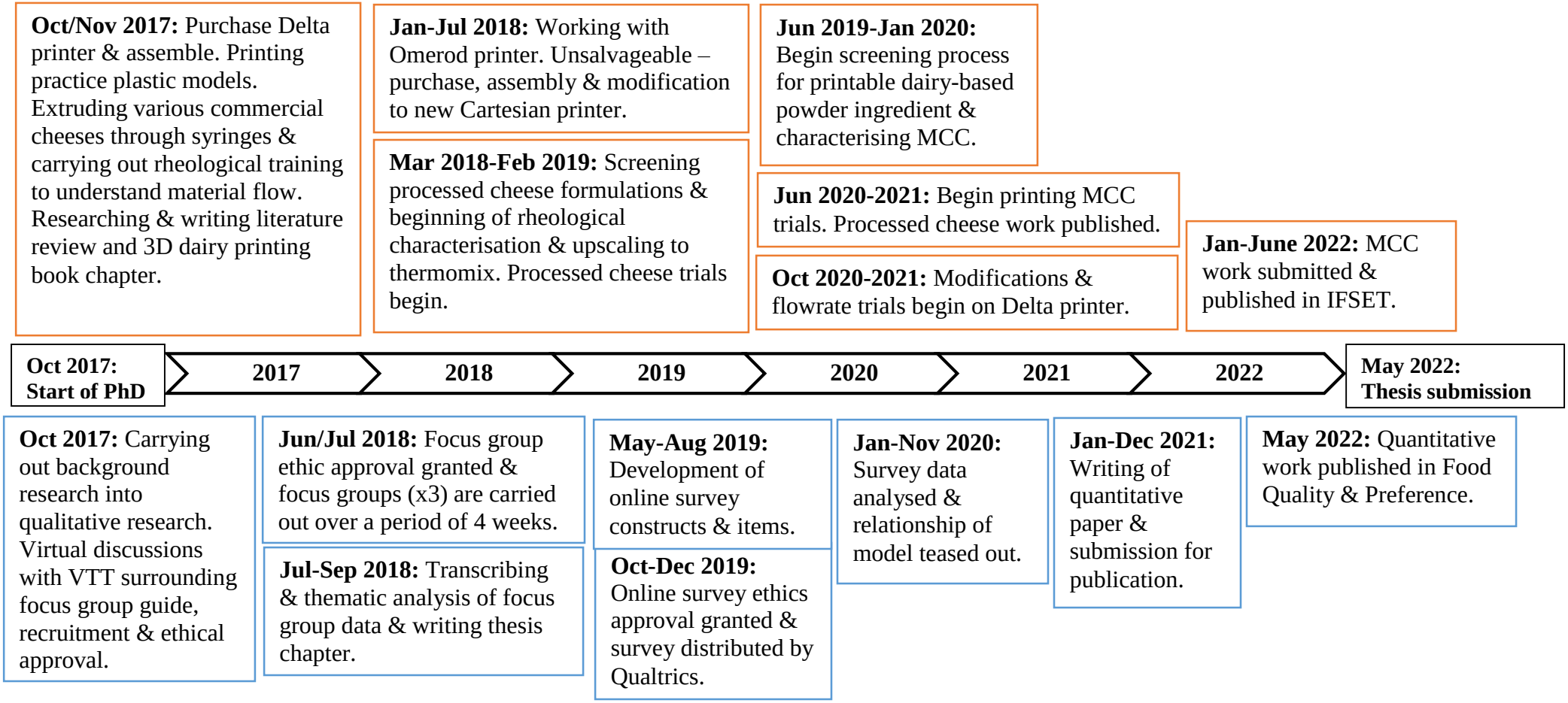
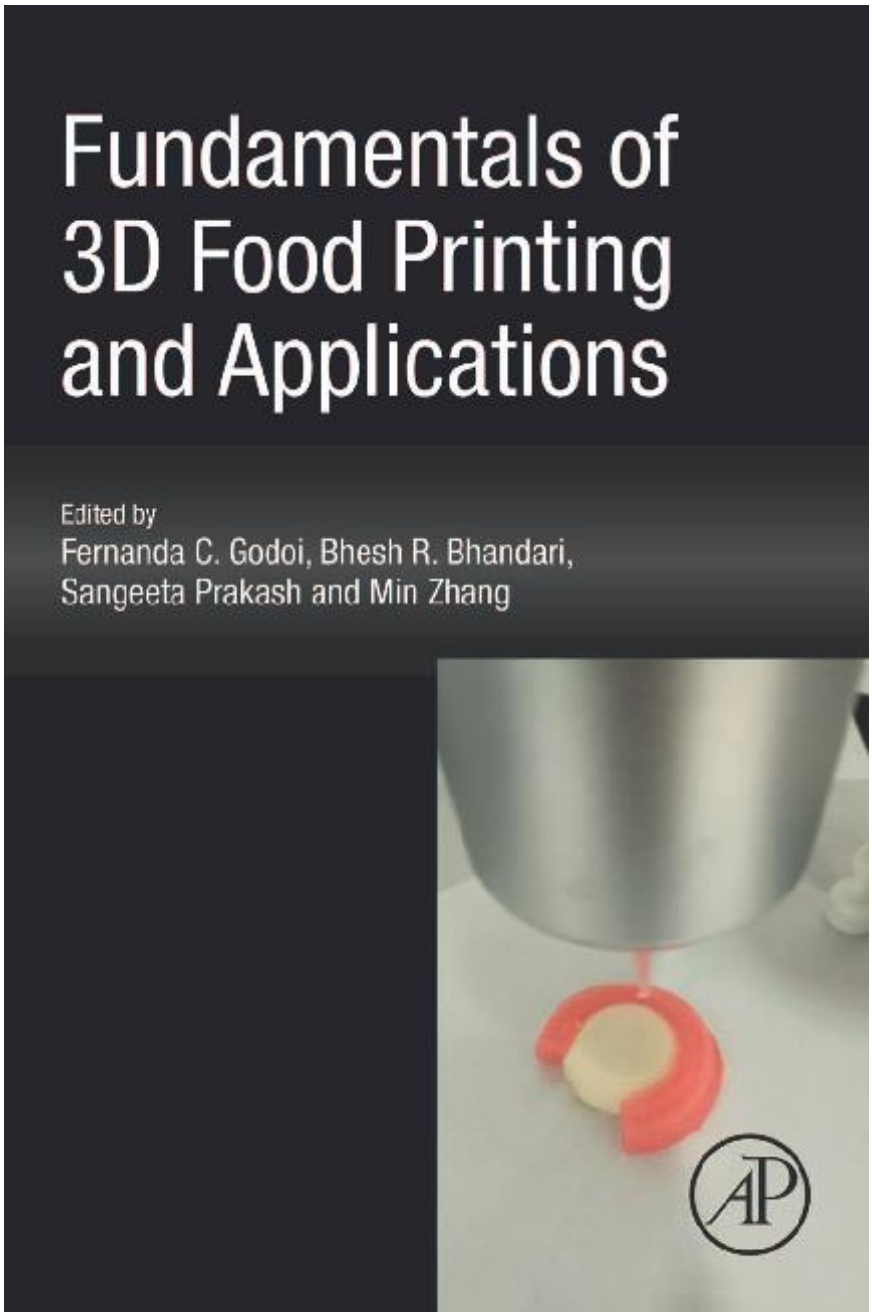


Fig. S7.1. Approx. timeline of PhD project from Oct 2017 til May 2022 which demonstrates how multiple projects of different disciplines (Orange outline = food science and engineering, blue outline = consumer science) often occurred concurrently

Appendix

Publications and additional research outputs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Chapter 7

Potential Applications of Dairy Products, Ingredients and Formulations in 3D Printing

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7.1 INTRODUCTION

3D printing is being investigated for a broad range of applications, including printing of replacement parts for automobiles (Schniederjans, 2017), printing of tissues and organs in regenerative medicine (Murphy and Atala, 2014) and, quite recently, the printing of food as solid structures with unique visual and textural attributes (Sol et al., 2015; Sun et al., 2018). As a novel technology, 3D printing has gathered some momentum in recent years, but has yet to penetrate most home kitchens and food businesses. 3D printing of certain foods, such as chocolate and other confections, has been the subject of both academic research (e.g., Mantihal et al., 2017) and commercial applications (Choc Edge, 2017). In comparison, few scientific studies have focused on the

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printing of dairy-based foods, little is known about optimising the printing of dairy formulations, and no current-generation 3D printer is specifically engineered to print these materials.

The dairy category spans a broad range of products and ingredients with diverse compositions, functionalities and structures, making dairy a promising source of raw materials for the 3D printing of food. The printability of dairy products (e.g., cheese, yoghurt, butter) and ingredients (e.g., protein isolates, edible lactose, milk minerals) is not well characterised despite the considerable opportunities they present for the preparation of foods that are both nutritious and flavoursome. There is significant scope to exploit the strong structure-forming properties of dairy systems during 3D printing through, for example, manipulation of gelling mechanisms, melting behaviour and ingredient interactions. To realise the potential of dairy in 3D printing, different model formulations will need to be tested and optimised for printability, with successfully printed objects subjected to multiscale characterisation to determine their post-printing integrity and functionality.

The evolution of 3D dairy will be interwoven with the broader narrative of food printing. Lupton (2017) studied online news outlets for their coverage of developments in 3D printing of food between 2013 and 2016 and organised results into the five major promissory themes of Futuristic (e.g., 'Kitchen of the future'), Creative (e.g., 'Customised foods'), Healthy (e.g., 'Foods for people with dysphagia and the elderly'), Efficient (e.g., 'Food for plane travellers') and Sustainable (e.g., 'Use of alternative edible substances in food products'). Indeed, the opportunities afforded by 3D printing of dairy can be said to fit within these thematic areas. The inherent 'promise' of 3D food printing should be tempered by the many practical challenges the technology presents to food researchers, which are not limited to issues of retail price and user friendliness. In terms of personalised nutrition, many formulated products used currently in 'healthy' nutrition (e.g., elderly, dysphagia) applications, for example, contain milk protein ingredients as their primary protein source, yet little is known about their printability.

As well as the processing-related challenges related to the effective and safe printing of such foods, there are questions associated with appropriate methodologies for analysing printed artefacts, which may have levels of geometrical complexity that are unprecedented for food. More broadly, much of the health-orientated imaginings of food printing are characteristic of a pervading discourse in food and nutritional science that has been strongly criticised as 'nutritionism' (Scrinis, 2008); for example, some imagine a dietary approach that merges the 'quantified self' movement with 3D food printing, in which self-tracking of biomarkers dictates the nutritional profile of the printed meal (Khot et al., 2017). Furthermore, 3D printing as 'additive manufacture' is frequently referred to as being 'waste free' and therefore sustainable, but this characterisation is not so straightforward. If dairy-derived ingredients are to be used in printing applications, then their entire life cycles,

encompassing associated by-products, emissions and consumer food waste behaviour, will dictate to what degree the printed object is indeed environmentally friendly. These and other issues need to be considered in parallel with technological developments in food printing. Nonetheless, efforts to realise the potential of 3D printing in the food industry continue apace. Many individuals are more concerned with diet, desiring greater control over what they eat without sacrificing modern conveniences (Petty, 2017). Three-dimensional-printed snack vending machines are being developed at present, with the object of delivering healthy printable snacks on the go (Gao et al., 2015). In this way, 3D printing is envisaged as facilitating the accurate 'metering' of ingredients/nutrients in printed meals.

In this chapter, an overview of printing technologies of potential relevance for dairy printing will be provided. The structural transformations that occur in dairy systems will then be discussed, with the aim of gaining insights into future possibilities in printable dairy. The relatively sparse literature on the topic of 3D printing of dairy systems will then be reviewed. Some analytical tools to analyse dairy systems before, during and after 3D printing will then be proposed. Finally, a case study on the 3D printing of processed cheese that integrates the preceding topics will be presented.

7.2 OVERVIEW OF RELEVANT 3D PRINTING TECHNOLOGIES

Over the past decade, the range of 3D printing technologies has expanded rapidly, particularly for the printing of nonedible materials, such as plastics and metal powders; however, food printing technologies are also being developed and are beginning to emerge in the consumer world. The equipment and materials available today are far more sophisticated than when 3D printing technology was first developed (Hull, 1986), when the first patent was issued for a stereolithography apparatus that could print plastics. A decade ago, 3D printers were an expensive hobby costing about the same price as a family car; in more recent times, a basic printer incurs roughly the same cost as a low-end laptop (Miller, 2016). This section will contain a brief overview of how a 3D-printed product is created from computer code and the printing technologies of potential relevance to dairy applications, as well as the advantages and disadvantages of these technologies.

The majority of printing techniques require modelling software such as computer-aided design (CAD) or 3D scanning equipment to create a digital file of the 3D design (3D Systems, 2017). This file will contain information pertaining to the dimensions of the object to be printed. As seen in Fig. 7.1, the most common file format for the 3D model is Standard Tessellation Language (STL), which can be transformed into 'G-code' using a slicing programme such as Slic3r or CuraEngine, both of which are commonly used open-source software applications in 3D printing. This slicing software works by taking the

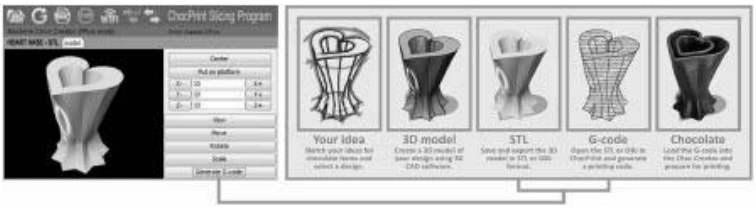

FIGURE 7.1 Example of slicing software used by Choc Creator. Displays step-by-step guide from basic sketch of an idea to final 3D printed product (Choc Edge, 2017).

object in the STL file and 'slicing' it into individual layers, which then act as printing instructions in the form of G-code, guiding where the printer must print the material in order to form the shape (Hamilton et al., 2018).

A common technology that has been used when printing desserts and confectionary is fused deposition modelling (FDM) (Fig. 7.2). This type of technology operates by extruding heated food material from a nozzle or syringe that is guided within the Cartesian coordinate space (X-, Y-, and Z-axis) during printing, with the rate of deposition being controlled by computer-regulated motors. This allows defined layer-by-layer geometries to be constructed using the most efficient pathway possible, with minimal excess material being deposited in the process. As each layer is extruded, the food material cools and hardens, providing a physical support for the subsequent layers (Additively Ltd., 2017).

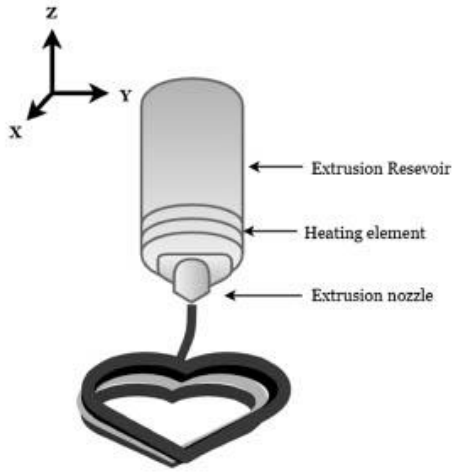

FIGURE 7.2 Fused deposition modelling (FDM). Food material in liquid/semiliquid state is extruded through a narrow orifice and deposited onto a preprogrammed design, layer by layer.

This form of printing often produces a visible layer-by-layer definition that may not be suitable for applications that, for example, require a smooth finish, such as detailed chocolate-based designs. Printing using FDM technology also has the advantage of being able to directly dose structure-building agents (e.g., hydrocolloids, transglutaminase) during printing by incorporating a side inlet embedded in the nozzle (Schutyser et al., 2018). Examples of FDM applications that are available on the market (at the time of writing) include Choc Creator and Foodini (Sun et al., 2018); these machines are claimed to be able to print confectionary-/chocolate-based structures and fruit-/vegetable-/cereal-based structures, respectively.

Another form of FDM printing technology is coextrusion printing. This technology was patented by Guillemette and Peters (2015), which mentions the potential for using coextrusion printing in food applications. In the food industry, extrusion technologies are commonly used to make pasta, dried snack foods, breakfast cereals and confectionary (Ding et al., 2006); however, extrusion technology can be combined with 3D printing to provide customisable layering, enrobing or filling capabilities in various food stuffs. The technology enables users to combine materials in different ways through different nozzle configurations. For example, a food material, such as semi-molten chocolate, could be extruded through a nozzle, while a different food material, such as strawberry jam filling, flows through a narrower nozzle embedded within the main nozzle. In another configuration, different ingredients or formulations could be extruded from adjacent nozzles, creating side-by-side layers in a visibly stratified product. Guillemette and Peters (2015) have claimed that their coextrusion technology has the potential to incorporate localised preservatives, alternating flavours or colours in layered food products as a useful method to build functional, sensorial and visual complexity.

This type of technology might also be useful in the pharmaceutical industry, where purified lactose, a dairy-based ingredient, is used as a bulking agent in pills and tablets. Three-dimensional printing pharmaceutical-grade lactose and medical drugs into tablets is a cost-effective production method for on-demand manufacturing, as well as individualised dosage (Sadia et al., 2016). Bilayer tablets are used as a controlled-release formulation to provide a way of successful drug delivery system into the body (Divya et al., 2011). Khaled et al. (2014) demonstrated the manufacture of relatively complex pharmaceutical formulations printed into bilayer tablets using an inexpensive desktop 3D printer. It was found that the 3D-printed formulation could match the controlled-release performance of a commercial manufactured bilayer tablet using conventional tablet compression methods. The authors proposed that there was clear potential for 3D printing to allow entirely new formulation types, for instance, new geometries and complex multilayer or multireservoir tablets and others.

Calcium phosphate is a mineral naturally present in milk and also in human bone. Xilloc (2017), a Dutch medical engineering company, developed a bone implant consisting of pure pharmaceutical-grade calcium phosphate that can be used in non-load bearing areas such as the skull. Calcium phosphate was found to be printable using bioceramic printing technology, a type of FDM where calcium phosphate is made into a wetted mixture which can then be 3D-printed. Calcium phosphate forms durable but porous structures when printed. This may be an interesting area to explore for food researchers, where edible-grade milk minerals (i.e., calcium phosphate, zine, magnesium, iron, etc.) may form hard, rigid structures using 3D printing technology.

When printing with dry, unwetted powders, however, a different form of technology called powder bed printing (PBP) is used to fuse or melt the particles together to create a solid structure. Selective hot air sintering and melting technology is a type of PBP and operates using hot air, which in turn fuses powdered food substances into a layered structure (The-CandyFab-Project, 2008). Initially, the powdered food material is spread out evenly and thinly using a levelling roller and subsequently undergoes melting when exposed to hot air. The roller then proceeds to level freshly introduced powder over the melted surface, fusing the layers together. This process is repeated to build the desired structure layer by layer in this fashion (Holland et al., 2018). This method can also use lasers in lieu of hot air to melt and fuse particles together (Fig. 7.3). This technology is called selective laser melting, also known as selective laser sintering (SLS). Diaz et al. (2014) patented this technology as a method of producing edible objects. The authors claim that in conjunction with a nonmelting powder component, a 'binder' can be used to

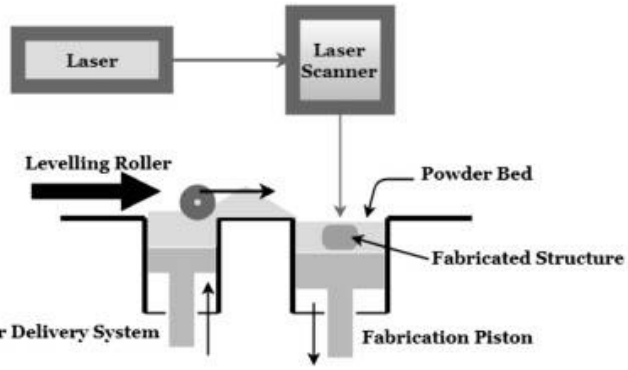

FIGURE 7.3 Principle of a laser sintering: a laser melts the fine layer of powdered material to cause it to fuse together into a solid structure. The levelling roller scrapes each layer to provide an even surface for the subsequent lasering.

provide structural support. The binder component will melt at the temperature of the laser (140–180°C) and connect the nonmelting component particles. Sources of binders that may be used in the SLS process include polysaccharides, fats and waxes, and also proteins like those found in milk (i.e., casein).

Liquid binding (LB) is another variation of PBP technology, which was originally patented by Bredt and Anderson (1999) as 3D printing. This 3D printing method, when applied to food, involves edible objects being produced by jetting a liquid food or 'binder' onto a print bed layered with edible powder. This powder can be composed of a water-soluble protein and/or a hydrocolloid to fabricate solid structures, as described by Godoi et al. (2016). The binder joins adjacent powder particles together due to dissolution–fusion or cross-linking of surface particles, thereby creating a 3D matrix. The technology can also be adapted to print flavoured edible binders which will allow the creation of interesting flavoured powdered structures, which can be rehydrated with a liquid if desired (Wegrzyn et al., 2012). There is limited evidence of this technology being applied in dairy formulations.

7.3 STRUCTURE-FORMING MECHANISMS INVOLVING MILK COMPONENTS

Milk is a complex biological fluid consisting of multiple macronutrients potentially suited to 3D printing due to their structure-forming capabilities. The solids in bovine milk are comprised of lactose, fat and protein, in order of decreasing abundance, in addition to minerals, vitamins and other minor compounds (Fox, 2008). The physicochemistry of major milk components will be described in this section to establish the principle mechanisms that could be exploited in the printing of solid dairy structures.

7.3.1 Milk Proteins

Two protein families are contained within the milk system, namely casein and whey protein. These proteins are present in bovine milk at a ratio of 80:20 casein:whey protein. Casein and whey protein have different structures and functionalities, with each family further subdividing into different proteins with unique properties (O'Mahony and Fox, 2013).

Whey products are widely used as ingredients in food formulations due to their excellent functional and nutritional properties. Native whey proteins are globular in structure and, following heating (>70°C), will denature and aggregate. In order to gel, whey proteins must be present at sufficient levels of protein (≥6%) and will gel on heating or cooling. However, many factors affect the gelation of whey proteins, such as pH, heating rate, temperature,

time and solutes (Lucey, 2008). It is generally agreed that whey protein gels can be classified as being either fine-stranded or particulate, or a mixture of both (Foegeding et al., 1998). These types of gel differ in functional properties. Fine-stranded gels are translucent and retain large quantities of water, while particulate gels are opaque and readily expel water. This influences mouthfeel in vivo where, for example, fine-stranded whey protein emulsion gels have smooth surfaces, express minimal fluid during chewing, with a low degree of cohesiveness and adhesiveness. In comparison, particulate gels have rougher surfaces, release higher levels of moisture during chewing and have a high degree of cohesiveness and adhesiveness (Gwartney et al., 2004). The type of gel formed can be controlled through certain factors and therefore manipulated to create a gel that will be 'printable', without being too thick or too liquid, in order to form a structure. For example, filament gels will form equally well at pH 2.0 and 7.0 in conditions of low ionic strength, whereas particulate gels will form at a higher ionic strength at or close to pH 7.0 (Foegeding, 2006). Changing the pH or salt concentration of the dairy formulation could thus enable the user to control the texture of the desired printed food. Fig. 7.4 shows these physiochemical differences between fine-stranded and filament gels in more detail.

Whey proteins, unlike caseins, are considered more ordered in their structure. Whey proteins form gels due to a combination of noncovalent (e.g., hydrophobic) and covalent (e.g., disulphide linkages) bonds between denatured proteins (Anema and Li, 2003). In yoghurt, for example, whey protein gelation is important, as milk must be heated to allow for denaturation of the protein, which, in turn facilitates interaction with casein and therefore incorporation of whey proteins into the casein-based gel matrix (Anema, 2008). Whey proteins can undergo extensive aggregation during heating and form strong gels on cooling (Damodaran and Parkin, 2017). This cold-set gelling mechanism is amenable to FDM, as the solution could be heated prior to

Filament Gel	Particulate Gel
Transparent	Opaque
Higher elasticity (less syneresis)	Lower elasticity (more syneresis)
Slow forming – also break down slower in mouth (good control of flavour release)	Random/unordered formation – faster forming – break down quickly in mouth
Formed at low ionic strength	Formed at high ionic strength
Can be used to fortify juices/water	Can be used to fortify milks
Formed at pH away from PI	Formed at pH close to PI
Whey proteins strongly charged	Whey proteins not strongly charged
Gels made up of strands of protein	Gel made up of protein particles
Strands are 10–20 nm thickness	Particles are micron sized (can be used as fat replacers
Brittle at low pH (rubbery at neutral pH)	Brittle
Good WHC	Poor WHC

FIGURE 7.4 Table with physiochemical property differences between fine-stranded and filament whey proteins.

printing to maintain a flowable consistency and promote denaturation and subsequently undergo cooling on the printer bed to initiate gelation. However, at temperatures of 70°C and above, an increased viscosity could lead to fouling issues on extruding through a narrow orifice, where the coagulated particles clog the printing nozzle (Chandan, 1997).

Caseins are large, self-associated assemblies of individual caseins linked together with calcium phosphate, forming casein micelles, which are porous and roughly spherical colloidal particles (Chandan, 1997). The casein micelle has a dynamic structure and is continuously exchanging components (i.e., ions) with the aqueous phase in which it is suspended to maintain chemical equilibria. Micelles also respond to changes in the surrounding environment, such as temperature, pH and pressure, accordingly. There are four subtypes of casein proteins, called α^{S1}-casein, α^{S2}-casein, β-casein and κ-casein. The κ-casein is located at the surface of the casein micelle and stabilises the structure sterically and electrostatically. This particular protein is easily accessed by chymosin, or other proteinases with comparable specificity, which cleaves a bond (Phe^{105}–Met^{106}) specific to κ-casein. This, in turn, initiates the gelation of milk by allowing coagulation of the caseins in the presence of calcium, which is the basis of cheesemaking (Huppertz, 2013).

Acid-induced gelation is also a method of coagulating casein and is commonly used in the manufacture of soft cheeses and yoghurt. Unlike rennet-induced gelation, bacterial cultures are added to liquid milk which ferment lactose into lactic acid, thereby lowering the natural pH of milk from pH 6.7 to pH 4.6, the isoelectric point of casein, where electrostatic repulsion is minimised (Lucey and Singh, 2003). Acidification may also be achieved through the addition of mineral acids (e.g., hydrochloric acid) in combination with warming, which is used to produce the ingredient acid casein.

Caseins are relatively heat stable as a consequence of their open structure and do not denature or unfold on heating (Holt et al., 2013). When combined with hydrocolloids (thickening and gelling agents derived from carbohydrates, animals and seaweeds) such as carrageenan, caseins can generate network structures which stabilise multiphase dispersions (Puvanenthiran et al., 2003). Verbeken et al. (2006) combined κ-carrageenan, milk proteins and modified starch to form gelled, sterilised dairy desserts. The study found that modified starch imparts a large influence on the complex modulus of the gel due to the exclusion effect of the swollen starch granules, which act as noninteracting fillers, which in turn concentrates the carrageenan–milk protein solution in the continuous aqueous phase, allowing a strong gel to form. Such approaches could potentially be applied to printed dairy products, depending on their desired attributes and functionality.

Oleogels are an interesting development in emulsion science, which have been proposed as an alternative replacement to trans and saturated fats (Dassanayake et al., 2011), and often rely on the structuring function of dairy proteins. Oleogels can be defined as an organic liquid (i.e., liquid fat)

entrapped within a thermoreversible 3D protein gel network (Stortz et al., 2012). According to Marangoni and Garti (2011), the major approach in producing oleogels is to incorporate specific molecules (i.e., polymers such as proteins) into the oil components to alter the physical properties of the oil. This leads to a decrease in fluidity, giving rheological properties that are similar to those of fats. Oleogels have been effectively used by Stortz et al. (2012) to replace hardstock fats (i.e., solid phase in margarine) in creams and cookies, and potentially other dairy-based foodstuffs such as cheese and ice cream. Oleogels could be a potentially printable substance to enhance nutritional profiles of food through encapsulation through the addition of plant sterols, lycopene or coenzyme Q10 to the protein−oil matrix (Zetzl and Marangoni, 2012; Zhang et al., 2015). This could be achieved using 3D printing technology where the nutrient can be added at the last step as the material leaves the nozzle and cools on the print bed.

7.3.2 Milk Fat

Raw milk is an oil-in-water emulsion, consisting of fat globules protected by a thin layer called the milk fat globule membrane (MFGM) suspended in the aqueous phase, which stabilises the emulsion through lowering of the interfacial tension surrounding the fat globule. In the manufacture of butter, the fat fraction of milk (i.e., cream: 40% milk fat) is separated from skim milk and pasteurised. The cream is then churned at cold temperatures (approximately 14°C) using an augur or similar disruptive force to disrupt the MFGM, leading to an inversion to a water-in-oil emulsion. Buttermilk is then separated from the butter grains, and the butter is subjected to a second churning stage in order to further remove excess buttermilk. At this stage, moisture levels can be adjusted and salt or cultures added if desired. Ghee is clear butter fat which originated from India, and is typically made by heating butter and evaporating the remaining moisture, resulting in a buttery-flavoured product with an extended shelf-life (Kratochvil and Vedlich, 1974).

Triglycerides (TG) are the primary fat component present in liquid milk, along with carotenoids and vitamins. TG content can be controlled though the diet of the animal, by feeding sources of unsaturated or saturated fatty acids. Milk fat has an extremely diverse fatty acid profile and therefore a varied TG composition, which contributes to a wide range of melting temperatures, spanning from −40°C to 40°C (Rønholt et al., 2014).

Butter has been used as decoration and ornamentation on hotel and restaurant tables for decades, as the unique texture of butter allows it to be easily moulded, shaped and sculpted. Schnell (2013) discussed the work of Pamela Simpson and Caroline Brooks, who were renowned for their famous butter sculptures, including a life-size bust of Teddy Roosevelt, the 26th US president.

There are many factors that can affect the structure of fat that may be relevant during printing of dairy products. This includes whether the butter has been subjected to thermal treatment, which can influence crystal polymorphism (change of crystal fat structure). In addition to this, the number and sizes of fat crystals created, as well as crystal–crystal interactions within the product, are critical determinants of texture (Rønholt et al., 2013). The process of fat crystal formation and growth are vital elements in developing the fat crystal network within a dairy fat product such as butter. Consequently, properties such as spreadability, mouthfeel, appearance and product functionality are affected by the fat crystal network. It is generally accepted that the presence of fat globules produces a softer product in comparison with milk fat-based products without any fat globules present (Mulder and Walstra, 1974). During printing, the food extruded is subjected to a certain stress when pushed through a narrow orifice or nozzle. Paragraph break Three-dimensional butter printing has never been studied to the authors' knowledge; however, the rheology of butter has been well characterised and may give some indication of how the butter will print. When a small stress is applied to butter, the crystal network may distort, resulting in reversible breakage of some crystal bonds. In contrast, when a large stress is applied, this will rearrange the network or cause fracturing, resulting in irreversible breaking of the bonds (Rønholt et al., 2014). Although studies have yet to be performed on the printability of butter and other dairy fat-based products, butter is known to be easily sculpted, holds its shape well at refrigeration and ambient temperatures, and has been well studied for its melting–solidification behaviour.

7.3.3 Lactose

Lactose, the sugar in milk, is a disaccharide of glucose and galactose joined by a glycosidic linkage. Lactose is present in whey and can be recovered in purified form through a crystallisation process for food or pharmaceutical use (Paterson, 2017). Lactose is used in a variety of different food applications, such as ice cream, to increase viscosity or improve texture without imparting an excess level of sweetness. Lactose has flavour-, aroma- and colour-absorbing properties, which can be beneficial in a product that requires the release of fragrant aromas or intense flavours over time (e.g., confectionary). In the baking industry, the role of lactose in the Maillard reaction or browning process is taken advantage of in creating a caramelised flavour and improved appearance of baked products. Lactose is primarily used in the production of humanised infant formula where its main role is to correct the balance between carbohydrate and protein of cow's milk to make it more comparable to human breastmilk. Lactose is relatively slow to digest and has bifogenic activity, meaning the infant has prolonged energy and maintains a healthy intestinal flora in the gut. Lactose can also be added to other powders in order to increase the free-flowing properties of powdered foods (Holsinger, 1997).

During an SLS process, powders must be free-flowing in order to create an even surface for the laser to penetrate a thinly layered powder. In this type of 3D printing, the powder bed process, sugars such as lactose provide the scaffolding or framework of the printed structure. Factors affecting the flowability include the compressibility and density of the powders themselves (Berretta et al., 2014; Godoi et al., 2016).

In comparison with other sugars, lactose has a relatively low level of solubility (Holsinger, 1997). This becomes important when lactose is added to a formulation and printed using FDM technology, as the solution must remain homogenous and flow readily without clogging the nozzle. However, solubility of lactose when dissolved can be controlled through mutorotation or interconversion between the two forms of lactose, α and β, each form having distinctly different solubilities. Of the two, α-lactose has a lower solubility in water at temperatures <93.5°C; therefore, when an excess quantity of α-lactose is added to water, the initial solubility will be limited. However, some α-lactose will be converted to β-lactose, resulting in an increase in solubility and enabling more α-lactose to be dissolved. This process will continue until the final equilibrium between α-lactose and β-lactose is achieved. The rate of mutorotation is affected by pH, where pH values < 2.0 and > 7.0 result in very rapid mutorotation. Temperatures above 70°C also allow mutorotation to occur in minutes rather than hours at room temperature (Huppertz and Gazi, 2016). Lille et al. (2018) studied the 3D printing properties of skimmed milk powder (SMP) which contains 53% lactose, and semiskimmed milk powder (SSMP), which was hydrolysed to create a lactose-free powder. Comparing the printing quality between these two food materials may be useful in determining how lactose may contribute to structure. Using 60% SSMP paste solution, the authors were able to print a square grid (25 mm × 25 mm) with excellent printing precision and shape retention (i.e., no warping or spreading of the layers). In contrast, 50% and 60% SMP prints sticky and dry, crumbly prints, respectively. However, it is not understood completely whether lactose is the contributing factor to a lower-quality print, and further research must be considered in this area.

7.4 STUDIES OF PRINTABLE DAIRY STRUCTURES

Despite recent progress with 3D food printing technology, only a small group of food products have been successfully printed. Dairy materials that may be applicable in 3D printing will be discussed in this section, including SSMP, SMP, sodium caseinate (NaCas), micellar casein concentrate (MCC) and processed cheese. Processed cheese will be the subject of a detailed case study later in this chapter.

SSMP is a dairy ingredient in which the fat/cream in whole milk (3.5% fat) is removed by centrifugation and then reincorporated at approximately half the level of the original milk; the reduced-fat milk is then evaporated and

spray-dried to generate a powder. SMP is a similar product except that the fat is not reincorporated after it has been removed (Lewis and Deeth, 2009). SSMP has been demonstrated to be compatible with 3D printing after reconstitution to a high solids content (60% w/v). Lille et al. (2018) conducted 3D printing experiments with protein- and fibre-rich foods. Print quality (e.g., precision, shape) was assessed visually from photographs using a scale, ranging from 1 = poor quality to 5 = good quality. The authors used lactose-free SSMP, in which the lactose, a disaccharide, was enzymatically hydrolysed into its component monosaccharides, glucose and galactose. SSMP at 60% solids scored 5 in print quality, SMP (60%) scored 1 and starch (15%) scored 3. The superior printability of SSMP was suggested to be due to its higher fat and lower carbohydrate content, allowing a more free-flowing print. Although SMP or starch were unsuitable for printing when either were used alone, Lille et al. (2018) observed that a mixture of starch (10%) and SMP (15%) was more printable (print quality value = 4).

Derossi et al. (2018) used SMP as an ingredient during a study on the 3D printing of fruit-based snacks for children. Fresh bananas, dried mushrooms, canned white beans, lemon juice, pectin and ascorbic acid were used in combination with the SMP to create a cube-shaped snack containing 5%−10% of the total daily energy requirement for a child. X-ray microtomography analysis of the snacks showed that the most structurally uniform sample was printed at a flow level of 130%, which indicates the amount of material extruded during printing, and a printing speeds of 70 mm/s compared to those at a lower flow rate (100% and 70%) and print speed (50 and 30 mm/s). A flow of 130% means a 30% increase in speed of the mechanism extruding the paste (e.g., motor driving extrusion pump) and therefore a 30% increase in millimetres of paste extruded from the nozzle. Printing speed refers to the rate at which the physical nozzle of the printer moves at. At a lower flow rate (i.e., 70% and 100%), the cube-shaped snack appeared irregular, depositing broken material line pathways and large undesirable cavities.

Many milk protein ingredients exhibit thermoreversible sol−gel transitions and could be used at lower concentrations than the previous SMP/SSMP examples by exploiting phase transitions induced by temperature changes. NaCas suspensions at high concentrations display such thermoreversible properties and have been studied in 3D printing applications (Schutyser et al., 2018). NaCas is manufactured by adding sodium hydroxide to acid casein (e.g., casein prepared from milk by acid precipitation) curds or powder to create a solution of pH ~6.6, under which conditions the caseins become more soluble and functional. NaCas displays a thermoreversible transition from a solution to a gel at or above a critical concentration (140 g/L) with added salt (1.53 M NaCl), existing as a liquid at >50°C and a solid gel at 5°C (Carr and Munro, 2004). Schutyser et al. (2018) 3D-printed hot NaCas-based emulsion systems that solidified on a cold printing stage. The authors used a nozzle with a side inlet to incorporate coloured vegetable oil droplets into the

protein matrix during printing. Using the dispenser with a side inlet created a unique structural design with a controlled spatial distribution of oil droplets. According to the authors, this technique may afford more freedom and creativity in the customisation of healthy foods, where vitamins and minerals can be encapsulated and incorporated into a protein matrix.

MCC is similar to NaCas in that it forms cold-set thermoreversible gels. MCC is manufactured using microfiltration of milk in order to concentrate the casein protein (without precipitation) and remove whey protein, lactose and soluble minerals (Schuck et al., 1994). Nutritionally, MCC is much higher in minerals (especially calcium and phosphorous) than NaCas, and, functionally, it generates more opaque gels. MCC will form a thermoreversible gel at low temperatures, and on subsequent 'melting', should not display any alteration in functionality compared to the initial liquid (U.S. Dairy Export Council, 2015). Amelia and Barbano (2003) confirmed the thermoreversible behaviour of MCC by using 18% MCC held in a suspension. A gel is formed when the solution is cooled to 4°C, but reverts back to a liquid when heated above from 4 to >22°C. Lu et al. (2015) also studied the thermoreversible properties of MCC, where MCC formed a cold-set gel at ≤38°C at 23% protein concentration.

Cheese has also been reported to be suited to 3D printing applications, with mozzarella or burrata cheese being printed on pizzas (BeeHex, 2017), for example, and processed cheese or mascarpone being printed into various shapes (Le Tohic et al., 2018). Certain cheeses may be more appropriate for 3D printing than others, which may depend on factors such as their composition (e.g., fat, protein, lactose) and functional properties (e.g., meltability, flowability). However, at the time of writing, published research on the 3D printing of different varieties of cheeses is solely limited to processed cheese (Le Tohic et al., 2018; Lipton et al., 2010).

7.5 ANALYTICAL TOOLS FOR 3D-PRINTED DAIRY APPLICATIONS

To print effectively, an understanding of the impact of heating/cooling and shearing forces on the macro- and microstructure within a food product is vital. Food formulations can be constructed using this information to add and subtract or manipulate the ingredients to suit the application. Using analytical techniques to examine the foodstuff is essential to gathering this information such as gel strength, textural properties (e.g., hardness, stickiness), colour, porosity, melting characteristics and viscosity. Analysis of 3D-printed food material may be divided into preprinting (e.g., melting, gelation and powder properties) and postprinting (e.g., texture profile analysis [TPA], colour, microscopy, X-rays) testing. Some tools used to measure these parameters, as well many other quantifiable measures, will be discussed in this section, beginning with rheology.

Understanding the rheological and mechanical properties of various dairy products is important in the design of flow processes in 3D printing (Foegeding et al., 2011). Rheometers are used to characterize the flow and deformation behaviour of a food material. They can apply oscillatory as well as rotational motion to the spindles, which can quantitatively measure parameters such as shear rate, percent deformation and shear stress. The gelation point of a dairy ingredient-based gel can be measured using small-amplitude oscillatory rheometry, for example. The solution is subjected to sinusoidal deformation or waves using a specific head geometry (e.g., cone and plate, parallel plate) to suit the viscosity of the solution. The gel point is defined as the point at which the storage modulus (G') becomes larger than the loss modulus (G''), indicating that the fluid has transitioned from fluid-like flow behaviour to solid elastic behaviour (Phillips et al., 1994).

Rheometers can also apply large step changes in stress and strain to determine viscoelastic properties as well as flow properties (Metzger, 2006). This can become useful in comparing flow properties of various dairy-based food ingredients and therefore determine optimum flow conditions suitable for printing that food type. Rheometers also tend to offer a wide range of sampling accessories, such as temperature control units to study materials under a wide range of conditions, which can simulate thermal processing or printing conditions over time (ATA Scientific Instruments, 2010). This may be applicable to printing in examining how food will flow through a nozzle, whether it will increase in viscosity over time with constant temperature and shear rate or whether the structure will be disrupted and become more liquid as more shear is applied.

In contrast, a rotational viscometer has a far more limited range of applications, where the equipment only measures viscosity (η) of various liquid/semisolid foods by rotating the spindle in one direction. The rotational or shear rate can be increased or decreased depending on the initial viscosity of the food. For example, in a chocolate printer used in a study by Mantihal et al. (2017), the chocolate was melted in a syringe whilst being mixed using a rotating auger within the reservoir. The temperature of the food can be altered or maintained through the use of a heating and cooling jacket surrounding the sample container. This allows the sample to be subject to temperature sweeps, mimicking the printing process, where the food may be heated in the syringe or reservoir, and cooled on a prechilled printer stage (ATA Scientific Instruments, 2010).

Melting properties are important attributes to measure, especially in the context of dairy-based foods, such as cheese. For example, a Discovery Hybrid Rheometer (TA Instruments, United States) was used in a processed cheese 3D printing study by Le Tohic et al. (2018) in order to assess the flow behaviour and melting properties of cheese samples in the linear viscoelastic region (LVR). Using a dynamic temperature sweep test and a stainless steel parallel plate attachment, the LVR was determined using a dynamic sweep test using

the method described by Prow and Metzger, (2005). It was found that the ratio of G′ to G″, called the loss tangent, tan (δ) represents the temperature at which the food material yields to a solid-to-liquid transition (Foegeding et al., 2011). This is useful information in terms of printing to determine the most suitable viscosity for a dairy product, both for extrusion without any issues of clogging, but also for determining the point where the product gets too fluid-like or 'runny' and is uncontrollable during the printing process (Lanaro et al., 2017).

In PBP applications, powder properties such as particle size, flowability and wettability are important to consider. Holland et al. (2018) suggests that a mixture of small and larger particles is the most suitable for use in 3D printing. Smaller particles will fill the gaps created between larger particles, therefore reducing unwanted porosity in the finished product. Particle size can be measured using a laser diffraction particle size analyser, which measures the angular variation of light intensity of a laser beam as it passes through a dispersed particulate sample. The sizes of the particles are inversely related to the measurement of the angles of light scattered (Levoguer, 2013).

Flowability also plays an important role in PBP, especially when the powder must be spread evenly, allowing the buildup of thin layers. Low flowability (i.e., restricted movement of the powder) will lead to insufficient recoating of the previous layer, therefore creating weak and porous structures (Berretta et al., 2014). Yang and Evans (2007) showed that high particle sphericity improves powder flowability. The authors also concluded that coarse powders flow easier than fine powders, and factors such as high temperature and humidity affected the flow negatively. Flowability of powders can be analysed using shear cell testers such as the Brookfield Powder Flow Tester, where a sample is sheared and compressed simultaneously using whilst measuring the torque resistance of the powder (Salehi et al., 2017).

Wettability of particles is also an important physicochemical property, especially in LB printing technology. The volume of binder dispensed onto the powder bed and the amount of binder absorbed by the particles dictates the quality of resolution and structural properties of the print. Low wettability of fine particles results in powder bed instability, and high wettability combined with low flowability may cause the particles to clump together, negatively affecting the quality of the print (Shirazi et al., 2015). The Washburn method is an analytical technique that can be used to determine wettability of a powder, where a column with powder lowered into contact with a liquid and the uptake of liquid are monitored as a function of time. Displacement or mass uptake is plotted against time to obtain the wettability (Thakker et al., 2013).

Accuracy and consistency of the print are important selling points of 3D printing. It is essential to be able to evaluate the performance and precision of the print in order to avoid any errors or discrepancies between samples. Lanaro et al. (2017) described a line test in which the calculated amount of extruded chocolate could be compared to the actual amount of extruded chocolate. Using a calculated flow equation integrating syringe plunger displacement,

nozzle diameter and syringe internal diameter values, the amount of extrudate was made to equal the inner diameter of the needle. To conduct the line test, the printer was programmed to extrude chocolate in a back-and-forth motion, where the diameter of the line could be measured. To do this, image analysis software was used, and as the food was extruded, the predicted could be compared to the actual diameter of chocolate extruded, which is a useful calibration and optimisation technique in 3D printing.

Texture analysis is the collective term for the destructive compression tests: TPA and uniaxial compression testing. TPA is an instrumental method of measuring critical sensory attributes (Steffe, 1996). TPA uses two uniaxial compressions and results in a plot from which parameters such as degree of firmness, cohesiveness, resilience, springiness and adhesiveness can be calculated (Chen and Opara, 2013). Uniaxial compression, however, works by only compressing the food sample once in one single direction. This method yields values for Young's modulus, fracture stress/strain, work of fracture and hardness (Hamann et al., 2006). Uniaxial compression can also be used to test structural stability and softness of a food substance postprinting, where a flatheaded probe attachment is used to compress the food to a certain point and then released. The force needed to compress the sample to a certain height is measured, where a larger force equals a harder, more solid food, and a lesser force equals softer, more elastic food. For example, Le Tohic et al. (2018) used this method to compare hardness between printed substances versus nonprinted processed cheese cylinders. It was found that the printed processed cheese required less force than nonprinted cheese, leading to the conclusion that printed cheese was softer than the control (see Section 7.6 for more detail).

This technology can also be used to determine optimal geometries to achieve certain sensory characteristics in printed chocolate (Mantihal et al., 2017). Snap quality is an important parameter in chocolate manufacture, with a stronger snap often being more desired, as it can suggest a higher-quality chocolate. In the above study, three different structural supports were tested for a printed hexagonal prism-shaped chocolate: a crossed biaxial support, a parallel support and no support present, as seen below in Fig 7.5. It was found that the chocolate design with cross-support required a higher force to break the chocolate structure, indicating a better snap quality than the other designs.

Another important group of methods that can be helpful in analysing 3D-printed products is microscopy. Microscopy allows the observation of the types of aggregates, location of nongelling components such as fat, structure porosity and homogeneity of the gel. Confocal scanning laser microscopy (CSLM) is a more recent development in microscopy technology, which is relatively noninvasive to the sample. The sample, in liquid, solid or powder format, is optically sectioned into X, Y and Z axes, which allows the viewer to observe each layer individually (Sozer, 2016). Foegeding (2007) used CSLM to view the effect varying the protein content of gels would have on the

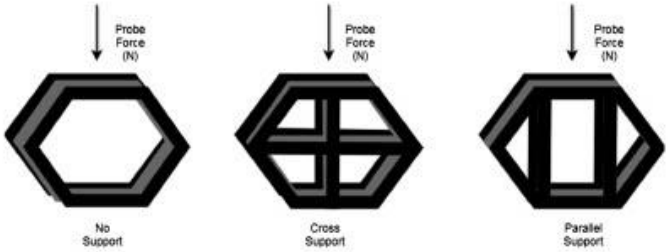

FIGURE 7.5 Three different structural designs of printed chocolates analysed for snap quality using uniaxial compression (Adapted from Mantihal *et al.*, 2017).

fracture strength. The authors found that gels containing 6% protein had a lower fracture stress, higher fracture strain and a porous structure, and protein clusters rotate during deformation. In comparison, gels containing 12% protein had higher fracture stress and lower fracture strain, and their microstructure showed considerably less change in deformation. Le Tohic et al. (2018) also used CSLM, in combination with staining to observe microstructural changes between printed processed cheese and untreated processed cheese. CSLM enables the users to stain particular items of interest (i.e., proteins, phospholipids, fat globules) to differentiate between different components within the structure (Keogh and Auty, 1998).

Porosity can influence flavour release, texture and degree of syneresis. This value can be measured using X-ray microtomography. X-ray microtomography is a powerful nondestructive technique which provides direct images of internal structures and is typically used to assess microstructural details (Babin et al., 2007). X-ray microtomography can be used to determine the air bubble/pocket size, distribution, orientation and their position within the food matrix. Defects such as altered support structures and abnormal porosities can be quantified through the use of this technology (Guillermic et al., 2018). Such defects can be caused by undesirable air pockets created within the food matrix which can cause gaps during printing travel across the print bed. Applying a vacuum to minimise air inclusion can remedy this issue (Liu et al., 2018). Kuo and Lee (2014) suggested that a more porous structure enabled a maximum rate of sodium release during initial gel compression, and flavour released at a faster rate. However, porous gels can also lead to greater degrees of syneresis which is typically undesirable in certain products such as yoghurt, but desirable in cheese (Harwalkar and Kalab, 1986). X-ray microtomography has also been used in non-3D-printed food applications by Laverse et al. (2011a,b) in both cream cheese and yoghurt, respectively. In these cases, this technology was used to determine an accurate percentage of the volume of the fat present and also the spatial distribution of those fat globules. Similar to the processed cheese study by Le Tohic et al. (2018), this

technology is useful in understanding how 3D printing might structurally alter morphology and distribution of fat globules and what implications this might have on texture.

Shape stability during postprinting processes are also important parameters to consider. The vast majority of 3D food printers are limited to single-step processes, and any cooking/baking steps require an additional separate step. Lipton et al. (2015) considers the possible implications of combining the printing and cooking process a single step on the food structure. In order to compare deformation and shape consistency, the authors measured the height, length and width of the 3D-printed cube-shaped dessert directly after printing and again after baking. It was found that an increase in butter quantity in the formulation led to a decrease in shape stability. The mixture became easily extrudable when butter quantities were increased by 150%; however, it was unable to retain its shape at room temperature.

Determining the colour change of dairy-based foods before and after printing can be meaningful. At times, this can be desirable if Maillard browning is required to achieve a caramel-like flavour; however, in many other applications, a change in colour may not be desirable. Colour measurements of food samples can be assessed using a Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) or similar colorimeter. The equipment must be calibrated using a referenced plate, and then Hunter L, a and b values can be recorded in triplicate at the surface of the food samples. L describes lightness and varies from 0 (black) to 100 (white). The magnitude of a and b both vary between −300 and 299, which describe green to magenta and blue to yellow, respectively. Relative differences for L (ΔL), a (Δa), and b (Δb) enable the user to determine overall colour difference, ΔE, using an non-3D-printed sample as a reference (Le Tohic et al., 2018).

7.6 CASE STUDY: 3D PRINTING OF PROCESSED CHEESE

In this section, a brief overview of the recently published findings of the paper, 'Effect of 3D printing on the structure and textural properties of processed cheese', will be presented and also the mixed media interest that accompanied this recent report.

Researchers at University College Cork, Ireland, first identified 3D printing potential in processed cheese in 2015 when trialling a variety of cheeses that may be suitable for the printing process. Processed cheese is manufactured using methods that in some way mimic the 3D printing technique, where ingredients are mixed at a high temperature under vacuum and then moulded into a cylindrical shape. The sample delivery system was modified from a traditional 3D printer designed to print plastic such as polylactic acid. Several design options were created, with the final construction being a 20-mL syringe where a rotating metal threaded rod pushed the syringe plunger downward and extruded the cheese sample from the nozzle at a constant flow rate.

Processed cheese was shown in preliminary tests to be suitably flowable to be distributed via a narrow nozzle, but also sufficiently viscous to set into a buildable structure during printing. The effect of speed of printing on the structure and textural properties of the cheese was studied, whereby samples were printed at flow rates of 4 or 12 mL/min, respectively. These were compared against an untreated processed cheese and melted (but not extruded) cheese sample to differentiate between the changes in properties. The melted cheese was heated at 75°C for 15 min and then cooled at room temperature for 30 min, followed by storage in refrigeration conditions. The samples for printing were treated in a similar fashion; however, they were heated for 12 min to achieve comparable melting characteristics as the melted cheese sample, as these samples would be subjected to further disruption when sheared by way of extrusion through the printer nozzle.

After printing, analysis was carried out on the cylindrical cheese samples to determine how the texture and structure was altered during printing. The cheese was allowed rest for 30 min postprinting and then refrigerated for 24 h before analytical evaluation. The textural analysis consisted of two compression−decompression cycles, separated by a time interval of 5 s at a rate of 1 mm/s, using a cylindrical aluminium head with a diameter of 35 mm. The probe compressed the sample to 25% (5 mm) of its initial height (20 mm) before decompression. The figures below indicate that the printed cheeses (C) and (D), required less force to compress the sample, meaning the cheese is softer. The untreated cheese (A) required almost twice as much force, implying that the sample was harder than printed cheese (Fig. 7.6).

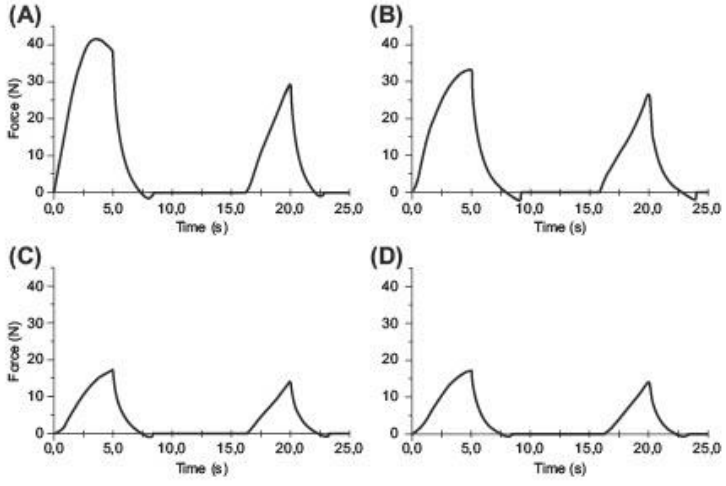

FIGURE 7.6 Texture profile analysis (TPA) profiles of (A) untreated cheese (UC), (B) melted cheese (MC), (C) low-speed printed cheese (LSPC), and (D) high-speed printed cheese (HSPC): two compression tests with 5-s intervals (Le Tohic et al., 2018).

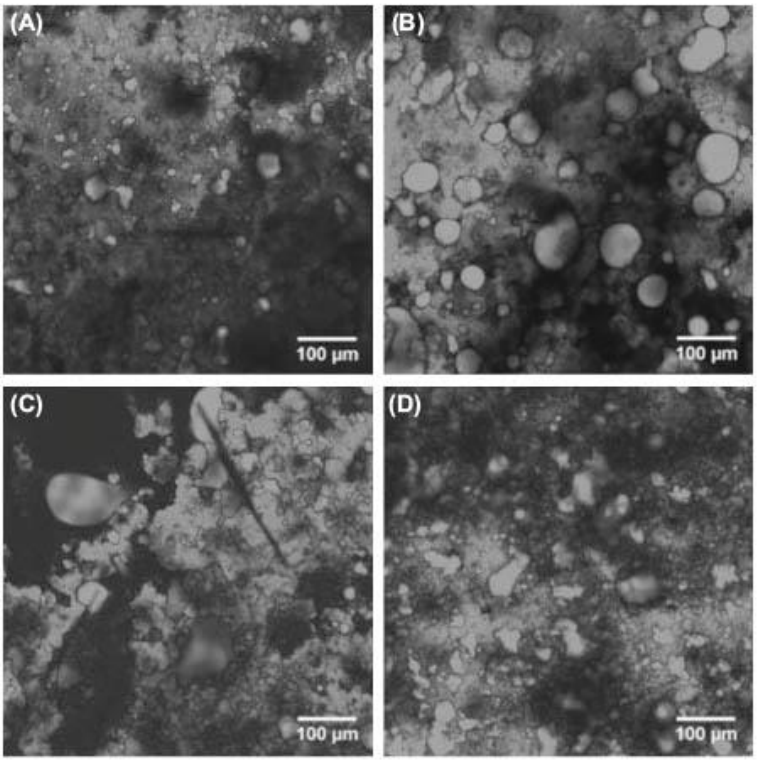

FIGURE 7.7 Confocal laser scanning micrographs of cheese samples. (A) Untreated cheese (UC), (B) melted cheese (MC), (C) low-speed printed cheese (LSPC), and (D) high-speed printed cheese (HSPC) (Le Tohic et al., 2018).

There was not a comparable difference between the two extruded cheeses in texture and softness; however, there was a more significant difference when the structures of the cheese samples were observed under a confocal laser scanning microscope. Here, a red stain shows fat structure, and a green stain illustrates the protein network within the cheese sample's structure. In Fig. 7.7, it is possible to make out the fat globules as the lighter white/grey areas and the darker, deeper grey/black areas as the protein network.

It can be seen that the untreated cheese (A) shows a well-characterised and uniform distribution of round fat droplets within the continuous protein phase. The melted cheese displays similar structural elements; however, the fat droplets are visibly larger than those of the untreated cheese, presumably due to the fat coalescing on melting. In contrast, the more slowly printed cheese (C) presents a discontinuous protein phase and random fat regions varying in

size and shape. The micrograph image of the faster printed cheese (D) suggests similar sized fat globules than those of the slower printed cheese, yet the lipid droplets appeared to be smaller and slightly more uniform (C). The higher shear rate being utilised during faster printing should contribute to greater fat globule disruption, resulting in this particular network formation. Consequently, the disruption of fat globules confirms the rheological and textural data shown in Fig. 7.7, where the interruption of the protein and fat phase produced a softer, more easily meltable cheese.

Another comparable difference found was that the printed cheese was notably darker in colour with a lightness value, L, of 80.8 for high-speed printed cheese than the untreated cheese sample with an L value of 83.3. This is also due to the modification to the fat globule size and morphology and nonuniform protein network formation, where the higher-speed printed cheese was slightly darker as a consequence of the presence of larger fat globules than the slower printed cheese (Le Tohic et al., 2018).

The media coverage on this topic began in early March 2017, when articles began to be published online on websites such as Dairy Reporter, 3D print.-com, Live Science and IFL Science, with headlines 'Study on 3D Cheese Printing Offers Possibilities' (Cornall, 2017) and 'Let's Cut to the Cheese: Researchers Publish Study on How 3D Printing Affects the Structure and Texture of Processed Cheese' (Saunders, 2017), to name a few. Within 10 days, there were over a dozen articles written, and video footage of the 3D-printed cheese went viral on social media platforms such as Facebook and Twitter. The video itself has been viewed 4800 times (at the time of writing in October 2017) on the CNN Facebook page alone, as well as many multiple thousands of views on other scientific- and technology-related pages. There were positive reactions from the online community to the concept in addition to sceptical comments relating to feasibility in everyday use, relevance of the technology to current global challenges and disappointment that processed cheese was the source material rather than cheese itself. Few commentators reflected on the possibilities for the technology to be adapted to other food systems beyond processed cheese. The mixed commentary echoed the report by Lupton and Turner (2016) in their survey of responses to the concept of 3D food printing by an online discussion group (titled 'Both Fascinating and Disturbing'). In general, most articles embraced the concept and its novelty while indicating that research for 3D-printed edible food is at an early stage of development.

Since the publication of the research paper and attention from the media, Professor Alan Kelly, as lead author of the research paper, has been invited to speak at multiple 3D printing-related events and conferences in 2017. It is now understood that more research will continue into how other dairy structures can be used for dairy printing applications and perhaps produce snack and meal concepts with a range of compositions and applications.

7.7 RESEARCH NEEDS, CHALLENGES AND OPPORTUNITIES

In spite of the most recent developments, the majority of technologies for 3D food printing are still undergoing research and in the growth phase. This is due to the challenges that researchers face in terms of creating a technology that will incorporate traditional methods of cooking and baking whilst modifying these foods to suit the 3D printing process and still remain appetising. Consumer acceptance of new technologies is another factor that creates obstacles for the emergence of 3D printing. Other considerations in terms of research needs, challenges and opportunities include how 3D printing will affect food supply chains, whether the food is considered safe and whether 3D printing will help or hinder our current relationship with food.

7.7.1 Safety and Acceptance

Food safety is one of the most important aspects of food manufacture. Due to 3D printing being a novel food technology, it will undoubtedly be met with a reasonable amount of scepticism by the general public. The name '3D printing' is another aspect that generates confusion amongst the general public. People may associate printing with ink and high level of processing if care is not taken to introduce the technology with due transparency and clarity regarding its operating principle (Brunner et al., 2018).

Consumers may view novel food technologies with suspicion (Popa and Popa, 2012); however, not all new food technologies and food trigger the same 'neophobia'. Both the technology itself and the individual's own experience and knowledge background play a determining role in the evaluation process. Repeated exposure to different types of novel food and visual representations of them can also contribute to making new processing techniques and new food more familiar to consumers, which may positively affect consumer attitudes (Brunner et al., 2018). The importance of 'naturalness' is also a key implication of how 3D printers will be accepted by consumers. Consumers may have conflicting interests in that they may view the potential convenience and creativity associated with 3D printing positively, but may also link the technology with undesirable, overly processed and unhealthy foods (Román et al., 2017). Nutrition-focused 3D food printers are being developed (Natural Machines, 2016), although much coverage has been given to food printers focused on convenience food products (BeeHex, 2017) and luxury food items (Choc Edge, 2017).

7.7.2 Research Challenges

In order for 3D printing technology to be feasible in industry and for everyday use, the individual food formulations must have suitable rheological characteristics for printing, such as a flowable consistency that will maintain a

constant viscosity during printing. At the time of writing, there has been very little research carried out on the fundamental principles of how the printing process affects dairy food microstructures. Food matrix interactions at a microscopic level are potentially important in order to understand physical and rheological behaviour and textural and sensorial traits of foods (Aguilera, 2005). Further research into the effects of 3D printing on both properties and texture is needed to allow greater diversification in more application areas. The lack of understanding of how structural change occurs during printing, which can influence printing accuracy and shape stability, is one of the biggest challenges in 3D food printing to date. For example, a study by Lipton et al. (2010) showed that when a dessert product with reduced fat was baked after printing, this led to increased shape stability after baking, whereas an increase in egg yolk concentration improved width/length stability but lowered height stability following baking. It was also reported by Lille et al. (2018) that a high yield stress was necessary to achieve acceptable shape stability postprinting, whilst a high solids content (samples with 60% SSMP) was shown to be most effective in retaining shape and structure in postprocessing by oven drying. This also shows the added challenge of compatibility of 3D printing technology with traditional food processing methods such as baking (Lille et al., 2018). The ideal situation is to incorporate cooking (e.g., baking, steaming) while the product is being printed to eliminate two-step print-then-cook processes. To the authors' knowledge, there are no such printers available on the market at present.

Furthermore, there are additional technological challenges to tackle, as illustrated by the printing of batters, where the food material is strongly dependent on oscillations during its pumping process to maintain viscosity, which will consequently have an impact on the final quality of the printing (Millen, 2012). Three-dimensional printing dairy-based products could benefit from the addition of novel additives, such as bacterial nanocellulose (BNC), a product of fermented waste coconut water, to improve shape stability and structural integrity of the food matrix. BNC could potentially be suited to 3D printing due to its own natural 3D nanofibrillar arrangement of pure cellulosic fibres, which will enable a stronger and more supported printed food structure (Gama et al., 2016). Other ingredient combinations have been used to create a multitude of texture varieties in order to recreate the texture that can be lost through the printing process. Cohen et al. (2009) printed various combinations of hydrocolloids to develop a certain 'mouth-feel matrix', measuring the strength and texture spectrum of a food material. This study showed that pure xanthan gum and gelatin added separately were more affected by the scale of strength of gel, rather than smoothness, with 0.5% gelatin resulting in a milk-like texture and 4% gelatin producing a mushroom-like texture. When two hydrocolloids were combined, the degree of textural smoothness was affected, whereby 1% gelatin and 4% xanthan gum brought about a risotto-like texture, and 1% gelatin and 8% xanthan gum created a tomato-like texture.

7.7.3 Industrial Advantages and Disadvantages

Other benefits predicted for 3D printing of food materials include flexibility, configurability and high-material-use efficiency (Hopkinson et al., 2006). The flexibility of 3D printing technology will enable the use of alternative dairy-based food ingredients in creating improved products with respect to nutritional content and health benefits. Broadly speaking, enterprises have been interested in the environmental benefits of 3D printing (SPI Laser LTD, 2017). This might apply more notably to the dairy industry, as it falls under increasing scrutiny and increased concern regarding its sustainability (Von Keyserlingk et al., 2013). Three-dimensional printing would potentially allow goods to be printed and sold close to their point of consumption. The novel technology presents an opportunity to 'reshape' the manufacturing supply chain to produce cheaper products through a reduction in the number of stages in the traditional supply chain (Berman, 2012). Additive manufacturing technology offers the opportunity to redesign products and with fewer components. The combined effect is the reduction in the need for storage, transportation and packaging (Gebler et al., 2014).

Several researchers have investigated the use of 3D printing in the spare parts supply chain, where printers would have the ability to print their own replacement parts and therefore reduce manufacturing downtime. According to Siddiqui et al. (2016), 3D printing is a viable option in printing parts for food processing operations, for example, feed spacers for membrane filtration technology, which is commonly used in to separate specific particles (e.g., fat, lactose, proteins, bacteria, salts) from milk depending on the pore sized used (Mercier-Bouchard et al., 2017). Feed channel spacers are placed on top of the filter membrane to facilitate feed flow and retentate recovery while promoting turbulence. Three-dimensional printing technology is considered a suitable tool in rapid, low-cost production of spacers with a thin and complex geometry design that may improve cleanability (Siddiqui et al., 2016). However, 3D food printing also has practical limitations. In the case of FDM printing, food materials must be converted to a paste, of which not all foods are suitable candidates. This can be due to potentially undesirable changes in texture or taste or negative interactions with other food contained within the paste (e.g., syneresis, browning, off flavours). Pastes might also be limited by their shelf-lives due to their high moisture content leading to a possible microbial risk. According to Lipton et al. (2015), regulatory agencies, mechanical engineers, chemists and other experts will have to collaborate to ensure that the right materials, processes and standards are in place.

Speed of printing is a common concern amongst industrial companies considering implementing the technology in their manufacturing lines. Printing speed has improved in recent years but has yet to reach speeds capable of efficient production standards. To put this into context, the Choc Creator chocolate 3D printer is capable of printing speeds of 20 mm/s (Choc Edge, 2017).

Choc Edge (2017) states that to create a design which is up to 3.75 cm in height and 4 cm in width/length will take between 20 and 45 min, depending on intricacy of the print.

One other final concept that is moving 3D printing a step further in terms of creativity and innovation is the term '4D printing'. This term is relatively new and much less developed than 3D printing. Wang et al. (2017) have introduced this concept through their experiments with pasta, where the initially flat-shaped pasta will twist and curl up in a unique pattern when hydrated and/or heated. The authors took 3D-printed edible films made of common food materials such as protein, cellulose or starch in order to achieve this '4D' effect. How such an effect could be achieved using dairy-based 3D printing is an interesting question.

Overall, 3D printing is an exciting technology at present, presenting researchers with many opportunities and challenges. Three-dimensional printing has captured the imaginations and attention of researchers, businesses and citizens. Successful exploitation of the diverse functional and nutritional properties of dairy ingredients could be an important contributor to progress in the field of 3D food printing.

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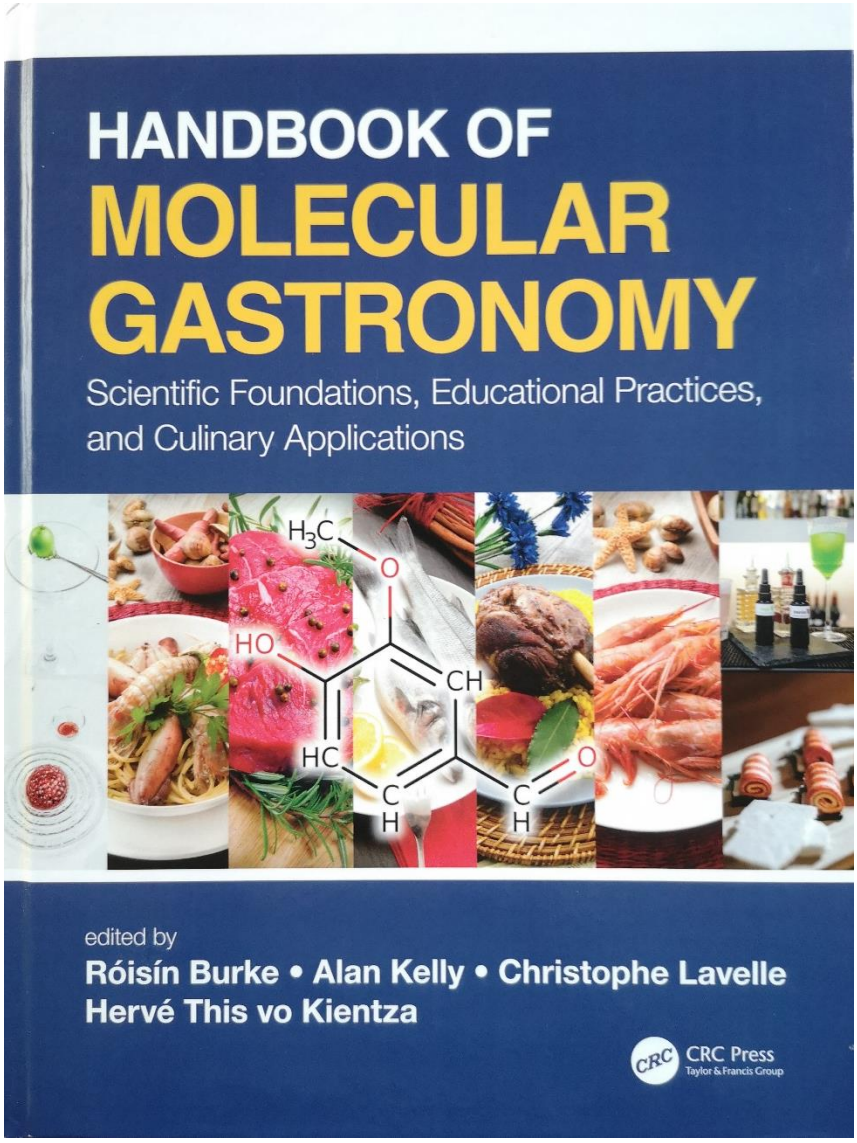
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3D Printing of Food

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Introduction and History

Three-dimensional (3D) printing is a process that creates 3D objects from a virtual model, usually designed through computer aided design (CAD) software. The principle of a 3D printer is analogous to that of a regular office printer, to which has been added a third dimension, giving the ability to print three-dimensional objects from a range of materials.

Charles W. Hull of 3D Systems Corporation created the first working 3D printer in 1984 (Hull, 1986). Since then, advancements in printing technology have widened the possibilities in terms of 3D-printed objects. A decade ago, 3D printers were an expensive hobby, costing about the same as a family car, but in more recent times, a basic printer is roughly the same cost as a low-end laptop (Miller, 2016).

3D printing has rapidly become a disruptive technology in many industrial areas around the globe, as seen in Figure 91.1 (Morris, 2014). Automotive, building construction, electronic manufacture, biomedical and aerospace are just a number of sectors where 3D printing has been implemented in order to increase automation and also decrease waste (Ngo *et al.*, 2018).

However, in more recent times, 3D printing has progressively appeared in the realm of food manufacture. Confectionary, chocolate and even pizza can now be printed from a machine. 3D food printing is beginning to emerge into society, and the idea of having a 3D food printer at home as a method of food preparation doesn't seem to be so unrealistic. Since 1988, various rapid prototyping techniques have emerged, which can be classified as liquid-based, solid-based and powder-based (Chua *et al.*, 2010).

Principle of 3D Printing of Food

3D food printing is the action of mechanically layering food material in such a way that a 3D shape is created. The process involves computer technology, which converts the blueprint of the shape and structure required into coordinates where the printer head must travel and deposit food material. A complex and unique 3D shape is created layer by layer, applying phase transitions (i.e., liquid to solid) or chemical or enzymatic reactions to fuse layers together. 3D food printing technologies have the potential to meet the growing demand for customised food. They have the potential to construct novel food forms and customise food textures, colours and flavours (Wegrzyn *et al.*, 2012).

The broad objective of 3D food printing is to create universal printers that will allow consumers to freely shape their food. This would offer the possibility to browse and download recipes from a mobile phone or any device and then order their 3D printer to deliver a proper meal. 3D food printing offers the possibility of customising food to suit one's dietary needs or health condition, but it also has the ability to change the shape and enhance the visual aspect of food. Ingredients like seaweed or insects can be incorporated in recipes and made to look appealing (Molitch-Hou, 2014).

Possible End-Use Scenarios

3D food printing has been seen to be used in a variety of applications, ranging from home-cooking with families to professional chefs at Michelin star restaurants (Table 91.1). This section explores how 3D printing can benefit each of these key food-related areas and why 3D printing is set to become the technology of the future.

Food Applications

Currently, 3D food printing has been established in food areas as diverse as the military, meals for the elderly, confectionery and food designed for space missions. The list of food materials that can be utilised in such applications using the 3D food printing process is growing at an incredible rate and includes ingredients such as chocolate, sugar, fruit and vegetables. In this section, relevant applications for everyday food ingredients that can be 3D printed using commercially available printers will be discussed in detail.

Chocolate

Chocolate extrusion has been by far the most common material to be used in creating 3D food structures. The Choc Creator V1 and V2 (Plus) became the first commercially available 3D chocolate printer in 2012 (Choc Edge, 2018). The Choc Creator is

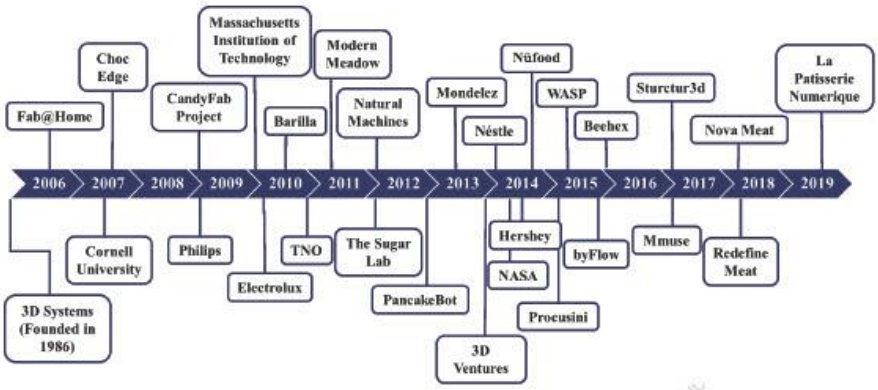

FIGURE 91.1 Timeline of companies involved in the 3D food printing sphere.

(Adapted from Van der Linden, 2015)

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TABLE 91.1

Examples of 3D Food Printing Applications in Different Use Scenarios

Scenario	Examples of applications
Domestic kitchen	• Enables creative, consistent designs to be created in bakery and/or decoration applications • Allows mass production for parties and large family events • Reduces food wastage by combining commonly discarded, but nutritious, parts of fruits and vegetables (e.g., stalks, skins, etc.) into purée to be used as sauce, topping, plate decoration, etc. • Allows control over portion size • Can encourage children to develop an interest in their own nutrition if allowed to create their own design • Can facilitate personal, precise and consistent nutrition
Restaurant kitchen	• Increases chef efficiency by delegating time-consuming and repetitive tasks to the printer • Produces consistent meals in terms of shape, portion size and precision placement • Creates shapes and structures that may be difficult or impossible to do by hand • Incorporates elements of 3D-printed foods into traditional dishes for a modern twist • Produces personalised cake toppers for special events • Creates unique and personalised culinary experiences (e.g., printing customers' name or custom designs on the plate or dessert)
Vending machines	• Potentially offers healthier snacking option • Offers convenience; order in advance online or through phone application and collect when completed • Allows customised nutrition, flavours and texture to suit each individual user • Can be placed in high-flow areas such as airports, train stations, shopping centres, cinemas, universities/colleges, work places, fitness centres, etc., for healthy, personalised convenience options
Nursing homes and hospitals	• Shearing effect of printing creates softer food material, which can be ingested more easily for individuals with swallowing difficulties (i.e., dysphagia) • Creates nutritious and attractive meals compared with traditional bland high-calorie pastes typically found in nursing homes to maintain weight • Nursing home residents can look forward to the nutritious and tasty meals that they used to enjoy pre-admittance. Encourages healthy appetite, which helps in maintaining muscle mass and weight • 3D-printed meals in hospitals might benefit patients who, due to surgery or treatment, might have a small appetite, be consuming strong medicines, and/or require high-calorie and nutrient-dense foods
Food processors	• Novel shapes can be created and changed as desired, with little or no changeover in equipment required • Only one production line is required for multiple products; changes to product design or size can be made immediately on the same production line, meaning that fewer lines are required, thereby saving factory floor space and capital cost on individual machines for individual products • Enables rapid in-house prototyping with minimal cost • Promotes production of mass customised products (i.e., personalised items have added value)

a three-axis Cartesian computer numerical control printer with a chocolate extruder head (Godoi *et al.*, 2016). The innovation relies on an extrusion system capable of handling chocolate, which requires accurate control of viscosity and temperature conditions. The printer is composed of three components: the first is a heat-controlled chamber, and the second is a pump that pumps the melted chocolate into the third component, which is the extrusion head and nozzle (Hao *et al.*, 2010).

Recently, on 7 July 2019, Cadbury launched the world's first Cadbury Dairy Milk 3D printer in Melbourne, Australia, for World Chocolate Day. According to Kopp (2019), chocolate-lovers were offered the chance to be among the first to take away their very own personal selection of 3D-printed chocolate pieces built from layer upon layer of delicious Cadbury Dairy Milk chocolate.

Pasta

Extrusion-based processes have been employed by TNO (Netherlands Organisation for Applied Scientific Research) researchers to print a large variety of foods using sugars, proteins, meat purees and other nutrients extracted from alternative sources, such as algae and insects (Van der Linden, 2015).

In 2012, TNO researcher Kjeld van Bommel announced that, in partnership with Barilla (an Italian pasta company), they had developed a 3D pasta printer that can print customised pasta shapes and colour. Most recently, TNO and Barilla created 3D-printed pasta in the shape of roses, using classical pasta recipes (ingredients: durum wheat semolina and water, without additives) (Van der Linden, 2015).

Massachusetts Institute of Technology (MIT) has brought 3D food printing another step further in terms of innovation by introducing the concept of '4D printing'. Wang *et al.* (2017) introduced this term through their experiments with pasta, where the initially flat-shaped pasta will twist and curl up in a unique pattern when hydrated and/or heated. The 4D effect is caused by the addition of carefully placed edible films made of common food materials such as protein, cellulose or starch, which contract when water and/or heat is incorporated.

Confectionery

In 2014, Print2Taste GmbH was established as a spin-off company from the University of Weihenstephan-Triesdorf in Freising (Germany). Procusini 3.0 was developed as an extrusion-based 3D printer that would be suitable for professionals in catering, event gastronomy and hotels, as well as bakery and confectionery. The company claims that the Procusini halves production time and can create 84 small structures in just 93 minutes, allowing professionals to complete other tasks whilst printing is in operation. The Procusini includes a temperature-controlled cartridge holder to maintain the temperature of food materials contained within. The printer can also operate through a Wi-Fi connection from an electronic device such as a tablet or phone. Procusini is a mobile printer with dimensions of 60 × 60 × 65 cm and weighs only 9 kg.

Food materials that have been printed using the Procusini include pasta (four colours available), chocolate, marzipan (Figure 91.2), fondant (five colours), goat's cheese, sausage meat, mashed potato and cassis (i.e., blackcurrant puree) (Procusini, 2018). In 2019, Procusini launced the Mycusini, which is a small, compact chocolate printer starting at €198; it will fit in any kitchen at 19 × 19.5 × 27 cm, which is described as being 'smaller than most coffee machines'.

In 2014, the American-based manufacturer 3D Systems developed Chefjet and Chefjet Pro, two NSF (National Sanitation Foundation) and UL (Underwriters Laboratory) certified kitchen-ready 3D food printers. The printer operates using liquid binding (LB) technology, which requires water and powder-based ingredients. A roller-component lays down a thin layer of powder (i.e., sugar) on the printing surface. A print head then sprays water or liquid binder in a pattern that draws a shape on the powder surface. The powder recrystallizes when in contact with the water or binder, which encourages a solidifying and binding process through the joining of adjacent particles. The piston supporting the print bed is progressively lowered at the completion of each layer until the object is completely formed. The process is repeated several times until the 3D structure is built (Figure 91.3) by cross-linking of the particle surfaces (Godoi *et al.*, 2016).

FIGURE 91.2 Procusini Printer printing chocolate pieces and an example for a marzipan-printed car cake topping.

(Procusini, 2018)

FIGURE 91.3 ChefJet and sugar sculpture made by ChefJet Pro.
(Brill 3D Culinary Studio – Powered by 3D Systems, 2020)

FIGURE 91.4 Example of 3D-printed sugar sculpture topping a custom-made cocktail.
(The Sugar Lab by 3D Systems, 2020)

The basic ChefJet printer can only print monochrome food decoration, whilst the ChefJet Pro prints in full colour and in a multitude of flavours, including sour cherry, chocolate, vanilla, mint and watermelon (Ngo, 2015). The ChefJet printer can print edible sculptures and confections of all shapes and sizes which were previously impossible to create by hand. An example of these complex and creative designs can be found in the collaboration between The Sugar Lab by 3D Systems and Modernist Cuisine, whereby, using the Chefjet Pro, a colourful and intricate swirling sugar sculpture (Figure 91.4) was 3D printed to balance across the rim of a cocktail glass for the 50th birthday celebration of renowned Spanish chef Ferran Adrià. The specially designed perforated shape of the sculpture allowed the absinthe to flow through the Gaudi-style structure while absorbing the sweetness of the sugar, creating a colourful, theatrical and sweet cocktail experience.

Meals

Natural Machines, a Barcelona-based start-up company, launched the Foodini food printer prototype in 2013. Foodini is a syringe-based deposition printer with a tactile user interface. The printer (10 kg) is a 43.8 × 43 × 43 cm cube with a touchscreen display powered by Android OS with Wi-Fi connection. The printer has a capsule receiver at the top where users can insert a cartridge with fresh pureed ingredients (Natural Machines, 2018).

The process proposed by Foodini is quite simple; users have to cook and prepare liquid-based food by mixing different kinds of ingredients. Then, they load the liquid-based mixture into capsules (five maximum). Each capsule can hold 123 mL of liquid, and the five combined can hold 615 mL. Users insert capsules in the Foodini and select printing programs through the touchscreen. The printer will then start extruding food one capsule at a time onto the print bed (Sevenson, 2014). Not all foods can be printed; however, the Foodini allows the user to print intricate designs that may not be possible to achieve by hand. This is accomplished through the use of a smaller nozzle diameter (0.5 mm) and computer-controlled print head. Different nozzles are also available to accommodate textural differences between food types (Chadwick, 2017).

The machine has successfully printed different kinds of food products, such as crackers, individually shaped cereal, gnocchi, ravioli, bread sticks, butter, pizza, cereal bars and biscuits (Figure 91.5). The machine is equipped with a heating element

FIGURE 91.5 Foodini printer and example of 3D-printed biscuit spoons.
(Natural Machines, 2018)

FIGURE 91.6 Examples of Focus printing capabilities.
(byFlow, 2020; Food Ink, 2016)

with a maximum temperature of 100 °C. The heating function can only keep food materials melted (like chocolate) or keep food warm. In some cases, where meals require cooking, users will have to cook the printed food in a separate process before eating (Hoopes, 2013).

The Foodini contains built-in 3D-scanner hardware capable of scanning an object, digitally saving the shape and then printing it. It can also scan an object such as a plate or a cupcake and print on top of this (Sevenson, 2014). The Wi-Fi connection allows users to share their recipes with the Foodini community. In addition, the founders of Foodini also propose to produce pre-prepared capsules and make them available in supermarkets. These capsules will contain all the ingredients required for a recipe pre-programmed by the printer (Natural Machines, 2018).

Netherlands-based company byFlow has specialised in 3D printing since 2009. The Portable 'Focus' 3D food printer, created by byFlow, is currently sold as a business-to-business (B2B) product, optimised for desserts, including chocolates and meringues (Figure 91.6). byFlow claims that chefs and patissiers use 'Focus' on a daily basis 'to experiment with textures and shapes, save time and money, create new designs, and amaze their customers' (Chadwick, 2017).

byFlow's clients in the restaurants space so far include La Boscana in Barcelona. This restaurant uses both Focus and Natural Machines' Foodini. The printer is not only based in the kitchen but placed in front of the customer's table so that they can see their dishes coming to life. Focus printers also appeared at Food Ink's 3D printing project in 2016 (Figure 91.7), a pop-up restaurant in London. Currently, the Focus printer has been used in printing applications such as marzipan, ganache, hummus, fondant, mango caviar, avocado, meringue, pureed tomato and mozzarella, and even pureed chicken and beef.

FIGURE 91.7 byFlow's Focus Printer.
(byFlow, 2020; Food Ink, 2016)

Specialist Nutritional Foods

One of the unique applications of 3D-printed food is producing foods with a soft texture for elderly customers who have difficulty swallowing (Deloitte, 2015). One in 25 adults is affected by chewing and swallowing difficulties, otherwise called dysphagia (Bhattacharyya, 2014).

The German company Biozoon launched the '3D Smoothfood Project' in 2014, which aims to design and create individualised nutritious meals. The meals will have a jelly-like texture that resembles solid food when printed but dissolves easily when ingested and swallowed by the consumer (Deloitte, 2015).

Biozoon originally specialised in a range of texturisers that change the consistency of food. Their SeneoPro powder is combined with pureed ingredients to form a smooth paste or gel. The mixture is then inserted into a cartridge in the printer and printed to resemble the solid shape of that ingredient (Figure 91.8).

Mathias Kück, owner of Biozoon, claims that the look and taste of the end product match the original food item: 'When eaten by a patient, the food can be destroyed without using the teeth and flow like a gel through the throat' (Chadwick, 2017).

Fruit

Dovetailed is a design studio and innovation lab founded in 2011 in Cambridge, UK. In 2014, Dovetailed created their first 3D food printer, 'nūfood', which is controlled using an app on the user's phone, allowing the user to design their own unique flavoured shape, which can be added to beverages, breakfasts and salads to provide added bursts of flavour. According to Vaiva Kalnikaitė, creative director and founder, the printing process is based on the spherification technique, a well-known molecular-cooking method (Figure 91.9).

This method involves combining liquids such as fruit purée or juices with sodium alginate. The combination is then dropped in a controlled fashion by the 3D printer into a bowl of cold calcium-based food-grade liquid, where it forms tiny caviar-like spheres. Fruits and other shapes are formed by layering the tiny spheres of juice until the desired shape is built. For example, nūfood explored unique flavour pairings such as balsamic and raspberries, which they combined in a 3D-printed cube and added to a whiskey sour cocktail.

Culinary Applications

Bakeries

The adoption of 3D printing is evolving rapidly in the baking scene (Duchêne *et al.*, 2016; Deloitte, 2018). For example, a large international company, CSM Bakery Solutions, which produces a broad range of products for customers in more than 100 countries (CSM Bakery Solutions, 2018), has entered into partnership with The Sugar Lab by 3D Systems to enable collaborative research and development, engineering, design and printer development, which will be focused on specific sourcing, food product development and go-to-market plans (Unrein, 2017). Initially, CSM Bakery Solutions will target how 3D printing may assist chefs creating high culinary art in venues such as casinos and cruise liners (CSM Bakery Solutions, 2018).

The artisanal field of confectionery that produces decorative bakery products (Figure 91.10), chocolates and other types of sweets is one of the food industry fields that can benefit from the dimensions that 3D printing can offer. However, 3D-printed chocolates and other decorations should not be confused with moulding, which has already been developed in the confectionery sector (Duchêne *et al.*, 2016).

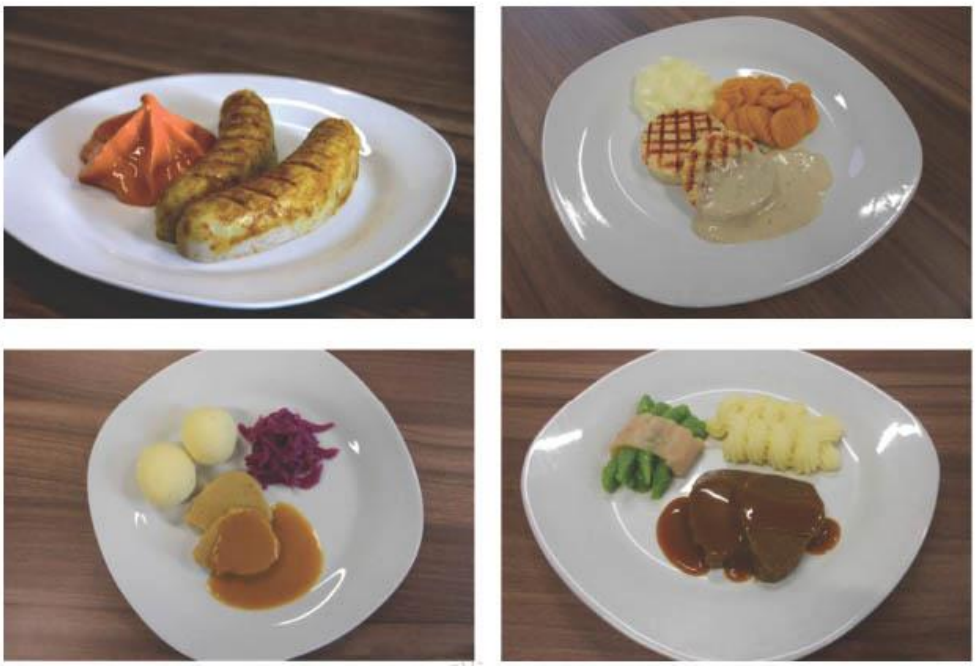

FIGURE 91.8 Examples of entire meals printed by Biozoon.
(Biozoon, 2018)

FIGURE 91.9 Dovetailed's 3D printer nūfood – printing a strawberry.
(nūfood, 2014)

Restaurants and Chefs Serving 3D-Printed Foods

Many smaller R&D service-focused companies are developing innovative concepts, such as 3D-printed dinner at pop-up restaurants. Additionally, pop-up fine dining concepts have been tested in Europe and the US (Duchêne *et al.*, 2016). In 2016, diners at the pop-up restaurant Food Ink were served a nine-course meal in which all the food was fabricated using 3D printing technology (Lupton, 2017). The dishes were made from pizza dough, hummus, mushy peas, chocolate mousse and goat's cheese (Figure 91.11). Two chefs of elBulli and La Boscana, Joel Castanye and Mateu Blanch, were behind the menu. The utensils and even the chairs were 3D printed (Hartman, 2016).

FIGURE 91.10 A ChefJet Pro 3-D printer from 3D Systems uses powdered sugar and hydrated food colouring to create products like cake decorations.

(The Sugar Lab by 3D Systems, 2020)

Figure 91.11 shows, on the left, 'Caesar's Flower of Life' (seasoned bread, assorted flowers and vegetables) and, on the right, '3D Boscana' (nocilla, chocolate cream, hazelnut polvoron, milk ice cream, Dutch chocolate leaves and 3D-printed centre spiral.

3D Printing and Fine Dining

One of the emerging regional areas for 3D printing of food is Barcelona. Here, restaurants such as Dos Cielos and La Boscana have been actively involved in experimenting (Duchêne *et al.*, 2016; Ahmed, 2017). At La Enoteca in the Hotel Arts in Barcelona, chef Paco Perez, who has won several Michelin stars for his restaurants, has created a new dish entitled 'Sea Coral', which is a 3D-printed 'coral' shape made of seafood purée (Figure 91.12). A report for the BBC website showed how Perez and his co-workers constructed this dish in one of his restaurants (Koenig, 2016).

Also in Catalonia is Reimagine Food, a research and design group who develop digital gastronomy ecosystems and robots in cooking (Duchêne *et al.*, 2016) and use disruptive technology, such as drones, artificial intelligence, robotics, wearable devices and big data, adapting them to the needs of consumers and the food industry (Reimagine Food, 2014).

In 2018, chef Jan Smink opened the first full-time 3D-printed food restaurant in the town of Wolvega in the Netherlands. Smink previously worked for De Librije, a triple Michelin star-winning restaurant. Now, after creating new recipes (Figure 91.13) and aiding in R&D, he is ready to provide a unique dining experience that takes advantage of 3D printing technology. Chef Jan Smink has stated: 'By using the Focus 3D Printer I'm able to make forms and shapes that would otherwise not be possible. I can surprise

FIGURE 91.11 3D-printed meals served at the pop-up restaurant Food Ink in London in 2016.

(Mendoza, 2016)

FIGURE 91.12 A 3D-printed coral.
(Koenig, 2016; Natural Machines, 2018)

FIGURE 91.13 3D-printed hazelnut, celery and cream cheese by Jan Smink.
(byFlow, 2020)

my guests with a unique experience that is very tasty as well. 3D Printing is the future!' (Smink, 2018).

In the United States, Melisse is a Michelin 2-star restaurant in Santa Monica, run by chef Josiah Citrin. The restaurant partnered with 3D systems and has, for example, developed a dish where the 3D printer crafted a fresh crouton using an aromatic onion powder, as seen in Figure 91.14 (Ahmed, 2017).

Collaborative Projects and Applications

The 3DS Culinary Lab in Los Angeles brings together 3D food printing technology developers with chefs and food industry representatives (Lupton, 2017). One such example is the dish created by Mei Lin, the winner of Bravo's Top Chef Season 12, which was based entirely on the flavours of Hawaii and inspired by the beauty of a passion fruit flower (Duchêne *et al.*, 2016; Lupton, 2017; The Sugar Lab by 3D Systems, 2020). The dish contained cylinders of passion fruit curd, caramelised banana crème anglaise chilled to a gelato consistency by liquid nitrogen, freeze-dried strawberry powder, bee pollen crumble, toasted yogurt, and sliced fresh bananas and strawberries (Figure 91.15a). A delicate, perforated 3D-printed interpretation of the passion fruit flower, flavoured with actual passion fruit, crowned each plating. Mei invited guests to shatter the delicate sugar passion

FIGURE 91.14 3D Systems partnered with Josiah Citrin of Melisse to create a 3D-printed crouton.
(The Sugar Lab by 3D Systems, 2020)

fruit flower with their spoons (Figure 91.15b) to incorporate the flavour and texture of the piece into each bite (The Sugar Lab by 3D Systems, 2020).

TNO has been involved in an interesting gastronomy project with food designer Marijn Roovers and chef Wouter van Laarhoven, who together created a chocolate shell just 0.8 millimetres thick using the Focus Printer. The chocolate's continent of origin is embossed in gold, whilst inside, it holds delicacies that symbolise that region (Figure 91.16). Roovers mentions that the chocolate globes take an hour to print and claims that they have the texture of aerated chocolate bars as a result of printing the globe in 200 layers of chocolate. The North America globe contains segments of crème of sweetened corn and bourbon whiskey. Meanwhile, the South America globe is filled with chocolate with allspice and popped corn. The African globe consists of portions of ras el hanout with cumin and yoghurt (TNO, 2015).

In Silicon Valley, a team of over 30 people, including 3D artists, designers, food scientists, chefs and engineers, created a way for people to immerse themselves in a new reality in which they enjoy different meals (The Project Nourished Initiative). All participants need is a VR headset (for stimulating vision), an aromatic diffuser (for smell), 3D-printed cubes (for texture), a bone conduction transducer (for chewing), a gyroscopic utensil (fork for the virtual and physical food), and a virtual cocktail glass (for intoxication). The 3D-printed cube is made of algae, which adds taste and texture. The texture of the cube, the aromatic diffuser and the sounds produced by the bone conduction transducer trick the consumer's mind into believing they are eating actual sushi (The Sensorama, 2017).

In 2014, Dutch industrial design student Chloé Rutzerveld presented her Edible Growth project in collaboration with Eindhoven University of Technology and TNO. For this project, she 3D printed an edible ecosystem made up of a carbohydrate matrix containing miniature plants and mushrooms. At the start, Chloé created the design for the carbohydrate structural matrix on a 3D modelling program, printed it out and then printed a combination of seeds, spores and yeast embedded in agar-agar into it. Agar-agar is a gelatinous paste, which functioned as a soil for the seeds and yeast to grow and feed on. After about five days, the plants had grown sufficiently, and the product was ready to be eaten (Figure 91.17). However, waiting a little longer gives the plants some more time to grow, and the taste of the food intensifies (Rutzerveld, 2014).

Applications of Molecular Gastronomy

In recent years, applications of molecular gastronomy such as 'molecular cooking' and 'note-by-note cooking' have emerged (This, 2008, 2013, 2014). Molecular cooking is defined as producing food in kitchens using 'new' tools, ingredients and methods (ttz-Bremerhaven, 2018); examples of this could include use of equipment such as siphons, ingredients such as sodium alginate, and methods such as sous-vide cooking. In the case of 'note by note cooking', meat, fish, vegetables or fruits are not used to make dishes, but instead compounds, either pure or in mixtures, are assembled by the chef to design the shapes, colours, tastes, odours, temperatures, trigeminal stimulation, textures, nutritional aspects and more of the desired dish (This, 2013).

In many instances, the ingredients used in these molecular gastronomy applications, e.g., hydrocolloids, including gelling agents such as agar-agar and gelatine, are suitable for 3D printing. This will allow freedom of design in terms of not only composition, structure and texture but also taste (Duchêne *et al.*, 2016). In particular, it is possible to design and print customised note-by-note foods. An example of a 3D-printed note-by-note prototype recipe can be found in Part III of this book. It is suitable for vegans and/or those who are lactose-intolerant.

Specialised Stores and Personalised Products

Specialised shops that offer 3D-printed products include the Amsterdam-based MELT Ice pops, which has offered custom-made ice pops since 2012 (Meltpops, 2018) and uses 3D-printed moulds that are printed using domestic 3D printer manufacturer Ultimaker's printers (Duchêne *et al.*, 2016). The Magic

FIGURE 91.15 The Flavours of Hawaii.
(The Sugar Lab by 3D Systems, 2020)

Candy Factory also specialises in personalised products and is the world's first 3D gummy candy printer, allowing anyone to create shapes, write messages and draw their own custom candies (Magic Candy Factory, 2017).

Limitations

Major challenges still to be solved relate to the need for multi-material printing systems and integration with traditional cooking processes, like baking or boiling. One of the major hindrances is the low printing speed (Duchêne *et al.*, 2016)

The cost of home food-printing machines has also been noted in some media reports as a potential barrier to consumer interest, as was the problem that these devices were still in development, so that people who might be interested in purchasing them would have to wait until they came onto the market (Lupton, 2017).

The expensive nature of the gourmet printed food featured in restaurants has also received attention, as in the following headline in an online newspaper article about the Food Ink pop-up restaurant: 'Is this the future of fine dining? Restaurant where all the food and even the table is 3D printed – but it will cost you £250 a head' (Best, 2016).

Some media reports also quoted experts in the food industry as voicing some reservations about whether 3D-printed food would be widely accepted by consumers. This was usually in response to the more speculative uses of the technology, as in the virtual reality meals served through the Project Nourished initiative or the use of cultured meat (Lupton, 2017).

Future Speculations

The global 3D printing food market is expected to expand during the period 2017–2024 and to reach USD 400 million by 2024. The market growth is driven by factors such as the growing demand for customised food (Researchnester, 2018). Richard Watson, futurist, writer and founder, predicted in *The Telegraph*'s feature on 'back to the future' that 'By 2045 many kitchens will feature a 3D Printer … a fun kitchen gadget to sit alongside the Soda stream and waffle maker' (Titcomb and Murgia, 2015).

FIGURE 91.16 TNO Michelin star chocolate dessert. (TNO, 2015)

FIGURE 91.17 Chloé Rutzerveld's award-winning Edible Growth Project. (Rutzerveld, 2014)

Companies like Natural Machines see the future of 3D printing food as a faster and more precise operation, possibly even including more textures. A US startup called Modern Meadow is working on a technique to 3D print meat without having to slaughter an animal at all. The process includes using stem cells to create what they call 'bio-ink', which is then inserted into a nozzle similar to that of a 2D inkjet printer. The 'live' bio-ink is then extruded into an agarose gel mould. After that, it matures in a 'bioreactor', resulting in organ tissue. The agarose gel is then removed, leaving the end product (Houser, 2017).

Production capacity can be increased through the use of a well-managed print farm or print cell of multiple 3D printers, allowing simultaneous production in multiple materials (Formlabs, 2017).

Conclusions

In recent times, the users of 3D-printed foods have become many and varied, ranging from domestic to professional kitchens to retailers and large food manufacturers. The use of this technology has opened up an array of applications allowing personalised food design and nutritional meal formulation. In the last few years, more and more companies have been developing 3D food printers that have specific design features, e.g., for printing doughs or even complete meals. As a result, it has become possible to diversify ingredients beyond those used by the pioneering confectioners. A number of collaborative 3D food printing projects are currently underway, involving multi-disciplinary teams (e.g., chefs, scientists, engineers and artists), who aim to

further improve our eating experiences. While there are some limitations to 3D printing technology, the pace of developments, together with the economic forecast for 3D food printing, signals a positive future ahead.

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Parameters affecting the printability of 3D-printed processed cheese

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ABSTRACT

In considering three-dimensional (3D) printing of food materials, fundamental understanding of the "printability" characteristics of different food materials is of vital importance to successfully meet user needs. In this study, a processed cheese formulation was 3D-printed with a modified 3D printer. Both intrinsic factors (i.e., pH and intact casein content) and measureable attributes (i.e., texture, printing accuracy, rheology and microstructure) were analysed. Using rheological methods, the optimal viscosity range (7.55–10.94 Pa.s) at which processed cheese will print successfully (i.e., simultaneously flow from the extrusion head uninhibited and build a layered structure) was identified. Processed cheese with a higher pH (5.8) was found to give a printed product that was significantly ($P < 0.05$) softer, gummier and more resilient in texture than the same recipe with a lower pH (5.4 and 5.6). Printed cheese containing exclusively fresh curd was significantly ($P < 0.05$) harder than that containing mild or mature Cheddar cheese. 3D printing at higher temperatures (60 °C) led to harder and more resilient cheese than printing at lower temperatures (40 °C). Overall, formulation and printing parameters for processed cheese significantly affect properties relating to "printability".

1. Introduction

Three-dimensional (3D) food printing has increasingly become a topic of discussion in the food sector in recent years as a disruptive and novel food technology (Dankar, Haddarah, Omar, Sepulcre, & Pujolà, 2018). 3D printing using traditional materials, such as metal, plastic and other artificial polymers, has been established for many years in other industrial sectors (i.e., automotive, medical and aeronautical) as a valid form of manufacturing. Up until recently, the food sector was a relatively unexplored area for 3D printing in comparison with the rate of progression in other fields (Lille, Nurmela, Nordlund, Metsä-Kortelainen, & Sozer, 2018). However, the number of studies investigating 3D-printed foods has increased at a steady rate, which has demonstrated the potential 3D printing has to offer to the food industry (Lipton, Cutler, Nigl, Cohen, & Lipson, 2015; Liu et al., 2019; Liu, Zhang, Bhandari, & Wang, 2017).

One common form of 3D food printing, Fused Deposition Modelling (FDM), involves the controlled layering of a flowable food material, like a paste or gel, until the desired 3D structure is built (Wegrzyn, Golding, & Archer, 2012). There are other types of 3D food printing technologies available involving the binding of powders (such as spray dried cocoa and/or milk powders) and sugar particles; these technologies have been described in more detail elsewhere (Le-Bail, Maniglia, & Le-Bail, 2020; Liu et al., 2017).

There are several potential advantages for the use of 3D food printers versus traditional food manufacturing methods, such as the ability to customise a consumer's meals according to their dietary needs and preferences (Pérez, Nykvist, Brøgger, Larsen, & Falkeborg, 2019). For example, individuals with dysphagia could potentially benefit from the development of 3D food printers, where the technology has been used to create creative and personalised specially textured foods to suit individual nutritional requirements without discomfort. However, it must be noted that, to the authors' knowledge, very few have carried out physical clinical trials with dysphagia patients (Dick, Bhandari, Dong, & Prakash, 2020; Dick, Bhandari, & Prakash, 2021; Kouzani et al., 2017). 3D printers may also be used to create different geometries and textures and which may be difficult or impossible to create by hand or by conventional manufacturing operations (Sun, Zhou, Huang, Fuh, & Hong, 2015).

Similar to 3D printing of traditional materials (i.e., plastic, metals

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etc.), there are many challenges associated with printing of food, one of which is obtaining the ideal consistency of printing material. According to Lille et al. (2018), the viscosity of a suitable food material for 3D printing should be low enough to permit extrusion through a nozzle, yet high enough to give a cohesive and stable structure when layered on a print bed. An increasing amount of published work has attempted to understand the link between shearing forces during 3D printing and textural changes to food stuffs (Dick, Bhandari, & Prakash, 2019; Lee, Won, Kim, & Parka, 2019; Pérez et al., 2019; Zhu, Stieger, Strieger Van Der Goot, & Schutyser, 2019). 3D printing parameters are also important elements to consider in determining the printability of certain foodstuffs. Derossi, Caporizzi, Azzollini, and Severini (2017) found that the dimensions and microstructural properties of a 3D printed fruit-based snack were affected by the flow rate of the material, which is an adjustable setting in the slicing software (i.e., computer software used in most printing processes which plays an active role in converting a 3D model into g-code or instructional code for the printer) of the printer, dictating the volume of material extruded over a period of time. It was observed that low flow rates resulted in an unstable and disrupted internal structure, whereas increased flow produced thicker structures with merged layers and decreased porosity.

Formulation of each 3D printed food material is also a vital consideration when 3D printing stable structures with high degrees of accuracy. The addition of a structure-building ingredient is important when considering the stability during and post-printing. Proteins which develop strong gel networks can be ideal additions to printing formulations, as seen in many studies involving 3D printing dairy ingredient-based structures. For example, Liu et al. (2019) reported that Milk Protein Concentrate (MPC) powder had a strengthening property when combined with sodium caseinate dispersions. This was achieved by increasing protein-protein interactions through inter- and intra- molecule disulphide bonds formed by sulfhydryl-disulphide interchange or sulfhydryl oxidation reactions; the ideal total protein content of the mixture was reported to be between 400 and 450 g/L. However, there appears to be a limit to the quantity of ingredients with a structural-aiding capacity that can be added to a formulation before negatively impacting the extrudability and printing quality of the food material. In the same study, formulations with a total protein content of more than 500 g/L showed compromised printing quality due to the lack of ductile strength of the printing material during printing.

Similar results have also been found in other dairy-based formulations, where Skim Milk Powder (SMP) (composed of 53% carbohydrate (primarily lactose), 35% protein and 0.6% fat) with a solids content of 50% produced viscous, but printable pastes. However, increasing the solids content to 60% resulted in extremely viscous and unprintable pastes, despite an increase in nozzle diameter from 0.41 mm to 1.19 mm (Lille et al., 2018). In the same study, lactose-free Semi-Skimmed Milk Powder (SSMP) (containing 38% carbohydrates, 37% protein and 15% fat) with a solids content of 60% was also found to exhibit excellent 3D printing qualities. It was suggested that the higher fat content in the lactose-free SSMP was the primary reason for improved printing characteristics, where the lipids in the formulation act as a lubricant, thereby improving flow during printing.

The objective of this study was to build on the work of (Le Tohic et al., 2018), who showed that commercial processed cheese can be 3D-printed using a modified 3D printer. In that study, commercial processed cheese was printed at high and low printing speeds, and compared to melted and untreated (i.e., non-heated/extruded) processed cheese for texture, colour and microstructural changes. In the current study, a formulated processed cheese recipe was used in place of commercial processed cheese, and both intrinsic factors (i.e., pH and intact casein content) and measurable attributes (i.e., texture, printing accuracy, rheology and microstructure) were analysed. The role of emulsifying salt (ES) (in this case Tri-Sodium Citrate (TSC), the most commonly used ES in processed cheese manufacture) concentration and moisture was also briefly studied in the preliminary trials of this work in order to optimise a workable food material to 3D print successfully (i.e., build a layered structure). According to Shimp (1985), ESs, such as TSC, as calcium-chelating agents, disperse and solubilise casein, thereby enabling the emulsification of fat in order to create a homogenous process cheese mixture. TSC is commonly used in sliced processed cheese applications where the body of cheese must ideally be robust to mechanical cutting and maintain its shape without crumbling or deforming (Purna, Pollard, & Metzger, 2006), a suitable property required for structure-building during 3D food printing.

2. Materials and methods

2.1. Materials

Two batches of Cheddar cheese used in the processed cheese recipes were obtained from Carbery Group Ltd. One batch was aged for 1–2 months and the other batch for 6–8 months, to give two different levels of intact structural protein, casein, as this decreases during ripening due to proteolysis. Alkali (3.12 M caustic solution – 15% sodium hydroxide and 15% potassium hydroxide mixture) used to adjust pH was obtained from Water Technology Ltd. (Ireland). A 3.12 M Hydrochloric Acid (HCl) solution (Sigma-Aldrich, USA) was used to adjust the fresh curd processed cheese recipe to pH 5.6. Trisodium Citrate (TSC), two fluorescent dyes, Nile Red and Fast Green FCF, and Polyethylene Glycol were purchased from Sigma-Aldrich (Arklow, Co.Wicklow, Ireland).

2.2. Methods

2.2.1. Printer modifications

A Tronxy X8 (Shenzhen Tronxy Technology Co., Ltd., China) 3D printer was used in this project, altered to print paste-like foods rather than plastic filament. This was achieved through modifications to the original extrusion system, by replacing the hot-end assembly with a custom-made stainless steel syringe mount and syringe barrel (Fig. 1). The original print head operated by feeding plastic filament (Fig. 1B) into the hot end (Fig. 1C) through the rotation of the stepper motor (Fig. 1A). However, in order to print processed cheese, the entire print head had to be remodelled to accommodate a syringe-based mechanism. An aluminium syringe mount (Fig. 1J) was manufactured to hold the syringe (Fig. 1I), and the original print head stepper motor (Fig. 1A) was reattached to the rear of the syringe mount, as shown in the right hand figure. In order to drive the helical screw (Fig. 1H) connected to the plunger housing (Fig. 1G), the stepper motor rotates a flexible drive shaft (Fig. 1F) which is connected to the helical screw driving the plunger mechanism, thereby pushing the plunger housing downwards (Fig. 1G) (or upwards) depending on the direction of travel (i.e., (anti)/clockwise). The syringe barrel was also fitted with heater and thermistor wires to allow the insulated syringe to be heated in a controlled manner. The heater wires were made from nichrome wire (0.5 mm diameter) and wrapped 34 turns around the stainless steel syringe barrel (22 mm external diameter). The coil resistance was found to be 14.4 Ω (Ohms) when 14.5 V were applied from the power supply (1 Amp current limit). A preliminary trial was carried out prior to use to test the temperature limits of the heating wire. The temperature of the wire was recorded every 10 s for 600 s, once the current was applied to the wire. It was observed that at 600 s the wire reached 90.6 °C (Measured using Type-K thermocouple). The thermistor provided feedback to the printer's controller to intermittently switch on/off the power applied to the nichrome heater wire by the printer's power supply to maintain the desired temperature of the syringe barrel, and therefore, by extension the contents within the syringe.

The printing temperature, flow rate (i.e., volume of fluid which passes through the nozzle per unit of time), printing speed (i.e., amount of printed material extruded over a given time, typically measured in mm/s), and layer height were controlled through the printing software (Repetier-Host, Hot-World GmbH & Co. KG).

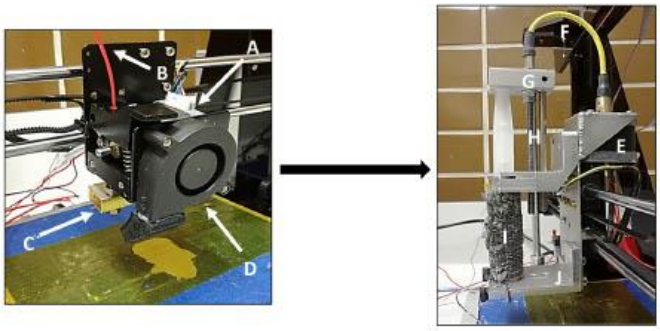

Fig. 1. A - Stepper Motor, B - Polylactic Acid (PLA) Filament, C - Hotend, D - Hotend Fan, E - Stepper Motor, F - Flexible Drive Shaft, G - Plunger Housing, H – Helical Screw, I- Syringe Barrel with Insulation, J - Syringe mount.

2.2.2. Compositional analysis

Three production runs of processed cheese were tested in order to eliminate the impact of batch-to-batch variation on the printing process. Moisture, fat, protein, and ash content of all Cheddar samples were analysed in triplicate. Carbohydrate content was calculated by difference. Compositional analysis of the eight recipes is shown in Table 1. Each recipe is hereafter designated by a code (A – H; Table 1).

2.2.3. Electrophoretic analysis

Polyacrylamide gel electrophoresis was performed on the raw Cheddar samples (i.e., fresh curd, 1–2 month and 6–8 month old Cheddar cheese) to determine the peak volumes of individual bands, thereby quantifying the amount of structural protein (i.e., caseins) within each sample (Fig. 2). Electrophoresis in polyacrylamide gels was performed using a Protean II xi vertical slab-gel unit (Bio-Rad Laboratories Ltd., Watford, Herts., UK) according to the method of Andrews (1983) with modifications. Gels were stained directly by method of Blakesley and Boezi (1977) and destained in deionized water.

2.2.4. Initial processed cheese formulation development

Some preliminary research into various processed cheese recipes from the literature was completed before selecting the final core ingredients. An adapted recipe was generated based on two sources (Berger, Klostermeyer, Merkenich, & Uhlmann, 1998; Kapoor & Metzger, 2005) and simplified into three core ingredients (Cheddar cheese, trisodium citrate (TSC) and water). With these basic ingredients, eight variations of processed cheese recipes were chosen. Three recipes varied in pH (i.e., pH 5.4 (A) (natural pH), 5.6 (B) and 5.8 (C)), where a 15% sodium hydroxide and 15% potassium hydroxide alkali solution was added to give the desired pH. The remaining five recipes differed in structural protein content, where the ratio of young and mature Cheddar cheese was varied to establish a range of high and low structural protein contents (i.e., 1–2-month old Cheddar: 6–8-month old Cheddar −25:75 (D), 50:50 (E), 75:25 (F), 100:0 (G) and the control – fresh curd (H)). These five recipes were all adjusted to pH 5.6 using alkali/acid solutions as required. A comparison sample using fresh cheese curd was used a control to compare the effects of intact protein content on the internal structure, printing accuracy and texture. The formulation for each recipe (per 100 g) are shown in Table 2.

Moisture levels and TSC content of the original processed cheese recipe (i.e., Recipe F) were chosen based on preliminary experiments, with samples that varied in moisture (55%, 50% and 47% total moisture content) and TSC (1.5, 2 and 2.5% TSC) content were printed into grids and cylinders (see Section 2.2.4 for expanded detail of each process). Texture Profile Analysis (TPA) was conducted on the printed cylinders according to the method outlined in Section 2.2.9. Textural attributes (i.e., adhesiveness, cohesiveness, springiness, hardness etc.) were compared between each sample.

2.2.5. Rheological profiles of processed cheese formulations

Rheological profiles for each recipe were generated using the viscosity data measured during each run (Fig. 3). This data reflected the typical viscosity profiles for each individual recipe and were used as a guide to predict how the sample would print (Section 3.3).

For initial small-scale processed cheese production, an AR-G2 rheometer (TA Instruments, New Castle, USA) with a Starch Pasting Cell (SPC) attachment was used for its heating and shearing function, making it ideal in mimicking the traditional manufacture of processed cheese. The raw ingredients (grated cheese, TSC, water and alkali/acid solution (if required)) were added to the cylindrical stainless steel barrel of the rheometer (capacity 30 g) at room temperature, and the mixture was heated to 80 °C (5 °C min^{-1}) from room temperature (25 °C) and held at 80 °C for a total of five minutes. The mixture was then cooled to 65 °C (5 °C min^{-1}) and held for 3 min to allow the mixture to thicken and become less liquid-like for printing. The mixture was sheared at a constant rate of 7.5 s^{-1} during the entire process, and the approximate

Table 1
Composition of eight experimental formulations, including a control recipe using fresh curd. (NB: Ratios used for the maturity part of the study, i.e., 75:25 refers to 75% 1–2 month old cheddar and 25% 6–8 month old cheddar). Carbohydrates were calculated by difference. Each sample were analysed in triplicate for $n = 3$ batches.

Code	Recipe	Fat	Protein	Ash	Moisture	Carbohydrate	pH
A	pH 5.4	17.69 ± 0.21	22.2 ± 0.1	4.36 ± 0.26	47.25 ± 1.27	8.45	5.4
B	pH 5.6	18.14 ± 0.07	22.4 ± 0.4	4.29 ± 0.23	47.13 ± 0.11	8.09	5.6
C	pH 5.8	17.78 ± 0.13	21.7 ± 0.4	4.25 ± 0.15	47.80 ± 0.62	8.43	5.8
D	Maturity 25:75	17.62 ± 0.30	22.6 ± 0.3	4.48 ± 0.03	45.60 ± 0.45	9.72	5.6
E	Maturity 50:50	17.68 ± 0.08	22.3 ± 0.5	4.37 ± 0.03	46.54 ± 0.91	9.07	5.6
F	Maturity 75:25	17.98 ± 0.86	22.0 ± 0.1	4.09 ± 0.05	46.34 ± 0.7	9.55	5.6
G	Maturity 100:0	18.28 ± 0.09	21.3 ± 0.4	4.51 ± 0.15	46.83 ± 0.62	9.06	5.6
H	Maturity 100:0 (Fresh curd)	18.40 ± 0.35	20.3 ± 0.64	4.81 ± 0.18	48.08 ± 0.42	8.41	5.6

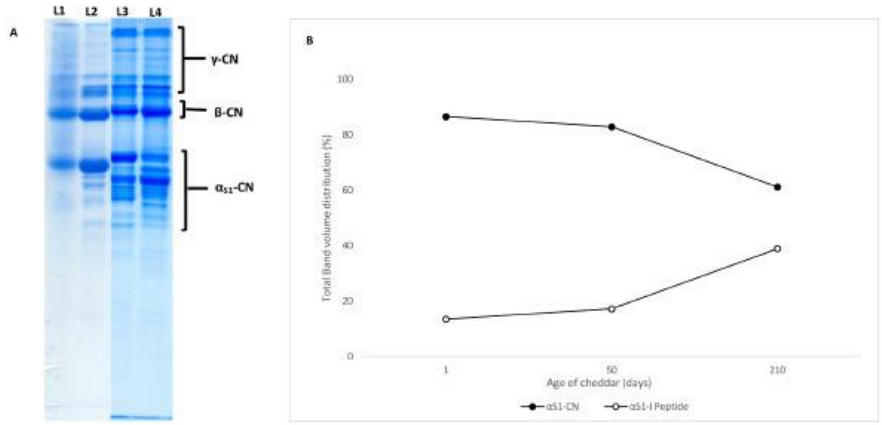

Fig. 2. a. Urea-PAGE electrophoretogram of cheddar cheese. L1 – sodium caseinate standard, L2 – fresh cheddar curd, L3–1-2 month old cheddar and L4–6-8 month cheddar (note bands from different gels are presented). Fig. 2b. Breakdown of α_{S1}-CN and production of α_{S1}-CN-I in cheddar cheese during ripening.

Table 2
Ingredient formulation of each processed cheese recipe (per 100 g).

	Recipe Code							
Ingredients	A	B	C	D	E	F	G	H
1–2 month old cheddar/*fresh curd (g)	58.13	58.13	58.13	58.13	38.75	19.38	77.5	77.5*
6–8 month old cheddar (g)	19.38	19.38	19.38	19.38	38.75	58.13	0	0
TSC (g)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Water (g)	20.0	19.82	19.52	19.82	19.9	19.89	19.8	19.76
3.12 HCl/Caustic Sol[a] (g)	0	0.17	0.47	0.17	0.1	0.1	0.2	0.24

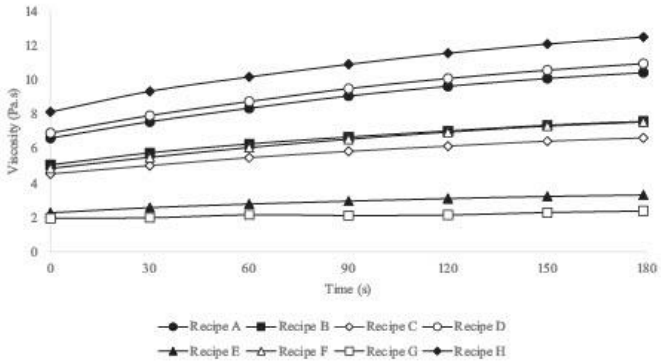

Fig. 3. Rheological profile of each processed cheese recipe. Table shows viscosity (Pa.s) of each processed cheese sample at the latter end of the processed cheese making cycle, where the mixture was held at 65 °C for 3 min.

time taken to complete this cycle was 22 min.

2.2.6. Preparation of processed cheese for printing

Two methods were used for preparation of samples of processed cheese, one on a small scale for characterisation of formulation rheology, and one on a larger scale for preparation of batches for printing. For the former purpose, the starch-pasting cell (SPC) geometry on the Rheometer proved to be comparable when producing small processed cheese batches. Then, to print multiple samples in quick succession, a larger heating and shearing unit was required to produce a large volume of processed cheese mixture (i.e., 200 g versus 30 g batches); a Thermomix (Vorwerk UK LTD, UK) food processor was thus used to mimic the heating and shearing effect of the traditional processed cheese production cooker and the SPC on the rheometer.

Eight processed cheese recipes intended for 3D printing were produced in 200 g batches using the Thermomix. The processed cheese ingredients were mixed at rotational speed 1 (1.67 s^{-1}) at room temperature until 80 °C was reached, and then held for five minutes to ensure the cheese was melted and the mixture fully homogenous. The mixture was then cooled to 65 °C (at 1.67 s^{-1}), held at this temperature for 3 min followed by an immediate transfer to the syringe barrel (approximately 75% filled) for printing. Processed cheese recipes intended to be used as controls (i.e., poured/non-printed) to evaluate the specific effect of printing were immediately poured into cylindrical silicone moulds, stored in an airtight container, and allowed to set at 4 °C overnight before analysis the following day.

2.2.7. 3D Printing Process of Processed Cheese (Cylinder and Grid Structure)

The shapes intended for printing were created using Tinkercad (Autodesk, California, United States), a free online Computer Aided Design (CAD) software used to model 3D designs. Twenty millimetre cylinders with an aspect ratio of 1 (Fig. 4A) were printed with each recipe in order to assess differences in texture and microscopic structure. Forty-millimetre squared grid structures (Fig. 4B) were printed to determine printing accuracy in conjunction with ImageJ analysis.

Immediately after the cheese mixture was cooled to 65 °C in the Thermomix, the syringe was filled to approximately 75% of the barrel volume, and then mounted onto the printer. To print the 20-mm cylinder, a 2 mm nozzle was used with 50%-grid infill at a print speed of 20 mm/s. When printing the 40-mm square grid structure, the print speed was set to 20 mm/s with no infill and the flow rate set to 50%. A single skirt line was added 5 mm away from the grid structure to avoid printing inconsistencies and broken lines during the initial print. All processed cheese recipes were printed between 40 and 45 °C, where the mixture was allowed to cool for a few seconds during filling of the syringe. During these trials, the heated barrel was set to the temperature of the cheese mixture as it was filled into the barrel.

Trials were also repeated using Recipe D at higher temperatures (65 °C) to determine the effect of printing temperature on the texture and microstructure of the sample. These printed samples were compared to the poured controls into silicone moulds. All printed structures were allowed to cool to room temperature and subsequently stored in an airtight container (to avoid moisture loss) under refrigerated conditions (4 °C) overnight before analysis the following day.

2.2.8. Texture profile analysis of processed cheese

TPA was conducted on the processed cheese cylinders immediately after their removal from refrigeration conditions (4 °C), using a texture analyzer TA-XT2i (Stable Microsystems Ltd., UK), as described by O'Mahony, McSweeney, and Lucey (2008). The TPA measurement consisted of two compression-decompression cycles, separated by a time interval of 5 s, at a rate of 1 mm/s, using a cylindrical aluminium geometry (diameter of 35 mm). The probe compressed the sample to 25% (5 mm) of its initial height (20 mm) before decompression. The TPA indicators recorded were hardness, adhesiveness, springiness, cohesiveness and resilience, and at least triplicate measurements were performed for each formulation (i.e., three printed cylinders and three poured cylinders per formulation).

2.2.9. Determination of printing accuracy using image analysis

ImageJ is a Java-based image processing programme that can be used for image quantification and descriptive analysis across a broad range of sample types originating from various disciplines (Harris et al., 2018; Scheuer, Ferreira, Mattioni, de Miranda, & de Francisco, 2015). Using ImageJ, the image of the grid was enhanced in order to accurately measure the perimeters of the gaps (i.e., non-printed areas) and the printed grid itself. This was done by selecting the 'Process > Find Edges' function; this function enables the use of a Sobel edge detector to highlight sharp changes intensity in the active image (Fig. 4C) (Ferreira & Rasband, 2012).

The 40-mm^2 grid structures were then measured using the 'Analyse > Measure' function of the software in order to determine the area of cheese printed. In order to measure the area accurately in the software, the width of the printed grid was measured prior to analysis and the distance value entered in the 'Analyse > Set Scale' function as the 'known distance'. Using the 'Polygon' shape measuring selection, a line was drawn around the perimeter of the grid structure and the area calculated using the 'Analyse > Measure' function. The areas of each 'non-printed' areas (i.e., the nine gaps shown in dark striped grey in Fig. 4A) were then measured using the same function and the sum of the area of the nine 'gaps' was calculated. In order to calculate the area of printed cheese (i.e., the solid light grey area shown in Fig. 4A), the total area of the nine 'gaps' was subtracted from the total area of the squared structure (including the non-printed area or 'gaps'). To determine the accuracy of the printed grid, the area value measured was compared to the area of the original CAD design measurements and expressed as a percentage (Table 3).

Table 3
Printing accuracy of 40 mm^2 grids of all experimental batches where values closest to 1.0 represent absolute precision (Value representing original CAD dimensions = 1.0) (* Though gap accuracy is highest in this category, the sample printed an incomplete grid – leading to large unprinted areas contributing to a larger gap area value).

Sample Code	Total printed area/grid accuracy	Total non-printed area/gap accuracy
A	1.6	0.3
B	1.7	0.3
C	1.8	0.3
D	1.6	0.4
E	1.7	0.3
F	1.6	0.3
G	1.8	0.2
H	(1.4)*	(0.4)*

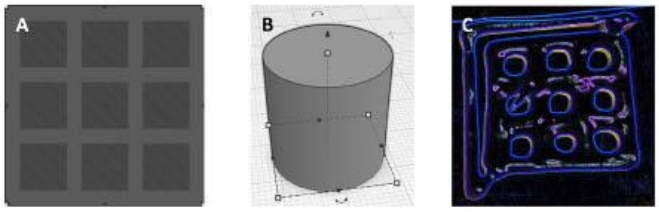

Fig. 4. A = CAD drawing of 40 mm square grid structure, B = CAD drawing of of 20 mm cylinder (AR = 1), C = Example of 'Find Edges' function of ImageJ analysis software. This function enables the user to measure the perimeter of the edges with increased accuracy in comparison to measurements using the original image without this function.

2.2.10. Microscopic imaging of processed cheese samples

Confocal Laser Scanning Microscopy (CLSM; Olympus FV1000 confocal laser scanning inverted microscope, Olympus Corporation, Japan) was used to view the structure of each processed cheese recipe (both control and printed samples) in closer detail. Each cheese sample was cut to the approximate dimensions of 5 × 5 × 2 mm using a sharp scalpel before being carefully positioned in a 35 mm diameter glass bottomed dish with a 0.17 mm coverslip base. A liquid mixture of Nile Red and Fast Green FCF fluorescent dyes were prepared to stain the fat and protein phases respectively in each sample. This was achieved by preparing a mixture of 200 μL Fast Green FSF aqueous solution (0.1 g/L) and 600 μL of Nile Red in Polyethylene Glycol (0.1 g/L). Fluorescent dye mix (50 μl) was applied to the surface of the sample before covering with the accompanying plastic lid, and allowing the sample to absorb the dye for 10 min at 4 °C before imaging. To achieve excitation of both fluorescent dyes, argon (488 nm) and helium/neon (633 nm) lasers were used to elicit fluorescence of Nile Red and Fast Green FCF, respectively, at their excitation wavelengths (Auty, Twomey, Guinee, & Mulvihill, 2001).

Scanning Electron Microscopy (SEM; Jeol JSM 5510 Scanning Electron Microscope, Jeol Ltd., Japan) was also used to visualise the microstructure of each processed cheese recipe (both control/poured and printed samples) in more detail. The samples were prepared using an adaption of SEM preparation methods from Tamime, Kalab, Davies, and Younis (1990) and Černíková, Nebesářová, Salek, Říháčková, and Buňka (2017). Each recipe was 3D-printed the day before the preparation stage for SEM and refrigerated overnight. The morning of preparation, the samples were cut into narrow three-dimensional rectangles of the approximate dimensions 1 × 1 × 15 mm using a sharp scalpel or blade. The samples were then fixed in 0.2 M Sodium Cacodylate Trihydrate buffer containing 25% *v*/v Electron Microscopy-grade glutaraldehyde for 2 h as a primary fixative, followed by a buffer wash (0.1 M Cacodylate buffer) for 15 min. The samples were subsequently dehydrated in a gradient (30, 50, 70, 90 and 100%) ethanol series for 15 min each. The final absolute ethanol step was repeated for a total of three changes. The samples were then defatted in chloroform (three changes of chloroform, 15 min for each change), and returned to the absolute ethanol for 15 min. The samples were then frozen and fractured in liquid nitrogen and dried using Tetramethylsilane (TMS) for 15 min, before allowing the TMS to evaporate from the sample containers in the fume hood. When the samples were entirely dry, they were mounted on labelled aluminium stubs using double-sided carbon tape and sputter-coated with 5 nm layer of of gold/palladium (80:20) using a Quorum Q150 RES Sputter Coating System (Quorum Technologies, UK). The samples are then held in a desiccator until they were examined at 5 kV using a Jeol JSM 5510 Scanning Electron Microscope (Jeol Ltd., Japan).

2.2.11. Statistical analysis

All recipes were printed in cylindrical and grid structures in triplicate, and analytical measurements were carried out at least in duplicate. Statistical analysis for this study was conducted in IBM SPSS Statistics Version 26 (IBM Corporation, US). Statistical analysis was carried out separately for the TPA measurements of both the pH and structural protein content datasets. Two-way Analysis of Variance (ANOVA) was carried out, with post-hoc analysis using the Tukey HSD test to compare treatments and assess the impact of changes in the product and process factors (i.e., printed or poured, pH, structural protein content). Differences were deemed statistically significance for $p < 0.05$ (5% or lower probability of the null hypothesis).

A Factor Analysis was performed on all datasets pooled together. As can be seen in the description of the methods, a large number of individual quality measurements were taken. A Factor Analysis (FA) is a variable reduction technique that replaces n-individual measurements (the responses, or output variables) by a few multilinear combinations of these, which have the feature of a maximum orthogonality between them, so as to capture as much information as possible from the individual output variables into a limited number of higher-level variable compositions, or Factors.

FA is commonly used for quality charts to provide integrated assessments of a large variety of individual quality measurements in a much more user-friendly way than considering each of them individually in turn. It also avoids redundant information arising from correlations between individual measurements or clusters of them. The physical meaning of one of these multilinear combinations is extracted from the clustering of individual measurements provided by those that have high loading factors with the respective Factor (loadings being the individual correlation of each quality measurement and each Factor). The first dimension, or Factor, corresponds to the multilinear combination able to explain the highest percentage of the total variability of the data (if all quality measurements could be correlated together by a simple linear combination and none of them had any errors of measurement, this Factor would explain 100% of the variance of the data and it would capture the entirety of information provided by the n-different measurements). The 2nd Factor will explain the greater percentage possible of the remaining variance of the data not explained by the 1st and that has a maximum possible orthogonality with it, and so forth. The number of Factors that captures the entirety of the data depends on a number of criteria, but one usually expects that all Factors selected to represent the data, when combined, would explain at least 80% of the variance of the data of all n-quality measurements. Calculations and graphical representations were performed using Statistica 13.0 (TIBCO, California, USA).

3. Results and discussion

3.1. Influence of TSC level and moisture content on printing accuracy and texture of 3D-printed processed cheese

During the initial formulation process, three TSC contents (1.5, 2, and 2% TSC) and three total moisture contents (47, 50 and 55% total moisture) were used as modifications of Recipe F to determine the optimum texture and printing accuracy for a standard recipe.

There was no statistically significant difference ($p > 0.05$) between the values measured for each textural parameter of the three TSC samples. However, there was a difference in printing accuracy between the grid samples with different TSC levels, where successful samples were selected by visually assessing for a lack of sample spreading during printing, and evenly printed lines with no gaps. Processed cheese containing 2.5% TSC (Fig. S1C) printed most evenly with minimal spreading, compared to 1.5% TSC (Fig. S1A), which produced a liquid-like and less precise print due to spreading; therefore, 2.5% TSC was selected for the final recipe. Shirashoji, Jaeggi, and Lucey (2006) found that, when heated from 5 to 85 °C, the storage modulus (G') of processed cheese mixtures with higher TSC concentrations (e.g., 2.75% TSC) tended to also have higher G' values compared to those with lower TSC concentrations (e.g., 1.5 and 0.25% TSC). Due to a larger quantity of the emulsifying salt present in the mixture, a larger amount of calcium was chelated allowing for fat emulsification and casein dispersion to be improved during the cooking phase and resulted in more solid-like, reinforced processed cheese structure (Shirashoji et al., 2006).

The effect of total moisture content on printability was then evaluated. Processed cheese samples (Recipe F) with 55%, 50% and 47% total moisture (the potential printability of processed cheese with 44% total moisture was also investigated, but the mixture was deemed too viscous to print) were 3D-printed into grids using the same methods mentioned previously. On visual assessment of the printed grid structures, the print clarity and lack of spreading of the lowest moisture sample (47% total moisture) (Fig. S1D) was deemed a more precise print than the highest moisture sample (Fig. S1F). For this reason, processed cheese samples with 47% total moisture were 3D-printed for the remaining portion of the study.

3.2. Effect of 3D printing on microstructure of 3D-printed processed cheese

Scanning Electron Microscopy (SEM) and Confocal Scanning Laser Microscopy (CSLM) were used to evaluate the effect of printing on the microstructure of processed cheese. In Fig. 5, the SEM micrographs show an example of the microstructure of two processed cheese recipes (Recipe A and F). Fig. 5A and B show the microstructure of the poured processed cheese recipes and Fig. 5C and D show the printed version of the corresponding recipes.

The microstructural differences between poured and printed samples varied between recipes. Certain poured samples (Fig. 5A) displayed distinctly spherical fat globules, whereas other poured samples (Fig. 5B) exhibit slight pooling and partial coalescence of the lipid fraction. These minor microstructural deviations within poured samples could be due to differences in recipe in terms of pH and structural protein content; processed cheese recipes with a higher pH or higher ratio of mature Cheddar to immature Cheddar could lead to increased fat pooling and reduced firmness in the final product (; Marchesseau, Gastaldi, Lagaude, & Cuq, 1997; Purna, Pollard, & Metzger, 2006). In comparison to the poured samples, the printed samples (Fig. 5C and D) appear to also have partially coalesced fat globules, but to a greater extent. This could be due to the longer heating times and shearing forces whilst the sample is extruded through the nozzle during printing, enabling the fat globules to interact and merge together, but not to such an extent that the fat is fully coalesced.

Fig. 6 shows micrographs of the same processed cheese recipes (Recipe A and F) using CSLM to further evaluate the microstructure of the printed versus poured processed cheese samples. The fat globules, seen in red, in the poured sample (Fig. 6A and B) showed a similar trend to those in the SEM micrographs; they appear partially coalesced to an extent, but not to the same magnitude as the printed samples (Fig. 6C and D), where the lipid fraction appears to have a higher rate of interaction and change following printing. Due to limited magnification in CSLM micrographs (x 40 magnification) compared to the SEM micrographs (from x 600 to × 1000 magnification), fat globules appear larger in collective images in Fig. 5 than in Fig. 6. However, it appears SEM images capture the coalescing effect of 3D printing more so than CLSM, so the combination of both microscopy techniques allows for a more comprehensive illustration of microstructural differences (both the surface level and more in depth view) between poured and printed processed cheese samples.

3.3. Change in structural protein content during Cheddar cheese aging

Urea-PAGE electrophoretograms of the raw Cheddar cheeses added in various quantities to the eight recipes are shown in Fig. 2. It was observed that α_{S1}-CN was intact in fresh curd samples, but gradually reduced in volume during ripening. It is generally accepted that exogenously added chymosin contributes to the breakdown of α_{S1}-CN, where the Phe_{23}-Phe_{24} or Phe_{24}-Phe_{25} bond is readily hydrolysed to produce fragment α_{S1}-CN-I (α_{S1}-CN f24/25–199) (Guerreiro, Barros, Fernandes, Pires, & Bardsley, 2013; McSweeney, Olson, Fox, Healy, & Højrup, 1993; Mulvihill & Fox, 1979). As shown in Fig. 2b, there was an inverse relationship between the parent α_{S1}-CN and α_{S1}-CN-I peptide during the period of aging in Cheddar cheese, with a gradual decrease in the parent band volume overtime whilst simultaneously increasing α_{S1}-CN-I peptide band volume. The higher levels of α_{S1}-CN-I in more mature samples

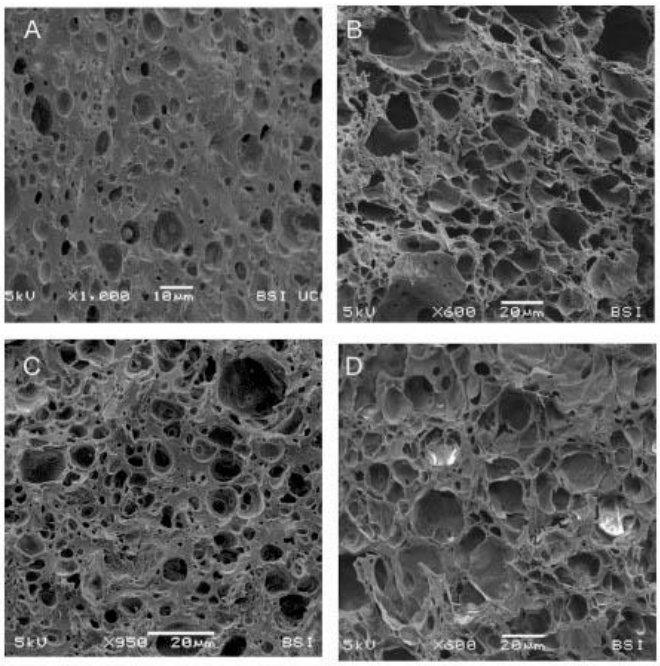

Fig. 5. SEM Micrographs: A = Poured recipe A (Magnification x 1000), B = Poured recipe F (Magnification x 950), C = Printed recipe A (Magnification x 600), D = Printed recipe F (Magnification x600).

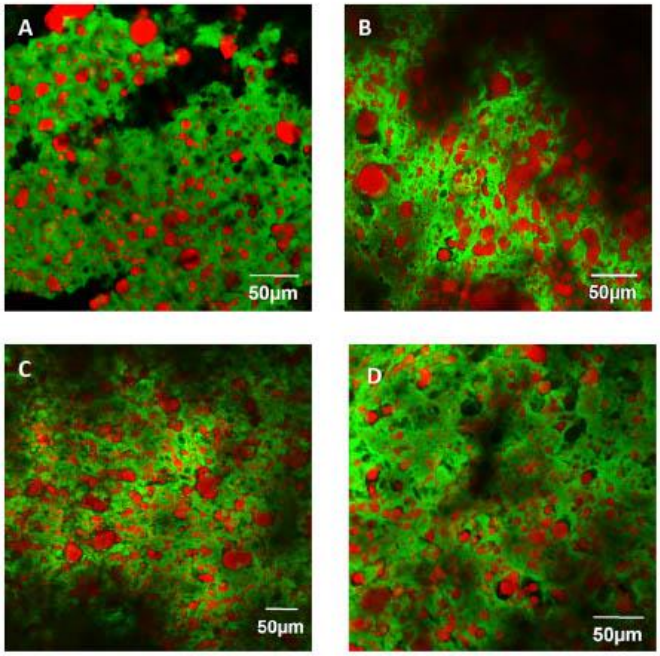

Fig. 6. CSLM Micrographs - A = Poured recipe A, B = Poured recipe F, C = Printed recipe A, and D = Printed recipe F (Images were taken using a 40× objective) (Red (Nile red stain) = Lipids, Green (Fast Green FCF) = Protein).

suggest that there is a reduction in structural protein (i.e., parent α_{s1}-CN), therefore producing a softer, more brittle Cheddar with improved meltability. Conversely, younger Cheddar samples appear to contain less breakdown peptides of the parent protein and more intact α_{s1}-CN, producing a harder Cheddar with poor meltability, though more suited to slicing applications than more mature Cheddar, which has a crumblier body (Creamer & Olson, 1982; Fenelon & Guinee, 2000; McMahon et al., 2014).

3.4. Effect of pH and structural protein content on the printing accuracy of 3D-printed processed cheese

To compare the printing accuracy of each recipe, and therefore, the impact of each parameter (i.e., pH and structural protein content), the area values of the printed grids were compared to the dimensions of the original CAD drawing (Fig. 4A) and expressed as a percentage (Table 3). Measurements of each printed grid were performed by ImageJ analysis software using the method described in Section 2.2.10. It should be noted that grid accuracy values deviating from a value of 1.0 indicate a loss of accuracy; therefore, values close to 1.0 indicate high accuracy. It should also be noted that the accuracy values of Recipe H are unrealistic (non-printed area accuracy value– 0.4, printed area accuracy value – 1.4), in comparison to the other sample values and should be omitted. This is due to the sample printing an incomplete grid (Fig. 7A), therefore leading to large unprinted areas and contributing to a larger than normal area value. The high final viscosity (Fig. 3) of Recipe H ($\eta = 12.48$ Pa.s) and poor meltability due to a lack of mature Cheddar content in comparison to the other recipes may have led to poor print bed adhesion

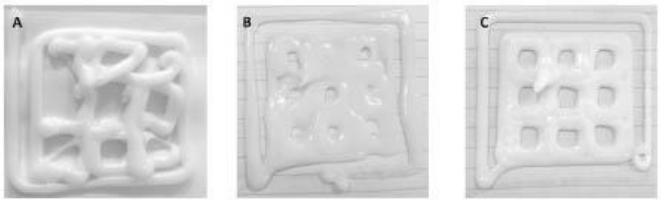

Fig. 7. 3D-printed 40 mm 2 grid: A, Recipe H: Fresh curd control (incomplete grid squares, dragging of mixture causing distorted lines); B, Recipe G: 100% 1–2 month old cheddar (spreading, suboptimal printing precision); C, Recipe D: 25% 1–2 month old cheddar and 75% 6–8 month old cheddar (accurate, clean lines, very little spreading, optimal printing precision).

leading to dragging and poor printing accuracy.

Sample pH had no significant effect ($p > 0.05$) on printing accuracy of Recipe A, B and C, where the accuracy value differences between the three pH ranges were negligible (between 1.23 and 2.78% difference in non-printed areas and 0.3–10.93% difference in printed areas). On visual assessment (Fig. 8), however, it was noted that processed cheese samples with a higher pH (Recipe C - pH 5.8) resulted in printed grids which had a soft, sticky consistency which contributed to the mixture spreading post-print. Marchesseau, Gastaldi, Lagaude, and Cuq (1997) reported that processed cheese produced at higher pH (pH 5.7–6.1) showed protein particles of a "string-like" quality, leading to a reduction in structural stability. Sołowiej (2007) also observed reduced firmness in processed cheese samples of pH 5.5 and above and concluded that increasing pH leads to an increase in casein hydration due to a decrease in protein-protein interactions.

In addition, there was no statistically significant difference ($p > 0.05$) between the accuracy values for the processed cheese recipes with varying structural protein content (Recipe D, E, F, G and H). However, from the accuracy values calculated, and from visual comparison between samples, Recipe G was the poorest performing in terms of printing accuracy. This recipe, which contained only 1–2 month old Cheddar as its cheese source, had the lowest gap (0.2 printing accuracy value) and printed grid area accuracy (1.8 printing accuracy value) due to spreading during printing (Fig. 7B). This may be due to the higher structural protein content in the younger cheese, making it difficult for the cheese to flow with a smooth consistency through the nozzle onto the print bed and, therefore, spreading occurred during printing.

It was also observed that Recipe D, which contains 25% 1–2 month old and 75% 6–8 month old Cheddar, performed best in terms of printing accuracy (Fig. 7C), showing the highest total gap area (0.4 printing accuracy value) and grid printing accuracy (1.6 printing accuracy value) closest to absolute accuracy (= 1.0) out of all eight formulations. During printing, there was very little spreading of the processed cheese mixture, which appeared to flow smoothly through the print nozzle during printing. This may have been due to the presence of a higher quantity of mature Cheddar in Recipe D, allowing the processed cheese mixture to flow in an uninhibited manner due to lower structural protein content and better meltability than recipes with larger quantities of younger cheese. However, the addition of small quantities of young cheese in Recipe D allowed the formation of a solid grid structure with very little spreading into gap area, leading to a cleaner, more accurate print.

Wang et al. (2011) and Fox, McSweeney, Cogan, and Guinee (2004) reported on the impact of ripening on Cheddar cheese before manufacture for processed cheese, noting changes in texture of young Cheddar during ripening, where the sample becomes softer, less rubbery and smoother as the cheese ages. Fox, McSweeney, Cogan, and Guinee (2000) also noted that processed cheese mixtures intended to produce as blocks with good sliceability requires predominantly young Cheddar (75–90% intact casein) in their recipe, whereas a recipe with predominantly mature Cheddar (60–75% intact casein) is more suited to cheese spreads.

During ripening, a 'softening' effect is observed in the texture of Cheddar cheese; these textural changes can be attributed in significant part to progressive proteolysis. In parallel, throughout ripening, a shift in calcium occurs as the Cheddar pH decreases due to increasing lactic acid content, and the reduction in cheese hardness can also be partly attributed to a reduction in levels of colloidal calcium phosphate (CCP) (Lucey, Mishra, Hassan, & Johnson, 2005; O'Mahony, Lucey, & McSweeney, 2005). These microstructural changes impact important functional properties such as melting, where mature Cheddar, which has less intact protein content and reduced CCP, exhibits better meltability than that of a younger cheese with larger quantities of intact protein, due to lack of proteolysis as a consequence of limited aging of the cheese (Kapoor & Metzger, 2008). Brickley, Auty, Piraino, and McSweeney (2007) also suggested that a reduction in firmness and increase in flowability in processed cheese recipes can be linked to a reduction in the levels of intact casein in the Cheddar cheese during ripening, thereby resulting in a reduction in the number of casein–casein interactions in the cheese matrix during processed cheese manufacture.

As previously mentioned in Section 2.2.5, rheological profiles (Fig. 3) were used as a guide to predict how each processed cheese recipe would perform during printing based on their final viscosity values. From the viscosity data shown in Fig. 3 for each recipe, recipe G had the lowest final viscosity value ($\eta = 2.14$ Pa.s). This sample was also one of the poorest performing recipes in terms of printing accuracy due to spreading of the mixture during printing and its thin consistency (Fig. 7B). In contrast, Recipe H displayed the highest final viscosity values ($\eta = 12.48$ Pa.s), yet also displayed poor printing accuracy due to the mixture dragging during printing, leading to large gaps and inconsistent prints (Fig. 7A). Therefore, it cannot be assumed that viscosity and printing accuracy are directly proportional. However, it could be suggested that there are a range of final viscosities for which processed cheese mixtures would be considered acceptable in terms of accurate printing. Recipes A, B, D and F all have final viscosity ranges between 7.55 and 10.94 Pa.s and, both from visual assessment and values of printing accuracy (Table 3), are deemed to yield a reasonable print. Any value that falls outside this range is considered suboptimal in terms of printing accuracy, due to line gaps, spreading or dragging during printing. Liu et al. (2019) also found, using a range of rheological methods (e.g., dynamic rheology and creep-recovery test), that a spectrum can be determined which allows a mixture of sodium caseinate and milk protein concentrate to print within the optimum printing precision range (e.g., when printed, flows uninhibited from the extrusion nozzle, but also holds a 3D structure without collapsing or spreading). Identifying this range for each food material also allows us to understand the function of each ingredient when added, omitted or when quantities are changed within the mixture, as was the case in a study involving the addition of whey protein (WP) powder to konjac flour (Yuwei, Zhang, & Chen, 2021). Those authors found that when adding WP powder at levels from 0% - 30% (*w/w*) to the konjac flour mixture, the addition of the milk protein up to 20% had a significant effect in printing performance in comparison to the control (no WP added) and lower concentrations of WP addition (i.e., better structural hold post-printing and printed layers were "cleaner").

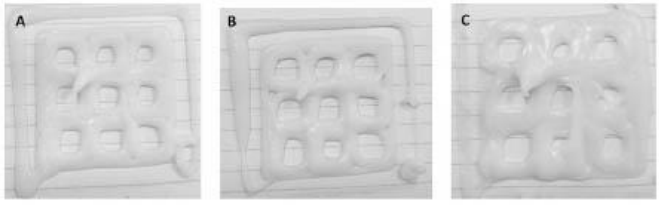

Fig. 8. 3D-printed 40 mm^2 grid, A (Recipe A: pH 5.4), B (Recipe B: pH 5.6), and C (Recipe C: pH 5.8).

3.5. Effect of pH and structural protein content on textural properties of 3D-printed processed cheese

Texture Profile Analysis (TPA) analysis was performed for each printed and poured processed cheese recipe sample using the methodology described in Section 2.2.9; for ease and clarity of discussion, results for pH and structural protein content are separated into two tables (Table 4 (pH) and Table 5 (structural protein content)).

3.5.1. Effect of pH

There was a statistically significant difference ($p < 0.05$) in hardness, gumminess, chewiness and resilience of recipe C in comparison to both Recipes A and B (Table 4). This confirms that altering the pH of the processed cheese formulation significantly affects the texture. Recipe C, which had pH 5.8 (i.e., the highest pH within the pH range tested), was found to be softer, less gummy, less chewy and less resilient than processed cheese samples of Recipe A and B, at pH 5.4 and pH 5.6, respectively. It was also found that Recipe C was significantly less cohesive ($P < 0.05$) than Recipe A, but there was no significant difference between cheese with Recipe C and Recipe B. These results compliment the grid analysis work mentioned in Section 3.3, where grids printed using Recipe C were observed to be sticky, soft and generally difficult to print accurately without spreading or dragging.

Adhesiveness of samples appeared to be the sole textural characteristic affected by the action of printing (in the pH dataset). Poured samples were found to be significantly ($P < 0.05$) less adhesive than printed samples. There are few studies in this area suggesting why this may be the case. Le Tohic et al. (2018) reported a decrease in adhesiveness in 3D printed samples in comparison to the control, and suggested that increased levels of surface oil were expressed during extrusion of the printed samples which in turn acted as a lubricant preventing adhesion to the TPA probe during testing.

3.5.2. Impact of structural protein content

With regards to the structural protein content data (Table 5), differences in cheese maturity (and hence structural protein content) result in statistically significant differences ($P < 0.05$) in hardness, gumminess and chewiness of processed cheese Recipes D, E, F and H (no significant effect on G). Recipe H, which contains 100% fresh curd, was found to be significantly harder than Recipes D (25% young Cheddar, 75% mature Cheddar), F (75% young Cheddar, 25% mature Cheddar) and G (100% mature Cheddar). It was also observed that processed cheese made using fresh curd (Recipe H) was also chewier and gummier than Recipes D and G. This is most likely due to the dense, fibrous protein network (i.e., greater levels of structural protein) of the fresh curd (see S2A in Supplementary Material) making up Recipe H. The lack of softening in texture in comparison to Recipes *E*-G, which contain varying ratios of young and mature Cheddar, could be due to the lack of proteolysis within the fresh curd due to limited ripening. The lack of proteolysis products can be seen in an absence of bands occurring in the lower sections of the Urea-PAGE gel of the fresh Cheddar curd (Fig. 2, L2). This profile is typical of a day 1 fresh curd sample, where there is limited breakdown of α_{S1}-CN (Fenelon & Guinee, 2000; McMahon et al., 2014).

Where most textural parameters within this dataset was affected by structural protein content, the only textural parameter affected by the action of printing was cohesiveness, where it was found that poured samples were more cohesive than printed samples. This may be due to the disrupted structure in the printed samples, where the processed cheese mixture was extruded into a ten-layered structure, thereby allowing ten potentially weakened points to be more susceptible to the compressive forces of the TPA probe. In contrast, in the poured samples, the processed cheese mixtures were allowed to settle evenly into one solid and even mass within the silicone mould in preparation for texture analysis. The lack of separate layers within the poured samples may allow the structure to withstand compressive forces, and therefore resist deformation and rupturing.

3.5.3. Factor analysis

A quality chart showing the scores of Factors 1 and 2 of the Factor Analysis of all TPA measurements provides an integrated view of the differences between the samples, and is shown in Figs. 9 and 10 for the data set with varying pH and with varying maturity, respectively; the points shown are the averages of three replicate measurements. It is noted that the factor scores in both graphs are from the same Factor Analysis and therefore can be compared. These factors are a multilinear combination of standardised values of the individual measurements, which implies that a score of 0 means an average value, a score of 1 a value above average by one standard deviation, a score of −1 a value below average by one standard deviation, etc. If the distribution of values was normal, 95% of observations would be within −2 and 2. The quality charts represent 2 graphs in each case, with Factor 2 in the y-axis of both, giving the 3D visualization of the Factor space in a projected plane form. The clustering of the TPA variables in the Factor Analysis is shown in Table 7, where it can be seen that just 3 Factors explain 92% of the variance of the data generated by the 7 different measurements for all samples (and 3 repeats in each case) and are therefore essentially capturing all of the TPA information of all samples (totalling 336 individual data points). It can also be seen that Factor 1 clusters measurements that quantify attributes of compression, whereas Factor 2 is essentially adhesion behaviour and Factor 3 relates to cohesion of the material. Thus, the terms "compression", "adhesion" and "cohesion" will be loosely used to designate Factors 1, 2 and 3, respectively.

Fig. 10 shows a clear difference between printed samples and the control, with the former having negative scores in adhesion and the latter positive ones, as already noted as factor 2 is essentially dominated by adhesiveness. One can also see a clear effect of pH in the scores of factor 1 that quantify compression behaviour, with the low pH at 5.4 at the center, the pH of 5.6 with high compression scores and the pH of 5.8

Table 4
TPA measurements of samples with varying pH level (5.4, 5.6 and 5.8) (poured and printed). Superscript letters indicate homogenous groups in a Tukey-HSD test at 95% confidence level. Those with differing superscript letters are statistically significantly different ($p < 0.05$).

	Hardness (N)		Adhesiveness (N.s)		Springiness (−)		Cohesiveness (−)	
Sample	Poured	Printed	Poured	Printed	Poured	Printed	Poured	Printed
A	7.94 ± 0.48^{b}	8.45 ± 0.31^{b}	-0.13 ± 0.19^{a}	-0.62 ± 0.47^{b}	0.84 ± 0.03^{a}	0.85 ± 0.02^{a}	0.80 ± 0.01^{b}	0.78 ± 0.02^{b}
B	8.28 ± 1.35^{b}	9.05 ± 1.16^{b}	-0.25 ± 0.13^{a}	-0.80 ± 0.31^{b}	0.82 ± 0.04^{a}	0.82 ± -0.01^{a}	0.79 ± 0.01^{ab}	0.77 ± 0.01^{ab}
C	5.97 ± 0.64^{a}	6.40 ± 0.36^{a}	-0.17 ± 0.28^{a}	-0.83 ± 0.49^{b}	0.81 ± 0.05^{a}	0.81 ± 0.03^{a}	0.77 ± 0.01^{a}	0.76 ± 0.01^{a}

	Gumminess (−)		Chewiness (−)		Resilience (−)	
Sample	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*
A	6.35 ± 0.45^{b}	6.60 ± 0.31^{b}	5.32 ± 0.39^{b}	5.60 ± 0.34^{b}	0.36 ± 0.01^{b}	0.35 ± 0.02^{b}
B	6.53 ± 0.98^{b}	7.01 ± 0.96^{b}	5.36 ± 0.69^{b}	5.76 ± 0.80^{b}	0.37 ± 0.02^{b}	0.35 ± 0.003^{b}
C	4.61 ± 0.49^{a}	4.86 ± 0.29^{a}	3.77 ± 0.61^{a}	3.93 ± 0.26^{a}	0.34 ± 0.01^{a}	0.32 ± 0.01^{a}

Table 5
TPA measurements of samples with varying structural protein content (poured and printed). Superscript letters indicate homogenous groups in a Tukey-HSD test at 95% confidence level. Those with differing superscript letters are statistically significantly different ($p < 0.05$).

	Hardness (N)		Adhesiveness (N.s)		Springiness (–)		Cohesiveness (–)	
Sample	Poured	Printed	Poured	Printed	Poured	Printed	Poured	Printed
D	7.23 ± 3.14 ^{a}	7.85 ± 1.73 ^{a}	−0.24 ± 0.21 ^{a}	−0.52 ± 0.52 ^{a}	0.83 ± 0.02 ^{a}	0.82 ± 0.01 ^{a}	0.79 ± 0.01 ^{a}	0.77 ± 0.01 ^{b}
E	7.36 ± 0.02 ^{a}	7.40 ± 1.55 ^{a}	−0.48 ± 0.36 ^{a}	−0.33 ± 0.24 ^{a}	0.82 ± 0.05 ^{a}	0.82 ± 0.03 ^{a}	0.78 ± 0.0004 ^{a}	0.77 ± 0.01 ^{b}
F	7.85 ± 3.26 ^{a}	7.91 ± 1.94 ^{a}	−0.20 ± 0.03 ^{a}	−0.44 ± 0.39 ^{a}	0.84 ± 0.01 ^{a}	0.83 ± 0.02 ^{a}	0.78 ± 0.01 ^{a}	0.77 ± 0.02 ^{b}
G	5.38 ± 1.96 ^{a}	6.20 ± 2.15 ^{a}	−0.02 ± 0.02 ^{a}	−0.51 ± 0.18 ^{a}	0.82 ± 0.05 ^{a}	0.80 ± 0.03 ^{a}	0.76 ± 0.03 ^{a}	0.75 ± 0.04 ^{b}
H	10.98 ± 0.66 ^{b}	11.79 ± 1.02 ^{b}	−0.22 ± 0.24 ^{a}	−0.08 ± 0.06 ^{a}	0.85 ± 0.01 ^{a}	0.84 ± 0.004 ^{a}	0.79 ± 0.004 ^{a}	0.77 ± 0.01 ^{b}

	Gumminess (–)		Chewiness (–)		Resilience (–)	
Sample	*Poured*	*Printed*	*Poured*	*Printed*	*Poured*	*Printed*
D	5.69 ± 2.52 ^{a}	6.06 ± 1.45 ^{a}	4.77 ± 2.20 ^{a}	4.94 ± 1.17 ^{a}	0.35 ± 0.04 ^{a}	0.35 ± 0.01 ^{a}
E	5.79 ± 0.01 ^{ab}	5.70 ± 1.26 ^{ab}	4.83 ± 0.25 ^{ab}	4.64 ± 0.91 ^{a}	0.35 ± 0.003 ^{a}	0.35 ± 0.01 ^{a}
F	6.16 ± 2.64 ^{ab}	6.08 ± 1.63 ^{ab}	5.21 ± 2.27 ^{ab}	5.08 ± 1.48 ^{a}	0.35 ± 0.04 ^{a}	0.34 ± 0.01 ^{a}
G	4.12 ± 1.63 ^{a}	4.67 ± 1.80 ^{a}	3.44 ± 1.50 ^{a}	3.75 ± 1.54 ^{a}	0.31 ± 0.04 ^{a}	0.33 ± 0.04 ^{a}
H	8.69 ± 0.50 ^{b}	9.09 ± 0.74 ^{b}	7.38 ± 0.47 ^{b}	7.60 ± 0.66 ^{b}	0.39 ± 0.004 ^{a}	0.35 ± 0.01 ^{a}

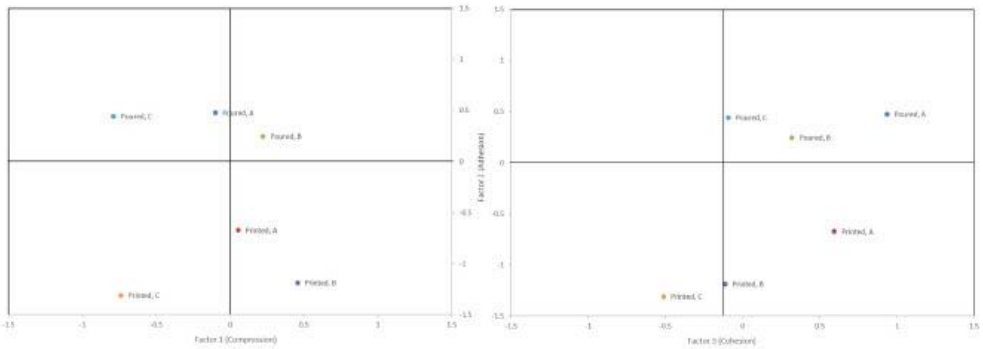

Fig. 9. Quality chart with the factor scores of the samples (averages of 3 independent repeat measurements) of the pH dataset (Recipes A-C).

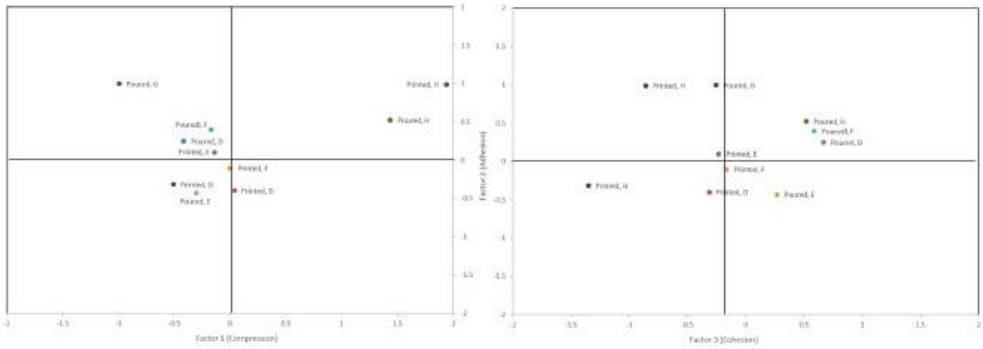

Fig. 10. Quality chart with the factor scores of the samples (averages of 3 independent repeat measurements) of the structural protein content dataset (Recipes D—H).

with the low compression scores, whether for printed or control samples.

Fig. 11 shows less clear differences between samples, with the exception of a significantly higher compression score of samples H, whether printed or control. It should be noted that these are scores of the same factor analysis as Fig. 9 and that the plots have different scales; sample H had a significantly higher compression score than all others, which means that the fresh curd itself gives a completely different compression behaviour to any of the matured cheese samples, whatever

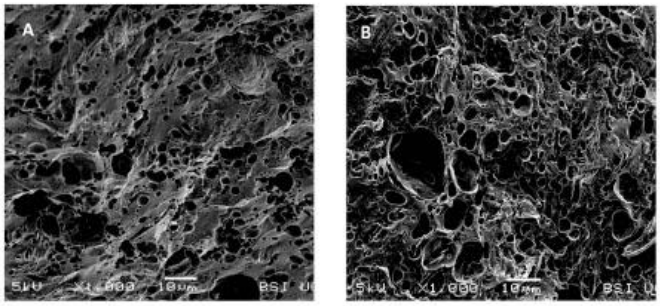

Fig. 11. SEM Micrographs showing Fig. 16A – recipe D, 3D printed at 40 °C (Magnification x 1000) and Fig. 16B – recipe D, 3D printed at 65 °C (Magnification x 1000).

its maturity. It can also be seen that, for the samples where maturity varied, the printed ones tend to generally have high cohesion scores whereas, printed ones have low scores. This is especially true for samples G and H.

The joint analysis of all data allows also to compare the magnitude of the effect of pH with that of maturity in the texture quality factors. A main effects ANOVA was applied to the whole set of data and results are shown in Table 8. It is noted that, in a main effects ANOVA, the error includes not only the white noise (variability between repeat measurements), but also all eventual interactive effects (quantifications of whether the impact of one variable depends on the values of another), but calculating interactive effects would have required a full factorial design. In any case, the results are very clear; printing did not affect the compression scores, with pH and maturity both causing statistically significant differences, and with maturity having the most significant impact. This is primarily due to the significantly lower compression score for samples of pH 5.8 and significantly higher score for sample H. On the other hand, printing had a statistically significant impact on adhesion and cohesion, where the effects of pH and of maturity were not significant. In both cases, the outcome was a significantly lower score for the printed samples.

3.6. Effect of printing temperature on textural properties and microstructure of 3D-printed processed cheese

The microstructure of processed cheese from Recipe D printed at a higher printing temperature (65 °C) compared to the same recipe printed at 40 °C was also investigated. TPA was carried out as described previously (See Section 2.2.9) to compare the potential differences in texture of the two samples (Table 6). The processed cheese samples printed at higher temperatures were harder, less adhesive, gummier and springier than samples printed at lower temperatures. This result is similar to that of Lee, Kilbertus, and Alais (1981), where it was found that the firmness of processed cheese increased with increased cooking temperature. The results of an independent *t*-test show that there is relatively little statistical significance to the textural differences outlined above, with the exception of the resilience of the samples ($P < 0.05$). Processed cheese samples (Recipe D) printed at the higher temperature (65 °C) were found to be more resilient than those printed at a lower temperature (40 °C), which correlates with the findings of Lee et al. (1981).

From SEM micrographs (Fig. 11A and B), it was observed that the sample printed at 40 °C (Fig. 11A) showed partially coalesced fat globules interspersed amongst a dense protein matrix. However, in the sample printed at 65 °C (Fig. 11B), the fat droplets are more numerous, are closer in proximity, and appear to have much higher incidence of coalescence. The higher printing temperatures would seem to accelerate

Table 7
Loadings of the Factor Analysis of the entire set of data showing the clustering of individual TPA measurements in the factors extracted with Varimax normalised rotation. The dominant TPA measurements in each factor are highlighted in bold.

	Factor 1	Factor 2	Factor 3
Hardness	**0.963**	−0.088	0.237
Adhesiveness	−0.085	**0.988**	0.056
Springiness	0.235	0.190	**0.834**
Cohesiveness	0.376	−0.155	**0.839**
Gumminess	**0.952**	−0.093	0.282
Chewiness	**0.929**	−0.066	0.344
Resilience	**0.701**	0.113	0.570
Variance explained	48.4%	15.3%	28.3%
Cumulative variance explained	48.4%	63.7%	92.0%

Table 8
Main effects Analysis of Variance of the totality of the data. SS are the raw sums of squares and *p*-values the probability of the null hypothesis (that the variable has no effect). The textural parameters which were found to be statistically signigicant ($P < 0.05$) are highlighted in bold.

	Compression		Adhesion		Cohesion	
Main effects	SS	p-value	SS	p-value	SS	p-value
Printing	0.928	0.196	**6.295**	**0.010**	**6.534**	**0.007**
pH	**3.796**	**0.038**	0.505	0.749	3.482	0.132
Maturity	**21.071**	**0.000**	5.491	0.297	4.350	0.394
Error	20.878		33.752		31.843	

Table 6
TPA measurements of Recipe D 3D-printed at 40 °C or 65 °C. Superscript letters indicate homogenous groups in a Tukey-HSD test at 95% confidence level. Those with differing superscript letters are statistically significantly different ($p < 0.05$).

Sample	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Unit of measurement	*N*	*N.s*	–	–	–	–	–
40 °C	7.91 ± 1.59 ^{a}	−0.44 ± 0.32 ^{a}	0.83 ± 0.02 ^{a}	0.77 ± 0.01 ^{a}	6.08 ± 1.33 ^{a}	5.08 ± 1.21 ^{a}	0.34 ± 0.01 ^{a}
65 °C	10.56 ± 0.30 ^{a}	−0.30 ± 0.19 ^{a}	0.87 ± 0.01 ^{a}	0.79 ± 0.01 ^{a}	8.35 ± 0.30 ^{a}	7.29 ± 0.32 ^{a}	0.38 ± 0.01 ^{b}

the coalescing effect on the fat globules in comparison to the sample printed at lower temperatures. Kapoor and Metzger (2008) reported that processing conditions such as cook time, cooking temperature, extent of agitation during cooking and rate of cooling of the mixture can have a significant effect on the functional properties of processed cheese. Processing temperatures affect the physical state of the lipid fraction within the processed cheese matrix, where increased temperatures liquefy milk fat, potentially leading to coalescence and formation of large fat globules.

4. Conclusions

There are multiple interrelated factors to take into consideration when 3D printing a food product. In the case of processed cheese, pH and structural protein content are key components in creating 'printable' recipes. These parameters can be optimised depending on users' preference for texture and printing accuracy subject to the desired product's attributes in terms of application. Rheological profiles are also useful when considering the printability of a potential processed cheese recipe; in this particular case, processed cheese mixtures with viscosity between 7.55 and 10.94 Pa.s were the most suitable for printing, meaning that the resulting mixtures were acceptable in terms of printing accuracy due to optimal texture and flow. Temperature is also important in terms of critical processing factors which can affect 'printability' in terms of optimum texture and printing accuracy; at higher printing temperatures (65 °C), the structure becomes harder and more resilient to compressive forces, where the coalescing effect of fat droplets is accelerated and there is an increase in droplet size, thereby dominating the overall structural composition, in comparison to fat droplets in samples printed at lower temperatures (40 °C). In conclusion, 3D food printing is a growing area of research and discussion. Understanding the fundamental effects of 3D printing on food systems, such as processed cheese, is important in order to progress to 3D printing more complex and intricate structures. Only then perhaps will 3D food printing become more of a common technique within the food sector.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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Overcoming barriers to consumer acceptance of 3D-printed foods in the food service sector

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ABSTRACT

The purpose of this study is to investigate the potential uptake and inhibitors of 3D-printed food applications in the food service market to provide market salient evidence to inform business investments. An online survey was designed and distributed to an adult Irish population and was completed by 1,045 participants. The collected data was analysed using Structural Equation Modelling to test a hypothesised model of willingness to try 3D-printed food applications. Results showed that perceived personal relevance of the technology is a strong positive determinant of willingness to try (Standardised $\beta = 0.614^{***}$). Novel Food Technology Neophobia (NFTN) represents a barrier to willingness to try 3D-printed food applications as evident from its significant negative direct effect (Standardised $\beta = -0.167^{***}$). NFTN is also found to have a depressing indirect effect when mediated through perceived personal relevance (Standardised $\beta = -0.202^{***}$), while the importance consumers attach to naturalness is yet another barrier (Standardised $\beta = 0.053^{*}$). Overall, considering its total effect, NFTN (Standardised $\beta = -0.369^{***}$) presents the greatest barrier to willingness to try 3D printed foods.

The role of trust in science by directly diminishing the effects of NFTN (Standardised $\beta = -0.445^{***}$) and the importance of naturalness also emerges (Standardised $-\beta = 0.137^{***}$). Consequently, this work has identified some of the major obstacles facing the technology in the forms of NFTN and the importance of naturalness but has pointed to possible resolutions in building continued support and trust in science, and a focus on designing and delivering both customisable consumer-focused food products and accompanying marketing strategies that communicate and emphasise the personal benefits that this novel food technology affords.

1. Introduction

The purpose of this paper is to investigate the potential for 3D printing technology in Ireland and the potential constraints and inhibitors to market acceptance and uptake of the technology. To address this question, the paper seeks to reveal the factors that either promote or inhibit consumers' acceptance of the technology in the foodservice market. Foodservice, or out-of-home dining, may be defined as "all food consumed and prepared out of home" (Bord Bia, 2019). The decision to focus on this market is due to the fact that it is a substantial market and has a high penetration and exposure rates among consumers given its spatial distribution and its attractiveness given today's consumer lifestyles. In 2019, pre-Covid 19, the market was worth in the region of €6.33B in terms of consumer expenditure. The foodservice sector in the Republic of Ireland is highly competitive with over 27,000 outlets distributed across a variety of business types, but faces considerable cost pressures due to a shortage of skilled staff and high labour content (Bord Bia, 2019). Given the conflicting force of the need to differentiate yet facing high operational costs, 3D printing offers enormous potential to operators within the sector. More importantly, given consumers' wide exposure to the foodservice sector, it provides an entrée for this novel technology and an opportunity for consumers to experience it for the first time, building a sense of familiarity with 3D printing and opening up further opportunities in the consumer market, the holy grail for commercialisation.

Benefits of 3D printing in the food environment present exciting possibilities in terms of customization to meet specific dietary requirements, managing food waste (Fuller, 2021) or, more generally, to appeal to consumers' desire for new hedonic and self-expressive benefits through the physical construction and presentation of food itself (Burke,

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Kelly, Lavelle, & vo Kientza, 2021). However, lack of familiarity with a novel food/ technology is often cited as a barrier to acceptance (Jung, Sharma, & Mattila, 2022; Schäufele, Albores, & Hamm, 2019). This has led to the recommendation, in the case of novel foods, to establish them in the context of the familiar (Lombardi, Vecchio, Borrello, Caracciolo, & Cembalo, 2019; Siegrist et al., 2008a). Consequently, our interest is to identify the determinants of consumers' willingness to try 3D printing applications situated within a familiar context, such as the food service market, where the person is aware of the technology being applied to create the food, but is not required to learn how to use it (Bos, van der Lans, van Klee, & van Trijp, 2018). Placing the technology in a familiar context enables the consumer to visualize the technology in their everyday lives in ways that offer benefits to them, such as, convenience, health and enjoyment. Additionally, understanding the determinants of willingness to try the 3D food printing applications set in this context provides a critical insight into responses to foods derived from this technology in more transparent supply chains (i.e., where the consumer knows that it is a 3D-printed food or contains a 3D-printed food component). Exposure to the technology through food service applications can also support broader uptake of application. This perspective aligns to the position that exposure is the main building block of familiarity, so it is reasonable to argue that food service has a potential role in driving consumer acceptance of the technology.

Consumer acceptance of new food technologies can be challenging due to its newness and unfamiliarity to end users. These concerns are likely to also exist with novel 3D food printing applications. 3D food printing is considered to be a relatively young and developing technology, having been in existence since the early 2000s, with the development of the first open-source 3D food printer called 'Fab@Home' (Malone & Lipson, 2007). Since then, the number of studies exploring the characteristics of 3D printed foods has climbed steadily, where foods such as fruit and vegetables (Derossi, Caporizzi, Azzollini, & Severini, 2018; Severini, Derossi, Ricci, Caporizzi, & Fiore, 2018b), pasta (Van der Linden, 2015), cheese (Le Tohic et al., 2017), meat (Dick, Bhandari, & Prakash, 2019), bread dough (Liu, Liang, Saeed, Lan, & Qin, 2019; Yang, Zhang, Prakash, & Liu, 2018), chocolate (Hao et al., 2010; Mantihal, Prakash, & Bhandari, 2018), and more novel products such as insect-based cereals (Severini et al., 2018a) have been investigated.

3D food printing may be described as the layering of food material using a computerised process (Huang et al., 2013a). Using this method, a multitude of intricate and complex 3D objects can be printed, limited only by the physical properties of the food material (i.e., sufficient structural integrity for layering) and stability of the design (i.e., one with a stable equilibrium or low centre of gravity is better suited) (Liu, Zhang, Bhandari, & Wang, 2017). 3D food printing is thought to be a beneficial concept in helping to improve an individual's health status in terms of personalised nutrition. Using 3D printing technology, a user can potentially 3D-print a meal or snack tailored to their specific health needs, as opposed to a generalised portion which is mass manufactured to suit the average person (Caulier, Doets, & Noort, 2020; Pérez, Nykvist, Brøgger, Larsen, & Falkeborga, 2019; Sun, Zhou, Huang, Fuh, & Hong, 2015).

Although there are many publications discussing the fundamental science behind 3D food printing technologies and printability of potential food materials (Le-Bail, Maniglia, & Le-Bail, 2020; Liu & Ciftci, 2021; Zhu, Stieger, van der Goot, & Schutyser, 2019), there are few available on consumer attitude and acceptance of the technology. This is an important knowledge gap and increases the risks of investment in this novel technology. Siegrist (2008) reported the vital importance of involving the consumer at the initial stages of product development in order to increase chances of success in the marketplace, as a multitude of factors, beyond stated benefits, can impact on consumer acceptance of novel food technologies and products which cannot be overlooked at the research and developmental phase.

Based on the few studies currently available on consumer acceptance of 3D food printing, the terminology used in association with the technology (e.g., "printer"), the degree of safety and palatability of the food produced by the printer, and the potentially impaired nutritional value of the food were some of the primary consumer concerns that negatively impacted acceptance (Brunner, Delley, & Denkel, 2018; Lupton & Turner, 2016). A common consensus across studies currently available involving consumer perceptions of 3D food printing shows that many participants were previously unaware of the technology and initially did not recognise any true value in using the technology for themselves (Brunner et al., 2018; Lupton & Turner, 2016). As argued by Caulier et al. (2020), an awareness of the perceived benefits of the technology did not have a significant effect on the consumer's acceptance or perception of 3D-printed foods; however, it was observed that repeated consumption (over a period of four weeks) was found to be much more compelling in positively impacting participant liking of the 3D-printed food product. Similar to other forms of innovations in food and food processing, factors such as neophobia (both of novel foods and novel food technologies) and degree of trust (in food regulatory bodies, scientists, government etc.) can also pose challenges to consumer acceptance and must be overcome in order to encourage consumers to be willing to purchase and/or try a product produced by novel food technologies in the first place (Lim et al., 2021; Losada-Lopez, Dopicoa, & Faína-Medín, 2021; Wezemael, Verbeke, Kügler, de Barcellos, & Grunert, 2010).

In keeping with the knowledge deficit perspective, much discussion has focused on whether or not the provision of risk benefit information to consumers can alleviate their concerns about novel food technologies and combat neophobic tendencies (Bruhn, 2007; Hansen, Holm, Frewer, Robinson, & Sandoe, 2003; Rollin, Kennedy, & Wills, 2011). This 'knowledge deficit' perspective has received some criticism as it represents a relatively flawed and simplistic approach to explaining the rational for risk aversion in consumers when faced with food produced using novel food technologies (Runge, Chung, Su, Brossard, & Scheufele, 2018). Rollin et al. (2011) argued that understanding and addressing consumer evaluations of, and responses to, novel technologies is a much more complex and multifaceted issue, involving an understanding of consumers' risk–benefit analysis, socio-demographic characteristics, established public knowledge and information, including the level in trust in the source of information). As Hansen et al. (2003) stated "[…] where trust is lacking, merely 'stating the facts' is not a viable strategy in risk communication, no matter how sound the underlying science is".

The present study aims to address the current paucity of research into the determinants of 3-D printed foods acceptance. It will achieve this by identifying the predominant factors determining the willingness of Irish consumers to try 3D-printed foods in the food service context. The research is based on an online representative sample of 1,045 Irish consumers. It is anticipated that the research findings will help inform global ingredient suppliers, manufacturers, and food service operators when designing, developing and implementing the use of 3D food printing technology in such a way that 3D food printers become an enduring and practical technology rather than a fleeting novelty. As 3D food printing has been shown to be beneficial in valorising food waste streams (Jagadiswaran et al., 2021) and providing personalised nutrition to enhance the health of individuals (Caulier et al., 2020; Sun, Zhou, Yan, Huang, & Lind, 2018), it is of current relevance to promote sustainable food technologies to global consumers and those operating in the food industry in order to address these immediate challenges. Whether that is by utilising the full potential of food products to avoid unnecessary food waste, or producing tailor-made snacks and meals to satisfy each consumers' unique nutritional needs instead of a mass manufacture of a generic product which only satisfies a portion of the population.

The structure of the paper is as follows. The next section will develop a series of hypotheses to explain the relationships between constructs implicated in the willingness to try variable based on findings in earlier studies. Considered together, these hypotheses form our overall model of willingness to try (Fig. 1) and will help reveal the mediating

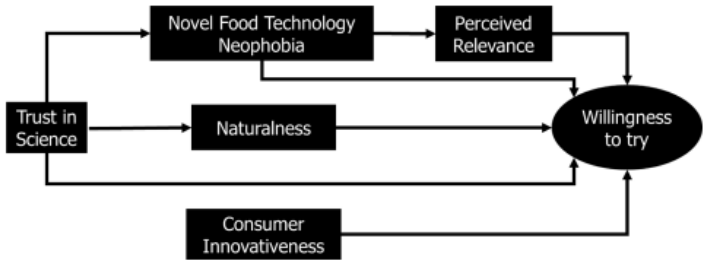

Fig. 1. A basic model of Irish consumers' willingness to try 3D food printing applications.

relationships that need to be understood in explaining the willingness to try 3D food printing applications. Following the hypotheses, we will progress to the methods applied and particular attention will be paid to our measurement model establishing convergent and discriminant validity use pre-validated items. Then, we test our model, before discussing the findings and implications for practitioners.

2. Hypotheses

Based on the literature available on the factors effecting general consumer acceptance of novel food technologies, the following section identifies the key factors which would be expected to influence consumer acceptance of 3D-printed food applications. A selection of these factors have also been accounted for in the few studies involving consumer acceptance of 3D-printed foods, where novel food technology neophobia (NFTN), perceived relevance/benefit, and attitudes towards naturalness have been shown to be significant factors in determining consumer acceptance (Brunner et al., 2018; Hassel, 2018; Lupton & Turner, 2016). The current work will establish a more complete model of the intention to use 3D printed technology through the development of a set of hypotheses that will reveal both the direct and indirect (mediated) effects between our independent and dependent variables.

2.1. Consumers' perceived personal relevance of novel food technologies

Perceived relevance is defined as the extent of which a person thinks something will contribute positively to their life (Celsi, Chow, Olson, & Walker, 1992; Davis, Bagozzi, & Warshaw, 1989). According to Greehy, McCarthy, Henchion, Dillon, and McCarthy (2013), individuals evaluate and prioritise products produced by novel food technologies according to their personal orientations and values, and perceived relevance of benefits offered by that product. As each individual has a unique life path and set of circumstances, personal values and benefits associated with technologies relevant to them personally can vary immensely (Henchion, McCarthy, Dillon, McCarthy, Greehy, Kavanagh, & Williams, 2013).

Other studies have shown that, when an individual perceives a product to be of high personal relevance to them, they are more likely to form a positive attitude towards that product and are more likely to purchase (Dean et al., 2012; Kang, Liu, & Kim, 2013). For example, individuals who are eco-conscious might perceive environmentally sustainable textiles as highly relevant to their own life and will therefore, feel a stronger desire to purchase in order to actively reinforce their interpretation of their projected self-image (Kang et al., 2013). In essence, individuals might be more willing to purchase items which they perceive as a way of enabling them to live the type of life for which they strive.

Therefore, it is proposed that:

H1:. *There is a direct and positive relationship between the perceived personal relevance of the novel technology and consumers' willingness to try 3D-printed foods applications.*

2.2. Novel food technology neophobic behaviours affect willingness to try 3D food printing technologies

Novel innovative technologies are often rejected by consumers as the technology enters the marketplace due to negative and suspicious feelings (Chen, Anders, & An, 2013; Wezemael, Verbeke, Kügler, & Scholderer, 2011). This can be due to a phenomenon known as 'Novel Food Technology Neophobia' (NFTN), which is a multidimensional concept comprised of perceived needs, choices, risks and media, as embedded in the scale developed by Cox and Evans (2008) to measure consumer acceptance of foods produced using novel technologies.

NFTN varies across different contexts. For example, Siegrist *et al.* (2008b) reported that consumer acceptance can fluctuate based on the technological application, where consumers were seen to be more accepting of nanotechnology being used in food packaging rather than in the food itself. Vidigal et al. (2015) also observed a difference in levels of consumer neophobia of different manufacturing processes of foods where Brazilian consumers were more accepting of processes which were seen as familiar and safe (e.g., traditional processing, pasteurisation); on the other hand, consumers were less accepting of these same products if they were produced using non-traditional and novel processes such as nanotechnology and Genetic Modification (GM), due to an increased level of perceived risk and negative perception of these technologies.

In the few studies of consumer attitudes towards 3D-printed foods studies available to date, it has been found that foods produced by 3D food printers are looked upon with suspicion by consumers, where fears generally derive from the uncertainty of the manufacture and nutritional quality of the printed foods (Brunner et al., 2018; Lupton & Turner, 2016). Hartmann and Siegrist (2017) suggested that consumers may regard foods which are 3D-printed as riskier to consume when compared to foods produced using gene technology, as the food as well as the technology can be seen as novel, meaning there is additional uncertainty and unease to comprehend. Similarly, in a quantitative study carried out by Brunner et al. (2018), it was observed that, despite the provision of targeted communication to their participants ($n = 260$) relating to the application of the technology, food technology neophobia persisted and even increased in some cases.

Therefore, it is proposed that:

H2:. *There is a direct and negative relationship between novel food technology neophobia and the consumer's willingness to try 3D-printed food applications.*

2.3. Consumer relationship with naturalness

In recent years, consumer demand for foods which are perceived as natural has been increasing (Staub, Michel, Bucher, & Siegrist, 2020). However, the term naturalness is not well-defined and tends to vary

depending on context and type of product (Schiano, Gerard, & Drake, 2021). Some have suggested that the degree of processing which food products are subjected to can be considered a measurement of naturalness, where foods which most resemble their native form are more natural than those which have been processed (Rozin, 2006). Whereas, others have proposed that physical changes (i.e., cutting, mashing etc.) are more natural than chemical changes (i.e., GM, additives) (Evans, de Challemaison, & Cox, 2010). According to Román, Sanchez-Siles, and Siegrist (2017), when food products are labelled as being natural, they are perceived to be better tasting, more nutritious and less harmful to the environment. In response to this, those involved in the manufacture and marketing of food have come to realise the considerable value which consumers place on perceived naturalness and have therefore made an increased effort to accentuate this particular aspect of their product over other characteristics on packaging labels and in marketing campaigns, further fuelling the narrative that natural is superior (Siegrist and Hartmann, 2020).

Studies involving consumer perception of 3D-printed foods have reported that there is a general sense of apprehension surrounding the levels of processing 3D printing would add to already processed (i.e., cooked, chopped, blended etc.) food purées (Brunner et al., 2018; Lupton & Turner, 2016). There is a general consensus that the consumers' perceived level of processing subjected to foods is inversely proportional to that of perceived naturalness (Etale & Siegrist, 2021; Evans et al., 2010; Rozin, Fischler, & Shields-Argelès, 2012; Rozin, 2005). This sentiment was also evident in works by Rozin (2005) and Rozin et al. (2012), where consumers were of the belief that where a food product had more steps involved in its manufacturing process, it was less 'natural'. Therefore, despite the possibility of 3D printing ingredients which should be perceived as natural, such as fruits and vegetables (b), consumers for whom naturalness is important may perceive 3D-printed foods as ultra-processed and unnatural due to the number of processing steps (e.g., blending of mixtures and sometimes addition of additives such as texturisers to create suitable textures for printing).

Therefore, it is proposed that:

H3:. *There is a direct and negative relationship between the importance of naturalness and consumers' willingness to try 3D-printed food applications.*

2.4. Consumer innovativeness and adoption behaviour

Consumer innovativeness has been described by Steenkamp, Hofstede, and Wedel (1999) as a consumer's predisposition towards the purchase of new or different products or brands, in favour of repeat purchasing of common or familiar products purchased previously. There is evidence (Rogers, 2010) that the degree of consumer acceptance of 'newness' can be a predictor of the probability of consumers adopting novel technologies, and the level of consumer innovativeness has been shown to have a direct and positive effect on innovation adoption behaviour (Arts, Frambach, & Bijmolt, 2011; Wang, Dacko, & Gad, 2008). Clearly, 3D food printing can be described as a novel, innovative and disruptive food technology (Berman, 2012; Kietzmann, Pitt, & Berthon, 2015), which can be attractive to innovators and early adopters who want to be the first in their social group to explore the newest creative culinary technology. Therefore, it is proposed that:

H4:. *There is a direct and positive relationship between consumer innovativeness and consumer willingness to try 3D-printed food applications.*

2.5. Consumers' level of trust in science impacts their willingness to try

Consumer trust in the industry, media, scientists and the government has been shown to play a decisive role in opinion and attitude formation towards acceptance of novel food technologies (Cvetkovich and Lofstedt, 1999; Søndergaard, Grunert, & Scholderer, 2005). It has been recognised by Roosen et al. (2015) that consumers with a high degree of trust generally exhibit lesser efforts of self-protection behaviour (i.e., are less risk-adverse) and are more willing to pay for novel foods than those with a lower degree of trust. Moreover, Bord and O'Conner (1990) observed that consumers who show trust in the food industry, government regulatory agencies or science have been more accepting and receptive of foods manufactured by novel food technologies.

Therefore, it is proposed that:

H5:. *There is a direct and positive relationship between consumers' level of trust in science and their willingness to try 3D-printed food applications.*

2.6. Relationship between trust in science and the importance of naturalness to consumers

According to Siegrist and Hartmann (2020), consumers often use a combination of cues and heuristics, such as perceived naturalness and or trust, to evaluate and form judgements of novel foods and foods produced using novel methods. Science has brought many advances beyond nature in terms of processes which have enabled the variety and availability of safe foods that characterise contemporary society (Chávez-Dulanto, Thiry, Glorio-Paulet, Vögler, & Carvalho, 2021). In doing so, trust in science has acted as a substitute for naturalness when making food-related decisions. However, a recent rise in consumers' perception of the importance of naturalness can be seen to coincide with a reduced level of trust in science, which has been amplified by high profile cases of food related dilemmas such as GM foods (Maghari & Ardekani, 2011) and E-numbers (Haen, 2013), and may have led to consumers preferring to form their opinions and judgements based on naturalness, rather than trust.

The emergence of more recent science-led modern food technologies, such as *in-vitro* meat production, can be seen as "extending human control" over what is considered natural and resonate in a negative light for consumers (Rozin, 2005). Despite many of the companies involved in *in-vitro* meat or GM foods production stating these technologies to be more environmentally friendly and to support sustainable food supplies (Farms, 2021; Phillips, 2008), consumers who have a 'natural preference' may view these technologies as destructive to what is natural and, therefore, dismiss those involved in the food chain, such as scientists, who may be associated with unnatural or ultra-processed food manufacture (Rozin et al., 2004). In addition to this, such as in the case of food irradiation or GM foods, consumers may perceive new food technologies to have unknown consequences which cannot be understood or prevented without long-term testing and observation (Frewer et al., 2011). To counteract this fear of human intervention, consumers have developed more of an affinity towards natural food products which are perceived to be lower risk (Frewer et al., 2011; Rozin et al., 2004). For example, Siegrist and Sütterlin (2017) reported that, despite being informed of a greater risk of developing colon cancer with the consumption of traditional meat, consumers will often choose traditional meat products over cultured meat (i.e., *in-vitro* meat) products due to issues of trust in the information given.

Conversely, Bearth, Cousin, and Siegrist (2014) suggested that, when trust in food regulators and risk communicators are high, consumers don't equate natural with being unequivocally safe. In the same study, it was observed that consumers who perceived more benefits in the use of artificial additives in foods were reported as being more trusting in regulators and had less preference for natural additives. Due to a higher level of trust in food regulators, consumers are able to remove or lessen the doubt that there are sufficient processes in place to ensure the safety of their food, thereby reducing the reliance on the natural heuristic (Bearth et al., 2014).

Therefore, with these factors in mind, it is proposed:

H6:. *There is a direct and negative relationship between the level of trust in science and the perceived importance of naturalness.*

Given H3, this leads to the proposition;

H7:. *There is an indirect and negative relationship between trust in science*

and consumer willingness to try 3D-printed food applications, mediated through the importance of naturalness.

2.7. Trust versus mistrust: Impact of technology neophobia on willingness to try

Cox and Evans (2008), conceptualise NFTN as comprised of risk, lack of benefit and quality or health concerns. Trust is often a substitute for knowledge in the process and safety of food manufacturing (Siegrist and Cvetkovich, 2000) and, therefore, the degree of trust in scientists and other actors within the food chain to do their job to the highest standard is an important factor influencing the level of perceived risk and quality concerns (Siegrist, 2000) and, as a consequence, their level of NFTN. Studies have established that scepticism in formal institutions involving the food industry are linked to higher incidences of novel food technology neophobia (Dolgopolova, Teuber, & Bruschi, 2015, Huang, Bai, Zhang, & Gong, 2019, Siegrist et al., 2008a). This is also supported by findings that people who exhibited higher trust in institutions also tended to associate new technologies with increased benefits and less risks (Siegrist et al., 2008b). Furthermore, without the combination of perceived benefit and trust in the actors behind the manufacture of that food product, consumers are less likely to be willing to purchase (Frewer, Scholderer, & Lambert, 2003).

Based on the argument above, the following hypotheses are proposed:

H8:. *There is a direct and negative relationship between the level of trust in science and consumers' level of novel food technology neophobia.*

Drawing upon H2 it is proposed:

H9:. *There is an indirect and positive relationship between trust in science and consumer willingness to try 3D-printed food applications, mediated through novel food technology neophobia.*

2.8. Barriers to willingness to try: Novel food technology Neophobia and perceived personal relevance

Certain studies have shown that the purchase of novel food products by consumers can be perceived as a way of building social status (Barrena & Sánchez, 2013; Perrea, Krystallis, Engelgreen, & Chrysochou, 2017; Rogers, 2010), which is a key element of the consumer value model (Papista & Krystallis, 2012) and perceived benefit that enhances perceived personal relevance. Thus, while novelty has been linked directly to personal relevance, it is to be expected that higher levels of NFTN based on perceptions of risks or the view that the technology is unnecessary or perhaps raises quality concerns (Cox & Evans, 2008) would be anticipated to have a negative relationship on perceived personal relevance. NFTN can lead to dissonance or incongruence in terms of association with the consumer's lifestyle and what they perceive as useful or relevant (Giordano, Clodoveo, De Gennaro, & FilomenaCorbo, 2018).

Others involved in similar areas of study have also suggested that, where consumers believe there to be a lack of necessity for new food technologies, they also perceive novel food technologies as high risk with low/no clear benefit and are less likely to be willing to try the product in the first place (Napier, Tucker, Henry, & Whaley, 2004). Therefore, we argue that, without personal relevance, consumers will not be willing to try 3D-printed food applications.

With these points in mind, the authors would propose the following hypotheses:

H10. *There is a direct and negative relationship between novel food technology neophobia and the consumer's level of perceived personal relevance of 3D food printing applications.*

H11. *There is an indirect and negative relationship between novel food technology neophobia and consumers' willingness to try 3D-printed food applications mediated through perceived personal relevance.*

3. Methods

3.1. Data collection and sample

In total, 1045 individuals who identify as Irish and are long-term residents in the Republic of Ireland were recruited to participate in completing the online survey during November/December 2020. The survey was delivered online via the Qualtrics platform (QualtricsXM, Dublin, Ireland) to their consumer panel. Quality control protocols were in place to ensure the integrity of the data (e.g., avoidance of over-surveying participants and professional survey takers). Participants who are currently working/studying or have had previous experience in the fields of food production, home appliances, production or consumer or market research were omitted from the recruitment process in order to exclusively gather data from the average lay citizen. The age bracket of the sample cohort recruited ranged from 24 to 65 years of age; 49.8 % of the total sample were male (n = 520) and 50.25 % were female (n = 525). The overall sample was representative of adults in Ireland with respect to age and gender distribution when compared to Census 2016 (Central Statistics Office, 2016. Details of the recruited sample characteristics can be found in Table 1. Ethical approval was sought and granted in October 2020 by the Social Research Ethics Committee at University College Cork.

Table 1
Demographic profile of respondents.

	Number	Percentage (%)
Total Sample	1045	–
Gender		
Female	520	49.8
Male	525	50.2
Subtotal	1045	100
Age (Years)		
24–34	263	25.2
35–44	263	25.2
45–54	256	24.5
55–65	263	25.2
Subtotal	1045	100
Education		
Primary	3	0.3
Secondary	344	34.1
Bachelors	453	44.9
Masters	189	18.8
Doctoral	19	1.9
Subtotal	1008	100
Employment		
Full time	531	52
Part-Time	172	17
Unemployed	111	11
Student	23	2
Retired	65	6
Self-employed	63	6
Unable to work	48	5
Subtotal	1013	100
Marital status		
Single	236	23
Married or living with partner	738	71
Widowed	11	1
Separated/divorced	60	6
Subtotal	1045	100

3.2. Measurement model

To test the series of hypotheses established above, measures for the focal constructs were identified and selected by drawing on pre-existing and validated scales and items. Novel Food Technology Neophobia (NFTN) drew upon the work of Cox and Evans (2008), the importance of naturalness drew on multiple sources (Hansen, Ingerslev Sørensen, & Riewerts Eriksen, 2018; Squires, Juric, & Cornwell, 2001; Tobler, Visschers, & Siegrist, 2011). Trust in science drew on the work of Bak (2001), indicators for perceived personal relevance were sourced from Kang et al. (2013), while measures of consumer innovativeness were drawn from Goldsmith and Flynn (1992).

While the original NFTN scale (Cox & Evans, 2008) was originally constructed with four dimensions, one item was employed for information and media. As a single item cannot be tested for reliability or validity this dimension of NFTN was dropped from our conceptualisation. Tables 2 and 3 presents the specific indicators (items) used to measure each latent construct.

All latent variables in the model were conceptualised and measured as reflective variables. All independent variables were measured using a 1–7 point (strongly disagree- strongly agree) Likert scale. The dependent variable, willingness to try 3D-printed food applications (adapted from Bäckström, Pirttilä-Backman, and Tuorila (2004)), was measured using a 1–7 point (extremely unlikely to use – extremely likely to use) Likert scale.

Table 2
CFA, factor loadings and reliability for dependent and independent variables.

Constructs	*p*-Value	Standard Loading
Willingness to try various 3D food printing applications (α = 0.84; CR = 0.81; source: adapted from Bäckström et al., 2004)		
Snacks freshly 3D printed on demand from a vending machine in public spaces (e.g., cinema, shopping centres, airports, train stations etc.)	[a]	0.811
3D food printer in the gym/fitness centre	0.001	0.793
3D-printed meal at a restaurant	0.001	0.701
Consumer Innovativeness (α = 0.75; CR = 0.70; source: adapted from Goldsmith & Flynn, 1992)		
In general, I am the last in my circle of friends to know the latest food brand names and new food products	[a]	0.661
In general, I am among the last in my circle of friends to buy a new food product when it appears in the shops	0.001	0.802
Trust in Science (α = 0.80; CR = 0.72; source: adapted from Bak, 2001)		
Most scientists want to work on things that will make life better for the average person	[a]	0.601
Science and technology are making our lives easier and more comfortable	0.001	0.667
Science and technology are helping to improve our health	0.001	0.759
Importance of Naturalness (α = 0.82; CR = 0.76; source: adapted from Hansen et al., 2018; Squires et al., 2001; and Tobler et al., 2011)		
When I purchase foods, I make sure that these are natural	[a]	0.775
I seek to choose food products which are minimally processed	0.001	0.642
I avoid foods that contain artificial preservatives	0.001	0.732
Perceived Relevance (α = 0.94; CR = 0.91; source: adapted from Kang et al., 2013)		
The purchase and/or use of 3D-printed food products would let others see me as I ideally would like them to see me	[a]	0.820
The purchase and use of 3D-printed food products could help me attain the type of life I strive for	0.001	0.843
I believe that I could make connections or associations between the purchase and use of 3D-printed products and other experiences and/or behaviours in my life	0.001	0.762
I think that the purchase and use of 3D-printed food products would be of personal importance to me	0.001	0.842
The purchase and use of 3D-printed food products would help me to express who I am	0.001	0.847
Novel Food Technology Neophobia (α = 0.87; CR = 0.95; source: Cox & Evans, 2008)		
Risk	[a]	0.952
Lack of benefits	0.001	0.925
Quality concerns	0.001	0.913

[a] *set to 1 for estimation purposes.*

Table 3
Items for the dimensions of Novel Food Technology Neophobia.

Measures for the dimensions of Novel Food Technology Neophobia	
Risk	New food technologies are something I am uncertain about
	New food technologies may have long term negative environmental effects
	It can be risky to switch to new food technologies too quickly
Lack of Benefits	There are plenty of new tasty foods around so we do not need to use food technologies to produce more
	Society should not depend heavily on technologies to solve its food problems
	There is no sense trying out high-tech food products because the ones I eat are already good enough
Quality Concerns	New food products using new technologies are not healthier than foods created using traditional methods
	The benefits of new food technologies are often grossly overstated
	New food technologies decrease the natural quality of food

A maximum likelihood confirmatory factor analysis was carried out to test the measurement model using AMOS 25. As proposed by Podsakoff, MacKenzie, Lee, and Podsakoff (2003), a common methods latent factor was introduced to ensure that our measures were free of common methods bias.

Consumer innovativeness drew on items used by Goldsmith and Flynn (1992). Initially, it contained six items; however, all but two items were deleted due to low factor loadings. Regarding Novel Food Technology Neophobia (NFTN), the model draws upon the conceptualisation of Cox and Evans (2008). The original NFTN scale identified 13 items which loaded into four factors, one as single item factor which we dropped as outlined earlier. During the initial stage of data analysis, NFTN was identified as a second order construct with three underlying sub-dimensions, risk, lack of benefits and quality concerns (Cox & Evans, 2008). This configuration was integrated into our measurement model.

The CFA for our measurement model yielded the following results; Chi-square (χ^2) = 678.5, df = 256, p =.000, (χ^2)/df = 2.651, goodness of fit index (GFI) = 0.949, adjusted goodness of fit index (AGFI) = 0.936 comparative fit index (CFI) = 0.967, the Tucker-Lewis index (TLI) = 0.961, and the root mean square error of approximation (RMSEA) = 0.040, demonstrating good fit (Byrne, 2001). Individual items, standardised loadings, P-values and reliability values are provided in Table 3. Reliability is demonstrated through the use of both Cronbach's alpha scores and composite reliability (Table 3) with all constructs meeting the desired 0.70 threshold (Nunally, 1981).

All standardised factor loadings demonstrate statistical significance (P <.01) on their corresponding construct. With the exception of Trust in Science (AVE = 0.47), the average variance extracted (AVE) for each construct (Table 4) meets the 0.50 threshold (Hair, Anderson, Thatham, & Black, 1995). To justify the retention our measure of trust in science we draw on the works of Acquila-Natale and Iglesias-Pradas, 2020 and Huang et al. (2013b) who argue that AVE values higher than 0.4 are acceptable if composite reliability (Trust in Science CR = 0.72) is higher than 0.6 (Fornell & Larcker, 1981). Furthermore, Cheung and Wang (2017) suggested that the case for convergent validity is stronger when the standardized factor loadings of all items are not significantly less than 0.5. Thus convergent validity is supported for all our latent constructs.

Discriminant validity is supported by the fact that the average variance extracted exceeds the squared correlation coefficient for each pair of latent factors (Table 4; Fornell & Larcker, 1981). Consequently, the measures of the constructs used in the model achieve satisfactory

Table 4
Convergent and Discriminant Validity.

	Correlations in the Upper Diagonal, Variance Extracted in the Diagonal, and R 2 in the Lower Diagona Correlations in the Upper Diagonal, Variance Extracted in the Diagonal, and R 2 in the Lower Diagona Correlations in the Upper Diagonal, Variance Extracted in the Diagonal, and R^2 in the Lower Diagonal					
	Willingness to try	Trust in Science	Perceived relevance	Naturalness	Consumer Innovativeness	Neophobia
Willingness to try	**0.59**	0.24	0.71	−0.22	−0.02	−0.54
Trust in Science	0.06	**0.46**	0.18	−0.15	−0.03	−0.60
Perceived relevance	0.51	0.03	**0.68**	−0.15	−0.07	−0.47
Naturalness	0.05	0.02	0.02	**0.51**	−0.18	0.25
Consumer Innovativeness	0.00	0.00	0.00	0.03	**0.54**	−0.73
Neophobia	0.30	0.36	0.22	0.06	0.53	**0.87**

reliability, convergent and discriminant validity while accounting for common methods bias.

4. The structural model and results

4.1. The structural model

The structural model, including three covariates, age, gender and education, was tested and yielded the following fit results; Chi-square ($\chi 2$) = 1061.658, df = 320, p = 0.000, ($\chi 2$)/df = 3.318, goodness of fit index (GFI) = 0.933, adjusted goodness of fit index (AGFI) = 0.915, the comparative fit index (CFI) = 0.943, the Tucker-Lewis index (TLI) = 0.933, and finally the Root Mean Square Error of approximation (RMSEA) = 0.047. All met accepted thresholds (Byrne, 2001), enabling us to proceed to test our hypotheses and interpret our results.

A descriptive account of the data is presented in Table 5. Consistent with the findings of others (Eurobarometer, 2005; Fell, Wilkins, Kivinen, Austin, & Fernandez, 2009), it was observed that males were more likely to use novel food technologies (e.g., 3D food printing applications) than females, though it must be noted the overall scores for both genders were relatively low. In the age category, a negative relationship was seen where willingness to try declined as the age of consumers increased. In the employment category, students were more likely to be interested in trying 3D food printing applications than those who were retired or fully/partially employed. A Eurobarometer report (2005) also found that students and other young people were generally more likely to have positive perceptions of novel technologies, such as biotechnologies.

4.2. Results

The model explains 57.0% of the variation in our dependent variable, willingness to try 3D-printed food products in out-of-home situations. Tables 6 and 7 present substantial support for our hypotheses, with the exception of H4 and H5. The standardized estimates for our direct effects are provided in Table 6. The first feature to note is that perceived personal relevance is the dominant factor that influences the willingness to try 3D-printed food applications in a foodservice context. Its standardized coefficient is substantial and significant (H1 β = 0.614***) and considerably larger than the direct effect of NFTN (β = −0.167***) (Table 6). However, NFTN also has a significant negative effect on perceived personal relevance (*H*10 β = −0.329***). Thus, NFTN had both direct and indirect effects on willingness to try. To estimate this indirect effect, we followed the approach of Preacher and Hayes (2004) and Zhao, Lynch, and Chen (2010) and applied bootstrapped tests (5000 samples). This approach revealed that the indirect effect of NFTN, mediated through perceived personal relevance, is negative and significant (β = −0.202***) (Table 8) while the total effect

Table 5
Mean scale values (mean of items) for age, gender, education and occupation categories (Scale: 1, low; 7, high).

	Naturalness	Trust in Science	Novel Food Technology Neophobia	Perceived Relevance	Consumer Innovativeness	Willingness to try 3D food printing applications
Mean	5.2	5.4	4.7	3.2	4.1	3.6
Gender						
Female	5.3	5.3	4.8	3.0	4.2	3.4
Male	5.1	5.5	4.6	3.4	4.0	3.8
Age (Years)						
24–34	5.1	5.5	4.7	3.7	4.1	4.0
35–44	5.3	5.6	4.5	3.6	4.3	3.9
45–54	5.1	5.2	4.7	3.0	4.2	3.5
55–65	5.3	5.3	4.7	2.7	3.9	3.0
Education						
Primary	4.8	5.1	4.4	4.0	4.3	3.7
Secondary	4.9	5.2	4.7	3.0	4.0	3.4
Bachelors	5.3	5.5	4.6	3.3	4.2	3.7
Masters	5.5	5.7	4.6	3.4	4.1	3.7
Doctoral	5.4	5.8	4.8	4.0	3.7	4.2
Employment						
Full time	5.2	5.5	4.7	3.5	4.2	3.8
Part-Time	5.4	5.3	4.7	3.0	4.1	3.5
Unemployed	5.0	5.3	4.6	3.2	4.2	3.6
Student	5.1	5.3	4.5	3.7	4.1	4.3
Retired	5.3	5.5	4.8	2.5	3.7	2.7
Self-employed	5.2	5.2	4.8	2.9	4.1	3.1
Unable to work	4.7	5.4	4.6	2.6	3.6	3.2

Table 6
Bootstrapped standardised direct effects.

Direct Effects	Standardised β	SE (Bootstrapped)	P
(H1) Perceived Relevance to Willingness to try	0.614	0.037	***
(H2) Novel Food Technology Neophobia to Willingness to try	−0.167	0.056	***
(H3) Naturalness to Willingness to try	−0.053	0.036	*
(H4) Consumer Innovativeness to Willingness to try	−0.022	0.042	NS
(H5) Trust in Science to Willingness to try	−0.055	0.046	NS
(H6) Trust in Science to Naturalness	−0.137	0.065	**
(H8) Trust in Science to Novel Food Technology Neophobia	−0.445	0.072	***
(H10) Novel Food Technology Neophobia to Perceived Relevance	−0.329	0.040	***

*** 99%, ** 95%, * 90%

Table 7
Unstandardized indirect effects.

Indirect Effects (Bootstrapped sample = 5,000)	Unstandardised β	SE (Bootstrapped)	P
(H7) Trust in Science through Naturalness to Willingness to try	0.013	0.013	*
(H9) Trust in Science through Novel Food Technology Neophobia to Willingness to try	0.137	0.059	***
(H11) Novel Food Technology Neophobia through Perceived Relevance to Willingness to try	−0.337	0.047	***

*** 99%, ** 95%, * 90%

of NFTN on willingness to try is also found to be substantial (β = −0.369***).

On analysing the relationship between trust in science and willingness to try 3D-printed food applications, we find the direct effect to be insignificant (H5 β = −0.054 ns). AMOS 25 provides unstandardized (standardized unavailable) estimates for specified indirect paths where there is more than one mediator. Consequently, we were able to test the proposed indirect effect between trust in science and willingness to try, mediated through NFTN. Table 7 presents the unstandardized estimates of this indirect path and reveals an indirect and mediated effect between trust in science, *via* NFTN, to willingness to try that was positive and significant (H9 β = 0.137***). Consequently, trust in science, by reducing NFTN, increased willingness to try 3D-printed food applications.

Trust in science also has a direct and negative effect on the importance of naturalness (H6 β = −0.137***), while the importance of naturalness reduced willingness to try 3D-printed foods (H3 β = −0.053*) (Table 6). Returning to Table 7, the unstandardized estimates reveal the mediating effect of naturalness, where we find that the path was positive, but only significant at the 90% confidence level (H7 β = 0.014*).

Considering the direct and both mediated effects of trust in science and willingness to try 3D-printed food applications, we can see (Table 8) the standardized total effect (β = 0.226***) is positive and significant. Trust in science matters when it comes to the future demand for 3D-printed foods. However, there is no evidence to support a direct effect; instead, the effect of trust in science was operant through its effects on NFTN and the importance of naturalness.

The analysis proceeded to examine the relationship between our co-variates, age, gender and education, on willingness to try using the standardized total effect to factor in both direct and indirect effects operant through our other variables. The results shown in Table 9 indicate that both age and gender impact willingness to try. Older consumers are less willing try 3D food printing applications than younger consumers. Gender was also found to be related with males displaying a greater willingness to try 3D food printing applications than females..

5. Discussion and conclusion

While many technologies involved the preparation and provision of food in the foodservice market have been silent and unobserved by the consumer, the use of 3D-printed food applications will reveal significant changes in the ways in which our foods are processed. Thus, the need to address our key findings on the impact of NFTN need to be considered. The evidence shows the direct negative effect of NFTN on willingness to use. In many respects this is what can be expected. However, by separating out indirect effects, the current analysis clearly shows the way NFTN undermines an individual's perception of relevance of the 3D food printing technology to them personally.

Marketing strategists need to be aware of this indirect effect in their endeavours to reach their target audiences by highlighting the personalisation factor of 3D food printing in order to promote the relevance of the technology. Introducing 3D food printing applications into the ubiquitous food service sector allows consumers the freedom and autonomy to try 3D-printed foods with relatively low risk (versus purchasing the technology for home use) which may help to negate initial neophobic inclinations towards 3D-printed foods. Others have also shown that contexts where consumers are perceived to be in control of their exposure to hazards are more accepted than those where exposure is not voluntary (Siegrist, Hubner, & Hartmann, 2018).

Although 3D food printing technologies are still relatively unknown among consumers (Brunner et al., 2018), it is very important to gain a deeper understanding of the thought processes and behaviours contributing to potential barriers to acceptance before attempting to extend the use of the technology into the mainstream marketplace. Findings from this study point to multiple, and sometimes interconnected, influences on consumer intention to use. In line with existing literature (e.g. Greehy et al., 2013, Kang et al., 2013) perceived

Table 9
Total effects of gender, age and education on willingness to try.

Co-variates	Standardised Total Effect	Standardised SE	Two-tailed Sig.
Gender	0.182	0.033	***
Age	−0.307	0.033	***
Education	−0.037	0.035	NS

*** 99%, ** 95%, * 90%

Table 8
Breakdown of the Total Effects (Bootstrapped 5,000 samples).

	Direct Effect			Indirect Effects			Total Effect		
	Standardised β	Bootstrapped SE	P	Standardised β	Bootstrapped SE	P	Standardised β	Bootstrapped SE	P
Trust in Science to Willingness to try	0.054	0.046	NS	0.172	0.045	***	0.226	0.040	***
Novel Food Technology Neophobia to Willingness to try	−0.167	0.056	***	−0.202	0.025	***	−0.369	0.057	***

*** 99%, ** 95%, * 90%

relevance was identified as a significant determinant of willingness to try 3D-printed food applications. Thus, a clearer understanding of the consumer through the application of a needs-based segmentation approach in the design of food service products and implementation of marketing and communication strategies will be critical. This reaffirms the importance of holding a consumer-oriented mind-set in the design and development of new products/technologies. Enhancing consumer experience through designing and communicating salient benefits is essential to offset any potential risks that may be perceived. Indeed, Henchion et al. (2013) suggests that off-setting risk is dependent on technology application, benefits offered and consumer segment needs. Applications of 3D food printing will be required to communicate the many benefits that the technology offers from the delivery of personalised nutrition snacks and meals where the technology can be used to help individuals reach their own unique nutrition goals in a convenient way (Burke-Shyne, Gallegos, & Williams, 2020; Caulier et al., 2020) to its ability to support consumers' sustainability concerns through waste reduction.

Extending beyond immediate personal relevance, to be successful the technology will need to be compatible with existing and evolving values and social norms (Rogers, 2010). In socialising these technologies, marketers may tap into consumer-innovators' drive for stimulation, novelty-seeking and/or a need to express identity (Roehrich, 2004). With this in mind, it would be beneficial for potential 3D food printing applications within the food service sector to emphasize the customisability and personalisation features of the technology to appeal to the consumers' individuality so that they feel as though they can enjoy a unique user experience. For example, the technology facilitates the physical construction of foods in novel ways to appeal to the consumer. This presents operators in the food service market, for example restaurants, to construct unique food items themed around special occasions without incurring prohibitive costs. There are emerging examples where these initiatives are being implemented by chefs in high-end European restaurants who have 3D-printed meals of a theatrical nature with novel flavours and textural combinations to showcase the full capabilities of the technology (Ahmed, 2017; byFlow, 2018a, 2018b; Restaurant, 2016).

It interesting to note that the hypothesised role of consumer innovativeness in determining willingness to try was not supported. This is contrary to much of the existing literature which suggests that consumer innovativeness has a positive and direct effect on willingness to try/adopt. In this study we used a domain level innovativeness measure that focused on food. However, given the nature of the application, participants may not have perceived the resultant foods as innovative (e.g., snacks), and thus, rather than food innovativeness being important, it is their perspective of the technology that was key. The analysis highlights the significant direct and indirect influence of NFTN on willingness to try the technology. Equally while the hypothesised direct effect of trust in science on willingness to try was not supported the significance of trust in science lies in its diminishing effects on NFTN and naturalness. These indirect effects have interesting consequences at a higher level and point to the potential role for various stakeholders in improving the prospects and consumer openness to innovative applications of technology in food production.

At a more general level, the paper highlights how the negative effect of NFTN might be mitigated. The findings clearly show that trust in science has a substantial and significant depressing effect. While our complete model addresses the specific application of 3D printing, this particular finding may be generalised to other novel food technologies. Increasing confidence in scientists and science will have a positive impact in the consumer environment and the willingness to embrace new technologies. Thus our findings have broader implications for the nature and content of marketing communications by industry and even policy makers. This will require a more long-term approach and a renewed focus on how food science is portrayed in consumer markets.

In conclusion, the evidence suggests that to successfully introduce 3D food printing applications into the food service sector a multifaceted approach will be required. This work has identified some of the major obstacles facing the technology in the forms of NFTN and the importance of naturalness and has pointed to possible resolution in building continued support and trust for science and a focus on designing and delivering both products and accompanying marketing strategies that communicate and emphasise the personal benefits that this novel food technology affords.

6. Limitations and future work

3D food printers are considered a novel and disruptive technology (Lupton & Turner, 2016). With that said, we are in the midst of experiencing a massive growth of technological advancements at an unprecedented rate and, consequently, the evolution of 3D food printer technologies can be predicted to continually change and adapt according to future trends and demands. Therefore, the results of this study are contingent on the fact that 3D food printers are not currently at the time of writing considered a mainstream technology or commonly used by consumers. However, we must be mindful that, in the near future, the factors affecting Irish consumer acceptance of 3D food printers may change if the technology becomes more readily available and commonplace in consumers' lives, and this must be considered when approaching future studies.

As exclusively Irish consumers were included in this study, this could be considered a limitation as cultural and societal norms tend to differ in other European countries and elsewhere in the world, therefore, attitudes towards the use of 3D food printing technologies might differ depending on the consumer's country of residence. It would an interesting investigation to compare the attitudes of other consumers worldwide and determine differences, if any, in the level of acceptance of 3D food printing applications.

Another aspect that could be explored further is to determine the relative importance of the mix of potential benefits that might be more or less salient among consumer segments. The technology offers so many opportunities through customisation in terms of functional, emotional and self-expressive benefits that determine those that are most likely to resonate in different consumer environments (out of home vs in-home) merits consideration. For example, it has been suggested by Sun et al. (2015) that 3D food printers could be utilised as a way of reducing food waste and increasing the use of sustainable food sources by disguising less appetising, but sustainable food ingredients such as algae and fungi, in appealing 3D-printed shapes in order to increase consumption of foods that would normally go to waste or alternatively to transform less attractive but healthier foods into forms that are more likely to be consumed by resistant consumer segments such as children.

In addition to this, there are reports of the potential use of 3D food printers as a means of portion control for individuals, where snacks and meals are 3D-printed in portion sizes unique to that individual based on biometric data supplied to the printer software (Lupton & Turner, 2016; Sun et al., 2018). Therefore, it would be of value to include items relating to sustainability and the environment in a future study, especially in more recent times when actions against climate change have become increasingly more urgent and compelling (Bouman et al., 2020; Mariam, Valerie, Karin, Angelika, & Nina, 2020).

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CRediT authorship contribution statement

Megan M. Ross: Writing – original draft, Conceptualization, Investigation, Methodology, Formal analysis. **Alan M. Collins:** Formal analysis, Writing – review & editing. **Mary B. McCarthy:** Supervision,

Conceptualization, Methodology, Writing – review & editing. **Alan L. Kelly:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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