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Head of School: Professor Mairead Kiely

Supervisors: Dr. Maurice G. O'Sullivan and Professor J.P. Kerry

The optimization of plant protein meat replacers and clean label water binders in processed meat (white pudding and chicken).

THESIS

Presented by

Seán Garvey O'Driscoll B.Sc (Nutritional Science)

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Abstract

Reformulations and the development of bespoke vegetarian/vegan products, are becoming increasingly popular for a host of reasons, including health, environmental, economic and ethical concerns. Processed meat products have been under the spotlight for much of the recent past, with particular regard to their typically higher fat and salt contents and the health consequences of these, as well as the impact of meat production on the environment. Therefore, the interest in plant-based alternatives is continuing to grow. A sequential reduction of meat and animal fat with either chickpea or red lentil protein was performed in white pudding, with the overall goal of producing an acceptable 100% vegan product, or failing this, identifying the optimal replacement level that was acceptable to consumers and would not compromise on technological quality. The technological, compositional and sensory quality of the samples were analysed. Replacement was performed in 10% increments from 10% to 100%. Samples that contained more chickpea or red lentil protein than meat and animal fat (50% + replacement) were significantly ($P<0.05$) less acceptable from a sensory perspective, while they were also significantly ($P<0.05$) higher in protein content, lower fat content and lower in pH. Overall, a vegan sample was not a viable possibility under the parameters due to significant deterioration in sensory and technological quality and the optimum replacement level was identified at 20% for both proteins. Replacement was possible up to 40% for CP and 30% for RLP before quality started to deteriorate. Further optimisation of the formula and/or production method to achieve further replacement of the meat and animal fat.

Recent years have seen an increase in demand for products that are perceived to be more “natural”, organic, containing less additives and preservatives or by utilising clean label ingredients. Phosphates, a common water binding agent, are one such food ingredient that consumers may actively seek to avoid. Sodium triphosphate (STPP) was sequentially replaced (25%, 50%, 75%, 100%) with Aquamin soluble, citrus fibre and carrageenan in a brine intended for injection into chicken breast fillets. The effect of the replacement on the technological, sensory and microbiological quality of the cooked chicken (in the form of restructured chicken hams) was investigated. The overall objective was to produce a phosphate-free brine system utilising Aquamin soluble as well as any other ingredients deemed necessary. Replacement yielded significantly ($P<0.05$) more acidic brines and cooked samples alongside a significant increase in WHC. The sensory quality was unaffected by replacement, with no significant differences in overall acceptability between samples. Similarly, no significant improvements or deterioration in microbiological quality were identified, though the acidic nature of the 75% and 100%

replacement samples may have had a slight statistically nonsignificant antibacterial effect. Ultimately, the complete replacement of STPP yielded a cooked chicken sample that performed largely on par with one or two exceptions, most notably cook yield, in the quality parameters to the control. Further optimisation could be performed to address cook yield and protein solubilisation of the 100% replacement sample, as well as to attempt to produce a completely clean label brine, as carrageenan is not considered a clean label ingredient.

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Declaration

This is to certify that the work I, Seán Garvey O'Driscoll, am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property

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List of Abbreviations

ADI – Acceptable daily intake

APLSR – ANOVA partial least squares regression

APP – Appearance

ARO – Aroma

CDC – Centers for Disease Control and Prevention

CFU – Colony forming unit

CLON VEG – Clonakilty veggie pudding

CON – Control

CP – Chickpea protein

CVD – Cardiovascular disease

D – Detected

EC – E. coli

EFSA – European Food Safety Authority

FAO – Food and Agriculture Organisation of the United Nations

FAT – Fatness

FDA – U.S. Food and Drug Administration

FLA – Flavour

FSAI – Food Safety Authority of Ireland

GRA – Grains

HPP – High pressure processing

IARC – International Agency for Research on Cancer

JUI – Juiciness

KCl – Potassium chloride

LAB – Lactic acid bacteria

MAP – Modified atmosphere packaging

NaCl – Sodium chloride (May be used in place of “Salt”)

NACMCF – National Advisory Committee on Microbiological Criteria for Foods

ND – Not detected

O-A – Overall acceptability

O-F – Off flavour

RES/GEL – Residue/Gel

RLP – Red lentil protein

RPM – Revolutions per minute

SAL – Saltiness

SD – Standard deviation

SFA – Saturated fatty acids

SL – Salmonella

SPI – Spiciness

STPP – Sodium triphosphate

TA – Texture analyser

TC – Total viable count

TEX – Texture

TOU – Toughness

TPA – Texture profile analysis

TSPP – Tetrasodium pyrophosphate

TVP – Texturized vegetable protein

US – Ultrasound

WBC – Water binding capacity

WHC – Water holding capacity

WHO – World Health Organisation

WMP – Winter mushroom powder

Chapter 1.

Literature Review

Section 1:

What are meat analogues? The motivation to replace meat and fat, the replacement ingredients currently in use and the scope for reduction and or replacement in white pudding.

Section 2:

Introduction to phosphate use and potential phosphate replacement in brines.

Section 1:

1.1 What is white pudding?

White pudding is a processed meat product popular in Ireland and the United Kingdom and a staple component of the traditional "full Irish" breakfast. It is a food item that has been manufactured for hundreds of years using ingredients that historically would have been readily available to the producer. The main ingredients used are lean pork meat, pork fat, grains, onions, salt, and other seasonings. Manufacture is similar to that of the traditional breakfast sausage, whereby the ingredients are chopped into an emulsion which is then filled into artificial casings before cooking, these casings are removed before eating and white pudding is usually reheated (typically via shallow frying) before consumption. There are many white puddings on offer on the Irish market from a range of brands. As is the case with many processed meat products, white pudding tends to have a high salt and fat content. The majority of the white pudding products on sale in Ireland up to 2015 contained between 12-18% fat and an average sodium level of 867mg/100g (Susann, G. et al. 2015).

1.2 Consumer concerns: Health impact of processed meat consumption.

Processed meat products have been and continue to be the subject of much scrutiny particularly in recent years, owing to their impact on the health of the consumer, largely due to the high fat and salt content. Salt is an important ingredient in food processing for both sensory and technological reasons, however, processed meats are the main contributor to salt intake in Ireland, with intakes averaging approximately 10g/day in adults, which is almost double the World Health Organisation's (WHO) daily recommended intake of 5g/day (FSAI 2016), (WHO 2012). On the back of accumulating evidence linking high salt intakes to negative health outcomes, the FSAI agreed guidelines with Irish food producers to limit the sodium levels in white and black puddings to 600mg/100g (FSAI 2016). Negative health outcomes that have been associated with overconsumption of processed meat products (particularly processed red meat products) include obesity, cardiovascular disease (CVD), and various cancers to name but a few (Steinfeld, Gerber et al. 2006), (IARC 2015), (Godfray, Aveyard et al. 2018). As an example, a high intake of processed red meat overtime can lead to an increased risk of developing colorectal cancer (IARC 2014). Dietary salt intake is a major contributing factor to hypertension and overtime, CVD. This is a major health concern in Ireland where CVD accounts for approximately 41% of all death (FSAI 2016). Processed meats are generally high in salt, with consumption of processed meats reported to be the second largest contributor to total dietary sodium intake (Pretorius and Schönfeldt 2018). WHO member states have agreed to reduce the global population's intake of salt by 30% by 2025 (WHO 2018) as dietary sodium is a major contributing factor to hypertension and chronic

cardiovascular disease. However, salt and fat play important technological roles in meat food systems including contributing to flavour and texture as well as characteristics such as water binding which will affect the juiciness of a meat product, all these impact heavily on a consumer's decision to purchase meat and/or meat products. Processed meat products also tend to be high in fat and depending on the cut/product, saturated fat. Saturated fat and its impact on health is a controversial subject, with the traditional view being that high SFA intakes have negative health impacts, however, in recent years there is an emergence of evidence to suggest otherwise, rendering this subject a grey area. Nevertheless, going by the traditional evidence, SFA impacts on human health in terms of the contributing role it plays in the development and onset of CVD, weight gain, and obesity, and if the opposite is indeed true, SFAs remain a calorie dense nutrient which can contribute to over consumption of calories, weight-gain and all the problems related to overweight and obesity (Astrup, Magkos et al. 2020), (Kaur, Tallman et al. 2020), (Rozanski, Arnson et al. 2021), (He and Fernandez 1998).

1.3 Consumer concerns. Environmental concerns of meat consumption.

In recent years environmental concerns arising from high meat consumption have been increasingly raised. The world's population has grown dramatically in the last 50 years and with it has the demand for food. To meet this demand farming practices have had to be scaled up and intensified. Intensive livestock farming practices (intensive beef production in particular) has negative environmental effects both with regards the emission of greenhouse gasses and mass-scale deforestation in the Amazon rainforest and other areas to make way for pastureland and to grow the vast quantities of animal feed needed to fuel production (Nijdam, Rood et al. 2012), (Vale, Gibbs et al. 2019). As you move up the food-chain there is a progressive loss of energy (Sabaté and Soret 2014) with a huge amount of plant protein needed to produce meat, with approximately 6Kg of plant protein equating to 1Kg of meat protein on average. The actual quantity of feed required obviously will depend on the animal involved, for example 3.3Kg, 6.4Kg and 25Kg of feed is required to "finish" poultry, pork and beef respectively (Alexander, Brown et al. 2016). There has been a large push for reform and change to farming practices in recent years, to sustainable carbon neutral and more environmentally friendly techniques, however, it is not possible to meet the current levels of demand using said techniques exclusively (Godfray, Aveyard et al. 2018). Consumers are also becoming more conscious of the environment in which the meat they eat was produced. Studies have shown that people are more willing to eat meat that comes from a production environment with a reduced capacity for animal suffering, looking towards organic, grass-fed, and free-range farming practices to provide their meat, while also adopting a

more plant-based dietary approach (Bratnova, Loughnan et al. 2011), (Hartmann and Siegrist 2020).

1.4 Consumer concerns. Antibiotic resistance.

Antibiotic resistance is another concern held by many consumers and can be a factor that influences their food choices surrounding consumption of meat and animal products. Antibiotics are administered to livestock to treat sick animals and prevent disease in animals in a herd where other animals are already sick (FDA 2020). Antibiotics are also controversially used to stimulate growth and feed efficiency in livestock. There is legislation banning this practice in many countries, for example the European Union banned the use of antibiotics as growth agents on January 1st, 2006 (European Commission 2003). In the US this practice was seen as a major problem and they have now banned the use of certain drugs for this purpose as opposed to the outright banning of using antibiotics as growth agents (CDC 2021). In countries where certain aspects of food legislation aren't as strict the practice continues and in some instances is growing. For example, in Brazil, Russia, India, China and South Africa antibiotic usage in livestock farming is projected to double by 2030, fuelled by the increased demand for meat and animal products, particularly in middle-income countries (Van Boeckel, Brower et al. 2015). The concern is that antibiotic resistant bacteria will develop and then proliferate in livestock, which can then be transmitted to humans who come into direct contact with the animal (farm workers for example) or enters the food chain via meat, milk and so on. Either mode of transmission offers the antibiotic resistant bacteria a chance to spread amongst humans and cause infection (Marshall and Levy 2011). A concern which has only become more prevalent given the global landscape since 2020.

1.5 The growing popularity of vegetarianism/veganism

For the above reasons many people have begun to limit their meat consumption and, in some cases, adopt vegetarian or vegan diets. The growth of this behaviour is evident in Ireland where 4.3% and 4.1% of a sample population surveyed in 2018 claiming to be vegetarian and vegan respectively in 2018, up from 3.7% and 0.2% of a population surveyed in a separate study in 2003 (Bord Bia 2018) , (Kelleher, Nic Gabhainn et al. 2003). Despite the increase in vegetarian and vegan diets in Ireland, it has also been found that while Irish consumers place high importance on health and nutrition, many are still reluctant to compromise on taste, meaning that while some consumers may reduce their meat intake more are simply not willing to compromise by reducing intake (Alliance 2011). This behavioural approach tends to lean itself towards individuals adopting a "flexitarian" approach over time, whereby they consume a vegetarian diet for the most part but do not strictly adhere to these dietary parameters and continue to consume meat and animal

products from time to time. A flexitarian is defined as an individual who limits his or her meat intake yet still includes meat in his or her diet (Rosenfeld 2018). This has become quite common in the 21st century with an increasing number of omnivores across a range of countries who are decreasing their meat intakes, citing reasons similar to those cited by vegetarians (Ruby 2012).

1.6 Meat substitutes and meat extenders

The willingness to change or perhaps more likely the adaption to consumer preferences has also been seen on the part of the food producer. Meat processing, while necessary in improving shelf life, reducing meat waste and so on, invariably leads to increases in the amounts of fat, salt, nitrates, and other additives present in our food (de Barcellos, Grunert et al. 2011). The negative publicity in terms of health has caused food producers to reformulate their products to offer lower levels of salt, saturated fat, and other additives, and increasing health omega-3 fats in some cases (Verbeke, Pérez-Cueto et al. 2010), (Fellendorf, O'Sullivan et al. 2015). Recent years has also seen an increase in the amount of vegetarian and vegan meat products on supermarket shelves. Meat substitutes also known as alternatives or analogues, are primarily vegetable-based food products that contain protein from plant sources, soy, cereal, pulses, and fungi being the main sources, which mimic muscle meats and processed meat products (Hoek, Luning et al. 2011). Such products may be a relatively new addition to the Western diet; however, such foods have been consumed in Asia for thousands of years with tofu and tempeh the most well-known examples. The most significant challenge in producing meat substitutes is replicating the sensory and technological attributes of meat and meat products. Meat is highly valued in the diet due to its high protein and nutritional value as well as its unique sensory attributes namely texture and taste (Grunert, Bredahl et al. 2004). One method of overcoming this challenge is by using meat extenders whereby, meat reduction and not complete replacement is the aim. Meat extenders are texturized vegetable proteins which are extruded and then mixed with meat before further processing to improve the overall technological properties of the product while simultaneously reducing the meat content (Riaz 2004). Therefore, extenders are a method to reduce meat content as opposed to completely replace it and have been used in “stealth reduction” strategies whereby, meat is gradually replaced in products with extenders over time so as not to overly change the sensory attributes of the product. This method was found to be effective in sodium reduction in meat in the UK (He and MacGregor 2009). Of all the vegetable proteins in use in such practices, the legume soy is by far the most popular as it is an extremely high-quality protein, characterized by a favourable amino acid profile, which of all the vegetable proteins is the most comparable to the profile of meat (Asgar, Fazilah et al. 2010), (Thirumdas, Brnčić et al. 2018). The processing techniques involved in the production of TVP, namely extrusion and texturization have also been

shown to increase essential amino acid availability and improve the colour and flavour of the final product (Bohrer 2019).

1.7 Legume proteins as replacement ingredients

Legume proteins aside from soy are growing in popularity for use as meat substitutes and extenders, particularly lentil, chickpea, pea, and lupine protein (Kyriakopoulou, Dekkers et al. 2019). Hence the interest in chickpea and red lentil as protein sources in this study. Traditionally, due to their high protein quality, nutritive quality and antioxidant content, chickpeas and lentils have held an important position in cuisine in the Mediterranean, Middle East, Pakistan, and India, in dishes like curries and stews as well as processed foods like falafel (Mitchell, Lawrence et al. 2009). Much like other vegetable proteins particularly cereal proteins, lentils and chickpeas have adequate amino acid levels, but they are limiting in tryptophan and the sulphur-containing amino acids cystine and methionine (Iqbal, Khalil et al. 2006). While legume proteins don't have protein digestibility scores comparable to soy or animal proteins, they remain a good source of protein in their own right and chickpea and lentil protein have similar or in some cases superior functional properties to soy protein and even some animal proteins in certain cases, notably they have excellent oil and water absorption capabilities and their ability to form stable emulsions (Bohrer 2019), (Aydemir and Yemenicioglu 2013). These functional characteristics are highly desirable when manufacturing a meat substitute. It is worth noting that when optimising oil and/or water absorption is the goal, lentil and chickpea proteins are best used combined together and when optimising the nutritional profile is the goal these proteins are best used in tandem with another protein source be that plant or animal derived. With regards meat replacement and reduction in white and black pudding there have been a limited number of trials conducted, with the focus in these trials focused on fat and salt replacement as opposed to overall meat reduction and replacement. One study looked at varying fat and salt levels in white pudding and found that critical acceptable limits were achieved at 0.6% sodium and 5% fat independently of each other, however, salt and fat interact synergistically with regards flavour, so the only sample from this study that was acceptable to assessors was the 15% fat and 0.6% sodium pudding (Fellendorf, O'Sullivan et al. 2015). The same authors conducted a similar trial in black pudding using ingredient replacers with the objective of reducing fat and sodium content once again. Through the use of various alternative ingredients including KCl, glycine, seaweed, and waxy maize starch the authors concluded that fat and salt reduction beyond what was achieved in the previous study albeit in a slightly different food system (Fellendorf, O'Sullivan et al. 2017).

1.8 Cereal proteins as replacement ingredients

Cereal proteins from wheat, rice, oats, and barley are other options for use in meat substitutes and extenders. Compositionally cereals are much higher in carbohydrate and lower in protein when compared to soy, and cereal proteins are also lower in many amino acids than conventional protein sources, with lysine being a major limiting amino acid (Joye 2019), (Mota, Santos et al. 2014). Despite the fact that other protein sources would appear to have superior protein profiles over their cereal counterparts, cereal proteins have visco-elastic protein structures that bind together to create fibrous textures, something that is extremely desirable in the production of muscle meat substitutes (Bohrer 2019). Seitan is an example of a cereal-based meat substitute which is produced from the wheat protein gluten. A recent study found that burgers made from either 100% beef, 50:50 beef and seitan, or 100% seitan, were all largely accepted and liked equally, with assessors declaring their willingness to purchase all 3 burgers (Tarrega, Rizo et al. 2020). An important point to note about the use of gluten is its unsuitability for coeliac and gluten intolerant individuals.

1.9 Fungi as replacement ingredients

Different fungi species are an integral part of many vegetarian and vegan diets and can be an attractive option for those looking to replace meat in their diet as they have a distinctive umami flavour which gives them a meaty quality. However, fungi can also be processed to form meat substitutes. In the 1960s the filamentous fungi Fusarium venatum was discovered to be able to aerobically convert carbohydrate into protein. This process was refined and commercialised to produce a food-grade protein known as mycoprotein. Mycoprotein is comparable to animal derived proteins in terms of protein availability and digestibility and is often combined with other proteins such as egg albumin or plant proteins to maximise or optimise certain aspects of its functionality (Bohrer 2019). The discovery of mycoprotein in the 1960s ultimately led to the conception of “Quorn”, which is quite possibly the most well-known and well-accepted meat substitute available in Europe and increasingly in America. Quorn first became commercially available in 1985 and has since become well established on supermarket and consumers shelves alike. The mycoprotein in Quorn has added vitamins and minerals to improve the nutritional profile and is also mixed with egg albumin in the vegetarian product and vegetable protein in the vegan product, to act as binding agents. The product is then processed into various forms such as mince, chunks, and forms resembling muscle meats (Quorn 2020). Quorn is marketed as a healthy eco-friendly product and in the UK, Quorn production has a carbon footprint 5x smaller than that of beef production, which is an appealing statistic for eco-conscious consumers (Quorn 2019).

1.10 Seaweeds as replacement ingredients

Seaweeds are another plant ingredient that can be used in reformulating meat products; however, it is worth noting that reformulation is the key main role here and not replacement (i.e., fat/salt reduction as opposed to direct meat substitution). This is mainly due to the sensory qualities of seaweeds. There are 3 classes of seaweeds; brown, red, and green, with brown being the most commonly consumed (Lorenzo, Agregán et al. 2017). As mentioned, seaweeds tend to have intense flavour profiles, so they are rarely consumed on their own, however due to their unique technological characteristics they have been shown to be ideal as ingredients in product reformulations, such as the lowering of fat and/or salt, or as a method of increasing the dietary fibre or omega-3 fatty acid profile of foods, including processed meat products (Cofrades, Benedí et al. 2017). Research in the use of seaweeds in foods and meat products is a relatively new development that has only really began in the last decade or so. 4 species in particular; nori, wakame, kombu, and sea spaghetti are the variants that tend to garner the most research attention. These species have been found to be high in essential amino acids (particularly aspartic and glutamic acid, both of which are important contributors to the umami flavour) as well as exhibiting excellent oil and water holding capacities (Fernández-Segovia, Lerma-García et al. 2018). The main polysaccharides found in brown seaweeds are alginates, which in the food industry are used as food colloids to thicken, gel, emulsify and stabilise food products (Brown, Allsopp et al. 2014). A study investigating the potential enhancement of the phytochemical and fibre content of beef burgers with sea spaghetti (10-40% w/w) showed improvements in mouthfeel and texture following a decrease in cook loss and an increase in tenderness. The burgers were also well accepted after sensory analysis while also displaying increased antioxidant activity (Cox and Abu-Ghannam 2013). A recent meta-analysis on the potential use of seaweeds in foods concluded that seaweeds are valuable food sources with a high nutritional value and high in bioactive compounds which can elicit functional enhancement such as improvement in water holding capacity and so on, in foods. However, the authors also concluded that these benefits are currently more evident from an economic point of view as opposed to the consumer's indicating that the sensory attributes remain an issue, so more work has to be done to garner widespread consumer acceptance of seaweed fortified meat and other food products (Afonso, Catarino et al. 2019).

Section 2:

1.1 Phosphates overview

Phosphates are derivatives of phosphoric acid (H_3PO_4), with positively charged ions of elements like sodium to form salts (inorganic phosphates) or with organic groups to form esters (organic phosphates). Phosphates are constituents in phosphoproteins and membrane phospholipids as well as facilitating physiological, biochemical and cell signalling roles in most living organisms and are therefore a dietary requirement for humans (Dykes, Coorey et al. 2019). Organic phosphates, found typically in whole foods (meat, dairy, grains, etc.) and inorganic phosphates from food additives are absorbed in the small intestine, with inorganic phosphates more readily absorbed (Glorieux, Goemaere et al. 2017).

1.2 Why phosphates are used in meat processing

Phosphates are added to processed meat because of their impact on the functional properties of meat including their ability to shift and buffer pH, dissociate proteins, increase the WBC and their antibacterial properties (Trout and Schmidt 1983). Diphosphates, particularly tetrasodium pyrophosphate (TSPP) but also sodium triphosphate (STPP) and others, have the ability to dissociate the actomyosin complex of meat proteins as well as to chelate ions bound to these same proteins, which will ultimately lead to the increased solubilisation of actin and myosin (Puolanne and Halonen 2010). Phosphates will also increase the pH of meat thus increasing the ionic strength and moving the meat's pH away from the isoelectric point, which is approximately pH 5.5 in meat proteins (Dykes, Coorey et al. 2019). These effects on the functional properties of meats ultimately increase the water holding capacity (WHC) of the meat, which is crucial factor in the succulence of the meat product which has a major impact on the sensory liking and acceptance of the product. Phosphates will also improve the texture of the meat product, as actin and myosin are able to form more stable gel structures following solubilisation (Dykes, Coorey et al. 2019). Improvement in WHC is the main reason for phosphate addition in meats, but they are also used to stabilise colour, inhibit oxidation and for antibacterial properties (Nguyen H. B. S. L, Gál et al. 2011). STPP is commonly used in meat and poultry processing and like most phosphates it is combined with NaCl in brine solutions or product formulations, due to the positive effect of NaCl on the solubility of actin and myosin as well as the combined effect of phosphate and NaCl on the emulsion stability of meat products (Thangavelu, Kerry et al. 2019) (Lampila 2013). Certain phosphates are also used for their antimicrobial properties, for example, STPP is used in poultry processing, as it is known to aid in preventing Campylobacter and Salmonella from attaching to

the poultry surface, with these bacteria the leading cause of food poisoning arising from poultry meat consumption (Dykes, Coorey et al. 2019), (Rouger, Tresse et al. 2017).

1.3 Brines and brining techniques

There are limits and restrictions on the use of phosphates in foods. According to European legislation phosphates are not permitted in fresh meat and may only be added up to a maximum concentration of 0.5% in finished processed meat products (EFSA 2013). At international level the WHO and FAO lead the way in regulating food additives. Phosphate use is covered in the Codex Standard 192, with 30 different phosphate compounds approved for use in foods, with the maximum permitted level permitted in meat products at approximately 5041 mg/kg (FAO and WHO 1995). Phosphates are typically added to meat products via curing which is one of the oldest meat preservation techniques known to man, whereby the meat would be treated with, or packed in salt to prevent spoilage. Present day curing involves the addition of various treatments to the meat including sodium nitrite, salt, phosphate, and ascorbates, to name but a few, to the meat in a curing mixture, so as to impart the desired technological, sensory and microbiological effects to the end product. There are 3 main curing methods; dry, direct addition and brine curing or where the curing ingredients are dissolved in water and incorporated or injected directly into the meat (Shahidi, Samaranayaka et al. 2014). Brine curing injection is the most common method used as it ensures a uniform distribution of the brine as well as being a fast method of incorporation. Several injection techniques can be used however, multineedle injection is the most commonly used, often in tandem with tumbling, where the meat is tumbled or massaged at low speed to aid in drawing out actin and myosin as well as to enhance the penetration and distribution of the brine cure (Shahidi, Samaranayaka et al. 2014). Tumbling can also be used as a curing method in its own right, exclusive of injection. Due to the advances in refrigeration technology in the last century the reliance on brining as a preservation method has dwindled and it is now a process to increase WHC, yield, and improve texture and so on of the meat being cured. From the consumer's point of view tenderness and juiciness are considered to be the most important quality parameters in fresh meat and processed meat products (Xiong 2004). Water content is the most important determinant of these sensory parameters and is therefore added (injected or otherwise) to meat, however, to minimise water losses upon cooking, binding agents like salt and phosphates but also fibres, starches and so on, must be added to the brine. The myofibrillar proteins, actin and myosin are the key meat proteins associated with WHC (Lopez , Schilling et al. 2012). In meats, water is retained in the space between actin and myosin. Myosin is solubilised when the ionic strength is raised, which in turn raises the pH slightly, increasing the gap between the ultimate pH and the isoelectric point of the meat. This dissociation of the acto-myosin complex expands the gap

between actin and myosin allowing more water into this gap where it becomes trapped, increasing the moisture content and influencing the juiciness or succulence of the meat product. To date only phosphates in combination with sodium chloride have been found to be the most effective in their ability to exert this effect on the myofibrillar proteins (Parsons and Knight 1989), (Offer, Knight et al. 1989), (Feiner 2006), (Petracci, Rimini et al. 2013). For example in cod fillets it was found that salt in combination with phosphate was the best formulation at attaining certain attributes including yield and WHC, while the brining of the cod fillets heightened the intensity of favourable sensory attributes while simultaneously decreasing certain unwanted attributes (Esaiassen, Østli et al. 2004). Another meat system that is commonly treated with phosphate and salt is poultry meat. This is done to increase yields and to ensure that the meat is tender and juicy regardless of how the consumer prepares and cooks the product. Consumers tend to show higher acceptance and liking of chicken breast fillets that have been enhanced with salt and phosphate and in most instances are preferred over non-treated control chicken breasts with regards aroma, texture, flavour and overall acceptability (Saha, Lee et al. 2009), (Lopez , Schilling et al. 2012).

1.4 Potential health concerns

In recent years there appears to have been a shift in consumer perception surrounding phosphate, with clear evidence being the large drop in phosphate usage in Italy and France in the last two decades or so seemingly driven by consumer demands (Petracci, Rimini et al. 2013). This reflects the growing demand for clean label foods, whereby consumers actively seek out foods that are considered to be more “natural” or positioned to be “free-from” artificial ingredients/additives, or to be more “organic” (Maruyama, Streletskaya et al. 2020). It is worth noting that the trend seen in Italy and France is not a universal decrease in usage, for example the average North American consumes >2x more phosphate coming from food additives compared to in 1990, with an estimated half of the U.S. population consuming in excess of the recommended daily phosphate allowance (Dykes, Coorey et al. 2019), (Thangavelu, Kerry et al. 2019). The acceptable daily phosphate intake is 40mg/kg bodyweight per day for adults, however in general higher quantities are commonly consumed due to how ubiquitous the use of phosphates as additives is (Younes, Aquilina et al. 2019). While there is no doubting the consumer trend of consumers seeking to reduce their phosphate intake, the basis for doing so from a health point of view is compelling but not completely clear cut. Phosphates form insoluble salts with calcium, iron and other metal ions which may lower the absorption of these minerals in the gastrointestinal tract leading to potential mineral deficiencies and as a result, long term may increase the risk of certain bone diseases (Sherman and Mehta 2009). However, a review done in 2011 found results that contradict this previous study where it was concluded that phosphate has no effect on bone health

due to the ease in which the kidneys excrete phosphate in healthy adults (Long, Gál et al. 2011). However, one area where phosphates may have an adverse effect on human health is in individuals with chronic renal disease, particularly in patients receiving dialysis. Studies have found that a high phosphate intake increases the potential risk of chronic renal disease, and an association has been seen between high phosphate intakes and cardiovascular morbidity and mortality, while elevated serum phosphate levels (hyperphosphatemia) appears to be a strong predictor of mortality in advanced chronic renal disease (Uribarri 2009), (Ritz, Hahn et al. 2012). More recently the link between cardiovascular disease (CVD) and hyperphosphatemia has been documented further, although the understanding of the mechanism is unclear. Nevertheless, people with chronic renal disease with excessive phosphate intakes have an increased risk of mortality from CVD as their kidneys cannot regulate their phosphate levels and the increased levels of serum phosphate have been associated with vascular calcification (Dykes, Coorey et al. 2019), (Thangavelu, Kerry et al. 2019).

1.5: Phosphate alternatives: Citrus fibre

So while there is no doubting the functional properties that phosphates can exert on meat, consumers are demanding a shift away from phosphate use, with additives such as sodium citrate, fibres, and carrageenans all touted as potential phosphate replacers (Alvardo and McKee 2007). One review found a blend that contained citrus fibre to be a promising phosphate replacer with results including higher cook yields, better fat and moisture retention and reduced cook losses, when compared to un-treated controls (Casco, Veluz et al. 2013). The functional role of citrus fibre in meat processed meat products is well documented in the literature, particularly in sausage-style products (Fernández-Ginés, Fernández-López et al. 2003), (Tomaschunas, Zörb et al. 2013). Citrus fibre is a hydrocolloid that has been extensively studied and utilised both in research and in industry. However, citrus fibre has now begun to be investigated as a potential phosphate replacer, due to its functional abilities with regards water retention and therefore texture improvement (Han and Bertram 2017). A study in pork Bologna sausage replaced STPP at various levels in the formulation, with the authors concluding that within the parameters of they tested, citrus fibre has the potential to replace some but not all of the STPP in that particular meat product (Powell, Sebranek et al. 2019). Citrus fibre is typically obtained from orange pulp and possess a high internal surface area, WHC, and apparent viscosity, which is why it is functionally similar to phosphates. This functionality is due to the two dominant polysaccharides present in citrus fibre which are pectin and cellulose, which when hydrated form a gel-like structure enabling them to enhance water retention and texture in processed meat products (Lundberg, Pan et al. 2014).

1.6: Phosphate alternatives: Citrates and bicarbonates

Citrates and bicarbonates are two more ingredients that show potential as phosphate replacers. Citrates are used most commonly in poultry processing to improve water binding, with alkaline citrates used predominantly to achieve this effect via their influence on the pH of the meat, similar to phosphate's influence on pH (Petracci, Rimini et al. 2013), (Feiner 2006). Similarly bicarbonates have seen use as potential phosphate replacers, with results including the enhancement of yields in poultry and reduction in drip loss and improvements in texture and yield in pork (Sen, Naveena et al. 2005) (Bertram, Meyer et al. 2008), (Lee, Sharma et al. 2015). In fish sodium bicarbonate was used to produce lightly-salted cod fillets with a high WHC and improved sensory characteristics, ergo fulfilling the role of phosphate which is not permitted for use in such products by European legislation (Åsli and Mørkøre 2011). Alkaline bicarbonates affect the myofibrillar proteins of meat in the same fashion as phosphate by moving the pH away from the isoelectric point, increasing the electrostatic repulsive forces between actin and myosin, which causes expansion and allows water to be immobilized in that space (Petracci, Laghi et al. 2012). In certain cases this effect appears to be greater than that of phosphate, for example, in the study conducted by Sen and colleagues, it was found that NaCl with sodium bicarbonate in brine was superior to brine containing tetrasodium pyrophosphates when WHC, cook loss and sensory results were compared in chicken (Sen, Naveena et al. 2005).

1.7: Phosphate alternatives: Other hydrocolloids

Other hydrocolloids that have been purported as potential phosphate replacers, include various starches both native and modified, and carrageenan. Something to note here is that modified food starches are classified as food additives, so therefore they do not fulfil the mould of "clean label" ingredients. In processed meat products, particularly poultry, starches have typically been used to improve texture as they influence gelling behaviour, water retention and are bulking agents. These effects mean that some starches are used to offset the negative sensory characteristics associated with low-fat products (Petracci, Rimini et al. 2013). However, these same technological attributes that make starches good fat mimetics may also allow them to be utilised as functional alternatives to phosphates. In low-salt, phosphate-free frankfurters, modified tapioca starch improved the water and fat binding properties of the samples, while modified tapioca starch in combination with sodium citrate decreased cook-loss in samples with NaCl concentrations lower than 1.5%, giving rise to a potential phosphate-free low-salt final product (Ruusunen, Vainionpaa et al. 2003). The nature of the final product in this study is noteworthy as there is no synergism present between NaCl and phosphate to impact on the myofibrillar proteins and a salt reduction in general

will negatively affect the water and fat binding properties. Carrageenans, are hydrocolloids that are extracted from red seaweeds that possess the ability to form thermoreversible gels and are typically used in the meat industry as binders and stabilisers (Campo, Kawano et al. 2009). In cooked sliced meat products such as deli hams and chicken rolls, carrageenan is used to improve moisture retention, cook yields, slicing properties and to improve certain sensory attributes (Imeson 2000). As a phosphate replacer carrageenans (iota and kappa) show promise in combination with other replacers in various meat systems, although it remains to be seen whether a reduction in phosphate or complete phosphate replacement which can be ultimately achieved (Park, Choi et al. 2008), (Tabak, Abadi et al. 2019), (Schutte, Marais et al. 2021).

1.8: Phosphate alternatives: Fungi

A further ingredient that has been garnering interest as a replacement for phosphates are edible mushrooms particularly winter mushroom, with their meat-like texture, high dietary fibre content and easily digestible protein profile all attractive attributes (Pérez Montes, Rangel-Vargas et al. 2021). Winter mushroom powder (WMP) has been used as a natural alternative to phosphate additives in numerous studies, in emulsion style sausages it was found that WMP at 1% addition was the most optimum addition concentration to increase the pH of the sausage (away from the isoelectric point) while having no adverse effects on colour or sensory properties (Choe, Lee et al. 2018). However, a different study concluded that WMP could be used a nitrite replacer in ground ham but it was not effective as a complete replacement for phosphate (Jo, Lee et al. 2020). In processed poultry meats, sodium pyrophosphate was replaced with WMP in low-salt chicken sausages. The WMP increased the pH of the samples compared to the negative controls (untreated), which was comparable to the role that phosphate would play in the same food system, furthermore sample hardness decreased, which can be perceived as positive or negative, and an increase in resistance to lipid oxidation was observed alongside no negative effects on colour or sensory quality (Jo, Lee et al. 2018). While the results from these studies show promise for WMP as a phosphate replacer it is worth noting that within these studies the phosphate-containing samples were the best performers overall, notably in the sensory aspects of the trials.

1.9: Alternative processing techniques to potentially replace phosphate

Research into phosphate replacement is not exclusively focused on using ingredients as replacers, with alternative processing techniques such as ultrasound (US) and high-pressure processing (HPP) potential options, which are either employed alone or in combination with phosphate alternative ingredients. US is a non-thermal processing technology that uses sound energy, which could be used to improve the functionality of certain phosphate replacement ingredients as a pre-

treatment or to improve ingredient distribution within the meat matrix itself (Thangavelu, Kerry et al. 2019). A defined level (18min at 25kHz) of US treatment was found to be able to achieve up to 50% reduction in phosphate levels in a beef and pork meat emulsion (Pinton, Correa et al. 2019). While this study in isolation seems promising, more research is needed on the potential of US as a mechanism in a phosphate replacement or reduction strategy as results from various studies are contradictory with regards WHC, texture, sensory scores and oxidative stability as has already been outlined in a comprehensive review (Thangavelu, Kerry et al. 2019). HPP is another non-thermal processing technique that could potentially be utilised in a phosphate replacement or reduction strategy. HPP is a technology commonly used as preservation technique as it has the advantage of inactivating microorganisms and enzymes within foods without compromising the colour, flavour and nutritional quality of the food (Hoover, Metrick et al. 1989). Pork sausages subjected to HPP with the goal of phosphate reduction, were found to be acceptable at a minimum phosphate level of 0.25% without any significant changes in functionality i.e. WHC and binding (O'Flynn, Cruz-Romero et al. 2014). Briefly, HPP can modify the protein structure of meats, which will impact on the functional properties of these same proteins, notably gelling, emulsifying and foaming properties, as well as the solubilisation of myofibrillar proteins, something which is particularly important in any phosphate replacement or reduction strategy (Hayashi 1989), (Chapleau, Mangavel et al. 2003).

1.10: The challenge surrounding phosphate replacement

As has been outlined here any potential phosphate alternatives, be they ingredients (clean label or not), alternative processing techniques, or a combination of the two, must have technological functionality on par or at the very least sufficiently comparable to phosphates. This is where the biggest difficulty lies with replacing phosphates as functional additives in processed meat products. While certain studies have shown that many different ingredients can increase the water-binding potential and emulsification of the meat matrix in which they are deployed, protein solubilisation and muscle-binding remain a significant challenge in the absence or reduced presence of phosphates. This is due to the fact that to date, alternative ingredients have not been found to act on the acto-myosin complex in the same capacity as phosphates (Prabhu and Husak 2014). One key specific challenge relating to dissociation of the acto-myosin complex is the binding of meat pieces to create reformed products such as deli ham rolls, as the ability to form a sticky exudate without phosphate's impact on the acto-myosin complex has yet to be discovered, without significant compromise to other quality parameters. To date while potential has been found for many ingredients and alternative processing technologies, in most studies identified here the phosphate-containing samples were the highest scoring in terms of sensory quality and

other technological parameters. To summarise, the main hurdle to overcome with phosphate replacement is the replication of their multi-functionality, which is extremely difficult to achieve (Thangavelu, Kerry et al. 2019). However, as evident in the literature, some ingredients show promise and could be optimised for use as phosphate alternatives and the consumer demand for "clean label" ingredients will inevitably drive innovation from food producers, the potential remains for the development of phosphate-free or at the very least phosphate-reduced processed meat products, without any losses to sensory and nutritional quality

Chapter 2.

The sequential replacement of meat and fat in white pudding with red lentil and chickpea protein.

2.1 Abstract

White pudding is a type of meat product made from pork that is popular in Ireland and the United Kingdom. A sequential reduction of meat and fat with either chickpea protein (CP) or red lentil protein (RLP) was performed, with replacement of both the meat and fat component of the formulation in 10% increments from 0% (control) to 100% replacement. 21 white pudding formulations were produced in triplicate (3 batches), 10 CP, 10 RLP and 1 control per batch, and sensory acceptance, optimised descriptive profiling, compositional analysis and physiochemical analysis was performed on these 21 samples. Samples were compared to Clonakilty Veggie Pudding as a commercial control in the sensory analysis. Samples with higher meat and fat replacement (50%+) were significantly ($P<0.05$) less accepted and were significantly ($P<0.05$) higher in protein content and lower in fat content. 20% CP and 20% RLP samples were the most acceptable samples ($P<0.05$) while replacement was possible up to 40% CP and 30% RLP without any significant decline in sensory or physiochemical quality.

2.2 Materials and Methods

2.2.1 *Production method*

The following steps were performed for each treatment in triplicate for a total of 3 batches (control to 100% rep) per replacement protein – 6 batches total (3 CP, 3RLP). Formulations containing CP will be referred to as CP treatment group or X% Rep from here on, the same applies to RLP. Lean pork shoulder and pork back fat was minced through 10mm and 5mm plates respectively, then weighed accordingly for each treatment, vacuum packed and frozen until production. 80g Pearl barley was brought to the boil in 250ml of water and then reduced to a simmer for 30 minutes or until all the water was boiled off, the barley was then drained and left to stand for 5 minutes. Pork and fat were left to defrost in the chiller (4°C) overnight. Pork, replacement protein (both CP and RLP are produced by Artura Proteins and were sourced from Deltagen, Highbridge UK), waxy maize starch, k-carrageenan, seasoning, salt and three quarters of the water were added to the mixer (Stephan UMC 5 electronic, Stephan Food Service Equipment GmbH, Hameln, Germany) and chopped at 1500rpm for 22 seconds. Fat was added and chopped for a further 22 seconds. The remaining water was then added and the chopped at 1500rpm for 15 seconds. Oatmeal and onion were then added and chopped at 1500rpm for 15 seconds. Finally, the pearl barley and rusk were added to the batter and chopped at 1500rpm for 30 seconds.

The batter was then transferred to the piston filler (MAINCA, Mod EB 12/25, Barcelona, Spain) and was then filled into a long length of white polyamide casing. The batter was filled into individual pudding chubs within the casings and each individual chub was secured at both ends with metal clips, using a U-shaped polyclip device (Niedecker Beteiligungen GmbH, Frankfurt, Germany). The pudding chubs were then cut into individual chubs before being cooled for 1 hour at 4° C before cooking.

Puddings were cooked in a Zanussi convection oven (C. Batassi, Conegliano, Italy) using a 2-phase cooking method. The Puddings were cooked at 85°C using 100% steam, until they reached an internal temperature of 74°C as measured by an internal temperature probe. The puddings were then held at 85°C for 15 minutes. Following cooking the puddings were allowed to cool to room temperature and one pudding from each treatment was used for pH, TPA and cook-loss analysis, while the rest of the puddings were frozen until compositional and sensory analysis.

Replacement protein information as follows: CP – is a clean label, GMO free, protein, produced from Turkish chickpeas, containing no less than 45% total protein with a good amino acid profile and low in saturated fat. RLP – is a clean label, GMO free, protein, produced from Canadian red lentils, containing 55-60% protein, low in saturated fat and high in dietary fibre. Both proteins are produced by Atura Proteins and distributed by Deltagen UK who are both subsidiaries of Marigot Ltd.

Table 2.1: White pudding formulations. All values in g to make up a 2Kg batch. % Replacement refers to both replacement of pork and back fat. Replacement protein in the first column can refer to either CP or RLP depending on the treatment group.

	Control	**10% Rep**	**20% Rep**	**30% Rep**	**40% Rep**	**50% Rep**	**60% Rep**	**70% Rep**	**80% Rep**	**90% Rep**	**100% Rep**
Pork	700	630	560	490	420	350	280	210	140	70	0
Back fat	300	270	240	210	180	150	120	90	60	30	0
Rep. protein	0	10	80	150	220	290	360	430	500	570	640
Water	540	560	590	620	650	680	710	740	770	800	830
Seasoning	30	30	30	30	30	30	30	30	30	30	30
Salt	30	30	30	30	30	30	30	30	30	30	30
Onion	50	50	50	50	50	50	50	50	50	50	50
Oatmeal	220	220	220	220	220	220	220	220	220	220	220
Rusk	50	50	50	50	50	50	50	50	50	50	50
Barley	80	80	80	80	80	80	80	80	80	80	80
WMS	0	60	60	60	60	60	60	60	60	60	60
Carrageenan	0	10	10	10	10	10	10	10	10	10	10

Water content increases with replacement as CP and RLP are high water binders and therefore, required more water to produce a white pudding that was not objectionably dry. WMS and carrageenan are included to mimic fat in the replacement treatments, these were standardised across all the replacement treatments as so that any technological, functional and sensory effects are as a direct result of the replacement protein.

2.2.2 Reheating procedure

The puddings were reheated for both cook-loss analysis and sensory analysis. Typically, in the home white puddings are sliced and then cooked on a frying pan or under a grill. For the purpose of this study and to remove variables such as oil absorption, the reheating was standardized and made replicable by removing the puddings from their casings and slicing them into 1.2cm slices. The slices were placed on aluminium trays and reheated in the oven using 100°C dry heat for 7 minutes. The pudding slices were then flipped over and cooked for a further 7 minutes under the same conditions.

2.2.3 Protein analysis

Protein content of white pudding samples was determined in duplicate using a slightly modified Kjeldahl method (Suhre, Corrao et al. 1982). The digestion block (Foss Tecator™ Digestor, HillerØd, Denmark) was pre-heated to 410°C. 0.5g of sample was weighed into digestion tubes along with two "kjeltabs" 15ml of sulphuric acid and 10ml of hydrogen peroxide. Two blank tubes (containing no sample) were prepared in the same way. The tubes were placed in the pre-heated digestion block for approximately 45min until the solution became colourless (completely digested) and the tubes were then left under the fume hood to cool. 50ml of distilled water was added to each tube before the tubes were transferred to the distillation unit (Foss Kjeltec 2100, HillerØd, Denmark) along with a receiver flask which contained 50ml of boric acid and indicator (Bromocresol green and methyl red). Once the distillation was complete, the receiver flask solution was titrated with 0.1 N hydrochloric acid until the green colour reverted back to the original red colour. The protein content was calculated using a nitrogen conversion factor of 6.25.

% Protein = titre wt. – blank wt. x 0.0014 x 100 / sample wt. = %N x 6.25

2.2.4 Ash analysis

Approximately 5g of homogenized sample was weighed into pre-weighed crucibles and dried in an oven at 135°C for 2 hours and were then pre-ashed over a hotplate at 300°C for approximately one hour or until the samples had stopped smoking. This was done in duplicate for each sample.

The ash content of the duplicate pudding samples was determined using a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) as per the AOAC method (Kolar 1992). The samples were placed into the muffle furnace at 550°C until a grey ash was produced (approximately 5.5 hours). Samples were removed from the furnace using tongs and placed in a desiccator to cool. Once cooled the samples were weighed and the ash content was calculated using the following method.

%Ash = [(Crucible and Ash wt. – Crucible wt.) ÷ Sample wt.] × 100

2.2.5 Salt (NaCl) content

The salt content was determined in duplicate by titration using a silver nitrate (Kirk and Sawyer 1991). Silver nitrate (0.1 N $AgNo_3$) solution was standardised against 0.1% sodium chloride (NaCl) solution. Samples were ashed in a muffle furnace as per the ash analysis method. The ash was washed into a conical flask with 20ml distilled water. 2ml of indicator (potassium chromate and potassium dichromate) was then added to the conical flask. Standardised silver nitrate was used

to titrate the solution from a clear yellow colour to an opaque light orange colour and the titre level (ml) was recorded. A blank titration was conducted using 20ml distilled water.

$$\%Salt = V_1 - V_2 / M \times \text{Molarity of } AgNO_3 \times 5.844$$

Where:

V_1 = Titre for test sample (ml)

V_2 = Titre for blank (ml)

M = Mass of sample (g)

Molarity of $AgNO_3$ = 0.1M

2.2.6 Fat and moisture content

A SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany) was used to measure the fat and moisture content of the white pudding samples. Moisture content was measured as follows. The SMART Trac instrument was tared using two CEM measuring pads. Approximately 5g of sample was weighed onto one of the measuring pads and then thinly spread across the pad before the second measuring pad was placed on top and gently squeezed together. The sample-containing pads were placed on the scale within the SMART Trac instrument, the lid closed, and the moisture percentage was generated. Following moisture analysis, the sample-containing pads were removed from the SMART Trac instrument, placed on a sheet of CEM film, folded and then rolled up within the CEM film, inserted and compacted into a SMART Trac tube and transferred to the fat analysis component of the instrument which generated the fat percentage of each sample (Bostian, Fish et al. 1985).

A correction factor had to be calculated as this particular SMART Trac instrument was underestimating fat content. Standardised fat samples were obtained, and fat analysis was carried out using out using both the SMART Trac instrument and the Soxhlet method. The correction factor was calculated from the difference between the Soxhlet result and the SMART Trac result.

2.2.7 Texture analysis

TPA analysis is a double compression test used for determining the textural properties of foods and is able to quantify multiple textural parameters in one test. The test is designed to mimic the mouth's biting action.

A texture analyser (16 TA-XT2i, Stable Micro Systems, Godalming, UK) was used to perform TPA on the white pudding samples, with each sample analysed 5 times. The pudding samples were placed in a 4°C for 1 hour prior to analysis. The puddings were sliced, guided by a ruler, into 12mm thick discs. The texture analyser (TA) was calibrated for force and height before commencing the test. The samples were placed on the TA platform and compressed at 40% strain in two cycles with a 10 second interval, using a 100mm diameter cylindrical probe (SMSP/100 Compression plate) attached to a 30Kg load cell. The probe was set to 1.5 mm/s crosshead speed. A time-force plot was generated by the software for texture profile analysis. Hardness (N), fracturability, cohesiveness (dimensionless), chewiness (N), springiness (mm) parameters were measured.

2.2.8 Cook loss analysis

Cook loss was determined by weighing samples in triplicate before and after reheating. Samples were sliced into 1.2cm slices weighed and reheated as per the reheating procedure. The samples were removed from the oven and let stand for 30 minutes before re-weighing. Cook loss was determined as the difference between the cooked (reheated) and raw weight (before reheating) and expressed as a percentage of the raw weight:

% Cook loss = [(Raw weight – Cooked weight) / Raw weight] x 100

2.2.9 pH analysis

The method used was adopted from (Câmara, Okuro et al. 2020). The pH meter (Seven Easy pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) was calibrated before any measurements were taken. The probe from the pH meter was inserted into the sample at different points to measure the pH. The probe was rinsed with distilled water between measuring samples. Samples were measured in triplicate.

2.2.10 Sensory analysis

The sensory evaluation was conducted using a panel of untrained assessors (n=25) in the age range of 18-60. They were chosen on the basis that they consumed meat products regularly. The experiment was conducted in sensory panel booths which conform to the International Standards (ISO 1998). The sensory analysis was split over 4 separate sessions whereby, 7 reheated white pudding samples were presented randomly to the assessors. Each sample was labelled with a randomly generated 3-digit code. The control was presented to the assessors during each session and in one session a commercially available vegetarian pudding product (Clonakilty Veggie Pudding) was presented to the assessors to act as a commercial control. The assessors were asked to mark on a 10cm line scale their liking of appearance, liking of flavour, liking of texture, liking of

aroma, and the overall acceptability of the samples (Hedonics). The assessors were then asked to mark on 10cm line scales their perception of intensity of the following attributes: grains, fatness, spiciness, saltiness, juiciness, toughness, and off-flavour as described in the method of (Silva, Casemiro et al. 2014). Samples were presented in duplicate (Stone, Bleibaum et al. 2012). The assessors were also asked to fill out a short questionnaire pertaining to their age, occupation, how often they consumed white pudding, and whether they would be more or less inclined to purchase a white pudding product containing reduced meat and a vegan white pudding product respectively.

2.2.11 Statistical analysis

Statistical analysis was carried out using SPSS version 22 for Windows (SPSS, Chicago, Illinois, USA) and Unscrambler software version 10.5 (CAMO ASA, Trondheim, Norway). One-way ANOVA was used to analyse the technological and compositional data, Tukey's post-hoc test was used to perform multiple comparisons between the treatment group's means, with significance defined as ($P<0.05$).

Sensory analysis data was analysed using Unscrambler software. The x-matrix was designated as 0/1 for treatment and the y-matrix designated as sensory variables. To identify significance for the relationships determined in the quantitative APLSR, regression coefficients were analysed by jack-knifing which is based on cross-validation and stability plots (Martens and Martens 2001).

2.3 Results and discussion

2.3.1 Compositional analysis results

Compositional results for the chickpea protein (CP) and red lentil protein (RLP) treatment groups are presented in Table 2.2 and 2.3 respectively. In general, for both treatment groups as the pork and pork fat were replaced samples had lower fat and moisture contents and higher ash, salt and protein contents compared to the control. These results were significantly ($P<0.05$) different from the control beyond a certain replacement level for each group (30% for CP, 40% for RLP). The only compositional parameter that did not yield any significant results was the moisture contents of both groups. However, a linear decrease in moisture content can be seen in both the CP and RLP treatments which was an approximate 10% decrease from control to 100% replacement. This decrease, while not statistically significant is nonetheless relevant and has considerable implications for textural and sensory results as will be shown. The ash content of a food reflects the total mineral content of said food, which includes inorganic compounds such as sodium chloride, therefore, as presented in Tables 2.2 and 2.3, the ash content of the samples increased

in tandem with the salt content, suggesting that both the CP and RLP have higher salt contents than the lean pork shoulder and pork back fat used in the formulation, as the salt in the formulation remained consistent (Table 2.1), (Liu 2019), (Fellendorf, O'Sullivan et al. 2017). As was to be expected a decrease in fat content is seen in parallel with an increase in protein content. Meat analogue products are traditionally low in fat content, particularly in the absence of additional plant-based fats or oils (Bohrer 2019). The protein increase is also to be expected given that the protein content of the CP and RLP powders is greater than that of the lean pork shoulder and pork fat used in the control (data not shown). Further evidence of this is the 10% replacement treatment in both groups where the protein content actually decreased, though not significantly ($P>0.05$), below the levels seen in the control. This is because at the replacement level of 10% for both treatments, the meat and the fat has been removed but CP or RLP is not the main replacement ingredient in terms of absolute weight here, with higher levels of water, waxy-maize starch and carrageenan than CP or RLP, so it stands to reason that the protein content of the 10% treatments would drop, given there are less protein-rich ingredients present in the formulation at this treatment level. Fat, moisture, salt and protein all play important roles in the technological quality of a meat product, influencing various attributes, such as water holding capacity, emulsion stability and texture. In general, fat reduction in meat and meat products leads to decreased emulsion stability, decreased water holding capacity and therefore, increased levels of cook loss, all which culminate in a lower moisture content in the finished product (Álvarez and Barbut 2013), (Kumar, Kairam et al. 2015). These issues are relevant because as the replacement level increases, moisture content sees an overall approximate 10% decrease for both CP and RLP samples from control to 100% replacement (Table 2.2 and 2.3) and while these moisture results were not statistically significant, they are relevant as the samples got progressively drier as replacement increased, which impacted on the sensory and TPA results. The emulsion also became less stable and crumblier as protein replacement increased, with this effect being more pronounced in the RLP treatments at higher replacement levels. Indeed, red lentil protein extracts have been reported to possess inferior technological functionality than chickpea protein extracts in previous research (Aydemir and Yemenicioglu 2013). Despite the obvious decreases in fat and moisture content, cook-loss remained largely unaffected across all treatments and no significant increases or decreases were detected (Table 2.4 and 2.5). This is in contrast to previous studies in reformulating processed meat products which have shown higher cook loss and lower moisture values in lower fat formulations (Fellendorf, O'Sullivan et al. 2017), (Fellendorf, O'Sullivan et al. 2015), (Mittal and Barbut 1994). Cook loss has previously been defined as the loss of water and fat after protein denaturation and aggregation during cooking (Hayes, Stepanyan et al. 2011).

Therefore, the higher the cook loss, the drier the finished cooked product will be, which remains one of the main stumbling blocks in terms of meat analogue development today, with dryness a commonly reported drawback (Sha and Xiong 2020). Why the cook loss of the samples in this study appear unchanged despite the drop in moisture content and obvious dryness of the higher replacement samples is unclear. However, it may be related to the way in which the samples were cooked. The white pudding emulsion was filled into high barrier non-permeable casings and then cooked in steam until the requisite internal temperature was reached. This cooking method yielded no change in pudding weight as all the fat and moisture was contained within the casings. The cook loss values here were taken from sliced discs of sample that were cooked for 7mins per side at 100°C. This method was chosen to standardise the reheating process as typically white pudding products are fried in oil in a home setting, which creates variables like pan temperature and oil/fat absorption. However, this method did not yield results that are consistent with previous research. It may be possible that the moisture content of the samples is the actual reflection of cook loss in this study. The moisture content of meat products is also heavily influenced by protein type and pH. Actin and myosin, which are the main two myofibrillar proteins present in muscle tissue as a complex, can be dissociated by salt and other ingredients like phosphates, or by increasing the pH away from acto-myosin's isoelectric point. This dissociation expands the gap between the actin and myosin filaments, allowing more water into the space where it then becomes trapped thus increasing the moisture content (Lopez , Schilling et al. 2012), (Thangavelu, Kerry et al. 2019), (Parsons and Knight 1989), (Offer, Knight et al. 1989). Unlike actin and myosin present in muscle tissue, the molecular association and structural configuration of legume proteins do not impart the same water binding ability exhibited by the myofibrillar proteins of the muscle cell (Sha and Xiong 2020). Previous work in the area suggests that legume proteins in their native forms must undergo processing such as extrusion and/or HPP to improve their functional properties, similar to how soy protein is processed into texturized vegetable protein (TVP) (Anzani, Boukid et al. 2020), (Smetana, Larki et al. 2018).

Table 2.2: Compositional analysis, chickpea treatment group. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05).

	Fat Content %	Moisture Content %	Ash Content %	Salt Content %	Protein Content %
Control	12.56 ± 0.47 ^{h}	63.29 ± 2.63 ^{a}	2.71 ± 0.01 ^{a}	1.93 ± 0.01 ^{a}	9.36 ± 0.27 ^{ab}
10% Chickpea	11.64 ± 1.71 ^{gh}	63.61 ± 4.97 ^{a}	2.83 ± 0.04 ^{a}	2.02 ± 0.02 ^{a}	8.70 ± 0.18 ^{a}
20% Chickpea	10.92 ± 0.27 ^{fgh}	60.51 ± 2.02 ^{a}	3.02 ± 0.09 ^{ab}	2.02 ± 0.08 ^{a}	9.23 ± 0.24 ^{ab}
30% Chickpea	9.15 ± 0.73 ^{def}	61.44 ± 4.33 ^{a}	3.36 ± 0.09 ^{bc}	2.16 ± 0.02 ^{b}	10.86 ± 0.83 ^{bc}
40% Chickpea	9.54 ± 1.01 ^{fgh}	59.04 ± 3.09 ^{a}	3.68 ± 0.12 ^{cd}	2.17 ± 0.05 ^{bc}	11.35 ± 0.78 ^{cd}
50% Chickpea	7.93 ± 0.37 ^{cde}	58.03 ± 2.83 ^{a}	3.94 ± 0.16 ^{de}	2.19 ± 0.02 ^{bc}	12.49 ± 0.48 ^{cd}
60% Chickpea	8.35 ± 0.63 ^{cde}	55.28 ± 3.27 ^{a}	4.17 ± 0.11 ^{ef}	2.21 ± 0.02 ^{bcd}	13.15 ± 0.77 ^{d}
70% Chickpea	7.07 ± 1.15 ^{bcd}	56.53 ± 7.88 ^{a}	4.38 ± 0.17 ^{fg}	2.21 ± 0.04 ^{bcd}	15.59 ± 0.24 ^{e}
80% Chickpea	6.13 ± 0.09 ^{abc}	54.37 ± 4.95 ^{a}	4.63 ± 0.16 ^{gh}	2.25 ± 0.05 ^{bcd}	15.98 ± 0.94 ^{e}
90% Chickpea	5.08 ± 0.68 ^{ab}	56.16 ± 6.14 ^{a}	4.83 ± 0.17 ^{h}	2.27 ± 0.04 ^{cd}	16.87 ± 0.43 ^{e}
100% Chickpea	4.05 ± 0.32 ^{a}	54.10 ± 8.20 ^{a}	5.04 ± 0.25 ^{h}	2.31 ± 0.01 ^{d}	16.90 ± 1.06 ^{e}

Table 2.3: Compositional analysis, red lentil treatment group. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05).

	Fat Content %	Moisture Content %	Ash Content %	Salt Content %	Protein Content %
Control	12.56 ± 0.47 ^{g}	63.29 ± 2.63 ^{a}	2.71 ± 0.02 ^{a}	1.93 ± 0.01 ^{a}	9.36 ± 0.27 ^{ab}
10% Red Lentil	11.52 ± 0.58 ^{fg}	59.24 ± 0.88 ^{a}	2.84 ± 0.03 ^{a}	1.97 ± 0.03 ^{ab}	8.67 ± 0.42 ^{a}
20% Red Lentil	10.30 ± 0.06 ^{f}	59.77 ± 0.83 ^{a}	3.06 ± 0.06 ^{ab}	2.04 ± 0.04 ^{abc}	9.97 ± 0.09 ^{ab}
30% Red Lentil	8.47 ± 1.04 ^{e}	59.27 ± 2.19 ^{a}	3.32 ± 0.09 ^{ab}	2.05 ± 0.04 ^{abc}	10.95 ± 0.36 ^{bc}
40% Red Lentil	8.34 ± 0.93 ^{e}	57.55 ± 0.87 ^{a}	3.72 ± 0.06 ^{bc}	2.16 ± 0.01 ^{bcd}	12.22 ± 0.59 ^{cd}
50% Red Lentil	7.36 ± 0.17 ^{de}	56.86 ± 2.10 ^{a}	4.23 ± 0.26 ^{cd}	2.17 ± 0.02 ^{bcd}	13.96 ± 0.50 ^{de}
60% Red Lentil	6.39 ± 0.58 ^{cd}	54.20 ± 0.51 ^{a}	4.20 ± 0.14 ^{cd}	2.21 ± 0.07 ^{cd}	15.36 ± 0.81 ^{ef}
70% Red Lentil	4.99 ± 0.30 ^{bc}	55.75 ± 4.64 ^{a}	4.83 ± 0.40 ^{de}	2.27 ± 0.08 ^{d}	17.08 ± 0.19 ^{fg}
80% Red Lentil	4.05 ± 0.19 ^{b}	55.29 ± 4.09 ^{a}	4.87 ± 0.29 ^{de}	2.29 ± 0.07 ^{d}	18.58 ± 1.01 ^{gh}
90% Red Lentil	3.44 ± 0.40 ^{b}	53.47 ± 6.53 ^{a}	5.09 ± 0.38 ^{e}	2.29 ± 0.18 ^{d}	19.38 ± 1.15 ^{h}
100% Red Lentil	1.72 ± 0.47 ^{a}	53.00 ± 6.68 ^{a}	5.51 ± 0.63 ^{e}	2.31 ± 0.07 ^{d}	20.60 ± 1.27 ^{h}

2.3.2 Technological results

Technological results for CP and RLP treatments are presented in Tables 2.4 and 2.5 respectively with pH results shown in Tables 2.6 and 2.7. As previously mentioned, no significant results were found in the cook loss results of both groups. The pH of the samples in both groups decreased from 6.04 in the control, with all samples from 20-100% CP and RLP significantly ($P<0.05$) lower in pH than the control. The lowest recorded pH in both treatment groups was 5.07 ± 0.03 for CP and 5.01 ± 0.01 for RLP. Typically, fresh pork has a pH in the range 5.7-6.1, with this value dictated by breeding, slaughter conditions, processing, storage, and temperature, with this value nonetheless being lower than the reported pH of the CP and RLP powders (Kim, Kim et al. 2016), (Pearce, Rosenvold et al. 2011), (Atura 2021). Therefore, it was anticipated that as replacement increases the pH of the samples will decrease. This drop in pH has implications on the overall quality of the product, not only with regards texture and WHC via the mechanisms outlined earlier, but also on sensory acceptability. Texture profile analysis (TPA) results are shown in Table 2.4 and 2.5. The replacement of meat and fat with CP and RLP led to increases in hardness and chewiness and decreases in cohesiveness and springiness. Significant ($P<0.05$) results are seen across all treatments for both replacement proteins, however, hardness and chewiness results fluctuate up and down considerably, in no discernible pattern. This may be due to the nature of the emulsion where grains of barley and chopped pieces of onion are dispersed throughout the batter, however, it is not possible to ensure uniform even dispersal and therefore, some samples may contain more barley and onion than others and indeed, this variance could be present within individual samples, potentially influencing the hardness and chewiness. Temperature may have also been an influencing factor on these results, as while the sample temperature was controlled, the environment temperature was not and most likely fluctuated up and down depending on the testing time and day. The cohesiveness and springiness values indicate that as pork and fat were replaced with either CP or RLP the samples became less cohesive and less springy. Visually this was observed, as the higher replacement levels yielded samples that were softer and had a more paste-like consistency than their lower replacement level and control counterparts, which had firmer, tighter gel-like structures. These TPA results are in line with previous work conducted in black pudding, which found that samples with lower fat content to be significantly ($P<0.05$) lower in springiness and cohesiveness, as was seen here with the lower fat, higher CP/RLP replacement samples (Fellendorf, O'Sullivan et al. 2017). Colour values for CP and RLP samples are presented in Tables 2.6 and 2.7 respectively. Briefly, lightness values (L*) did not yield any significant results for either treatment group, however, both CP and RLP L* values trended downwards towards the darker end of the scale as replacement increased, with the 100% replacement samples in both

groups being visually the darkest of all the samples. The a* values for both CP and RLP groups trended upwards towards the red end of the scale, however, the only sample to be significantly redder ($P<0.05$) than the control was 100% CP with the RLP group not yielding any significant results. The b* values in both groups trended upwards towards the yellow end of the scale. 60-100% CP were all significantly ($P<0.05$) more yellow than the control as were 40-100% RLP. Thus, the replacement of meat and fat yielded a slightly darker more significantly yellow (visually more of an orange) sample than the control, which itself was a pale white/grey colour. These results are similar to findings in a previous study conducted on white pudding, which found that lower fat (and lower salt) samples to have a more intense ($P<0.05$) yellow colour (Fellendorf, O'Sullivan et al. 2015). In terms of sample colour impact on liking of appearance, assessors tended to significantly prefer the appearance of the lower replacement level samples, up to 40% for CP and 20% for RLP, however, there is more involved here than just colour, with visible fat particles and familiarity to conventional white pudding products more pronounced at the lower replacement levels.

Table 2.4: Technological analysis, chickpea treatment group. Mean values (±SD) in the same column bearing different superscripts are significantly different ($P<0.05$).

	Cook Loss %	**Hardness (N)**	**Cohesiveness**	**Chewiness (N)**	**Springiness (mm)**
Control	13.47 ± 0.99 ^{a}	54.05 ± 1.04 ^{a}	0.72 ± 0.04 ^{bc}	33.16 ± 2.83 ^{a}	0.85 ± 0.04 ^{d}
10% Chickpea	12.85 ± 3.72 ^{a}	67.60 ± 1.12 ^{a}	0.73 ± 0.02 ^{bc}	40.72 ± 1.33 ^{ab}	0.82 ± 0.01 ^{cd}
20% Chickpea	12.63 ± 2.93 ^{a}	120.30 ± 6.65 ^{de}	0.77 ± 0.01 ^{c}	81.07 ± 5.87 ^{c}	0.88 ± 0.02 ^{d}
30% Chickpea	13.14 ± 1.94 ^{a}	106.33 ± 4.89 ^{cd}	0.75 ± 0.01 ^{bc}	69.05 ± 3.42 ^{c}	0.87 ± 0.05 ^{d}
40% Chickpea	12.94 ± 3.09 ^{a}	132.28 ± 1.77 ^{e}	0.72 ± 0.03 ^{bc}	75.72 ± 1.60 ^{c}	0.80 ± 0.01 ^{bcd}
50% Chickpea	15.33 ± 3.97 ^{a}	111.49 ± 3.36 ^{cd}	0.61 ± 0.03 ^{a}	49.66 ± 1.57 ^{b}	0.74 ± 0.05 ^{abcd}
60% Chickpea	14.65 ± 1.52 ^{a}	62.66 ± 3.02 ^{a}	0.69 ± 0.01 ^{b}	28.79 ± 2.05 ^{a}	0.67 ± 0.02 ^{abc}
70% Chickpea	14.38 ± 1.86 ^{a}	99.46 ± 3.03 ^{c}	0.71 ± 0.02 ^{bc}	52.08 ± 4.95 ^{b}	0.75 ± 0.10 ^{abcd}
80% Chickpea	13.29 ± 1.22 ^{a}	81.94 ± 13.07 ^{b}	0.71 ± 0.05 ^{bc}	35.55 ± 9.14 ^{a}	0.61 ± 0.10 ^{a}
90% Chickpea	12.37 ± 2.09 ^{a}	66.33 ± 2.27 ^{a}	0.72 ± 0.01 ^{bc}	28.43 ± 1.93 ^{a}	0.59 ± 0.04 ^{a}
100% Chickpea	10.90 ± 1.09 ^{a}	119.87 ± 3.37 ^{de}	0.61 ± 0.01 ^{a}	48.66 ± 5.63 ^{b}	0.66 ± 0.05 ^{ab}

Table 2.5: Technological analysis, red lentil treatment group. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05).

	Cook Loss %	Hardness (N)	Cohesiveness	Chewiness (N)	Springiness (mm)
Control	13.47 ± 0.99 ^{a}	54.05 ± 1.04 ^{abc}	0.72 ± 0.04 ^{bc}	33.16 ± 2.83 ^{cd}	0.85 ± 0.04 ^{d}
10% Red Lentil	13.96 ± 2.93 ^{a}	66.26 ± 5.23 ^{cd}	0.76 ± 0.03 ^{c}	43.24 ± 1.95 ^{e}	0.87 ± 0.01 ^{d}
20% Red Lentil	15.39 ± 3.87 ^{a}	59.23 ± 3.20 ^{bcd}	0.59 ± 0.03 ^{a}	26.53 ± 2.98 ^{abc}	0.75 ± 0.01 ^{cd}
30% Red Lentil	13.99 ± 1.06 ^{a}	44.27 ± 1.30 ^{a}	0.57 ± 0.03 ^{a}	17.11 ± 2.16 ^{a}	0.67 ± 0.04 ^{abc}
40% Red Lentil	15.08 ± 2.57 ^{a}	48.55 ± 0.71 ^{ab}	0.61 ± 0.02 ^{ab}	20.77 ± 1.09 ^{ab}	0.70 ± 0.04 ^{bc}
50% Red Lentil	13.08 ± 1.60 ^{a}	51.63 ± 1.77 ^{ab}	0.60 ± 0.05 ^{ab}	19.51 ± 2.07 ^{a}	0.63 ± 0.03 ^{abc}
60% Red Lentil	15.36 ± 2.28 ^{a}	70.10 ± 1.75 ^{d}	0.67 ± 0.02 ^{abc}	30.08 ± 2.91 ^{bcd}	0.64 ± 0.07 ^{abc}
70% Red Lentil	14.69 ± 1.23 ^{a}	65.72 ± 2.45 ^{cd}	0.55 ± 0.04 ^{a}	23.16 ± 3.53 ^{ab}	0.63 ± 0.07 ^{abc}
80% Red Lentil	15.83 ± 2.53 ^{a}	60.91 ± 6.87 ^{bcd}	0.57 ± 0.06 ^{a}	20.32 ± 4.70 ^{a}	0.58 ± 0.05 ^{a}
90% Red Lentil	13.21 ± 0.96 ^{a}	105.31 ± 4.62 ^{e}	0.60 ± 0.05 ^{a}	38.09 ± 6.60 ^{de}	0.60 ± 0.04 ^{ab}
100% Red Lentil	13.94 ± 2.47 ^{a}	122.65 ± 10.41 ^{f}	0.67 ± 0.06 ^{abc}	55.85 ± 1.22 ^{f}	0.69 ± 1.01 ^{abc}

Table 2.6: CIELAB colour and pH results, chickpea treatment group. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05).

	L*	a*	b*	pH
Control	69.53 ± 2.25 ^{a}	1.31 ± 0.24 ^{ab}	16.81 ± 0.95 ^{ab}	6.04 ± 0.04 ^{e}
10% Chickpea	68.85 ± 3.07 ^{a}	1.20 ± 0.17 ^{a}	15.45 ± 0.52 ^{a}	5.97 ± 0.05 ^{e}
20% Chickpea	68.33 ± 3.04 ^{a}	1.37 ± 0.42 ^{abc}	17.87 ± 0.67 ^{abc}	5.77 ± 0.03 ^{d}
30% Chickpea	69.57 ± 2.67 ^{a}	1.54 ± 0.32 ^{abc}	19.10 ± 0.51 ^{abc}	5.67 ± 0.03 ^{cd}
40% Chickpea	68.74 ± 3.94 ^{a}	1.60 ± 0.51 ^{abc}	19.46 ± 0.90 ^{abc}	5.62 ± 0.01 ^{c}
50% Chickpea	68.75 ± 3.30 ^{a}	1.75 ± 0.53 ^{abc}	21.65 ± 1.55 ^{abc}	5.27 ± 0.05 ^{b}
60% Chickpea	66.21 ± 4.09 ^{a}	2.40 ± 0.49 ^{abc}	22.26 ± 1.57 ^{bc}	5.09 ± 0.02 ^{a}
70% Chickpea	66.44 ± 4.86 ^{a}	2.44 ± 0.62 ^{abc}	23.85 ± 4.14 ^{c}	5.12 ± 0.02 ^{a}
80% Chickpea	64.91 ± 2.96 ^{a}	2.49 ± 0.38 ^{bc}	23.57 ± 3.46 ^{bc}	5.07 ± 0.03 ^{a}
90% Chickpea	65.08 ± 4.24 ^{a}	2.41 ± 0.42 ^{abc}	24.14 ± 3.10 ^{c}	5.11 ± 0.02 ^{a}
100% Red Lentil	64.23 ± 3.29 ^{a}	2.59 ± 0.98 ^{a}	23.26 ± 1.68 ^{c}	5.10 ± 0.05 ^{a}

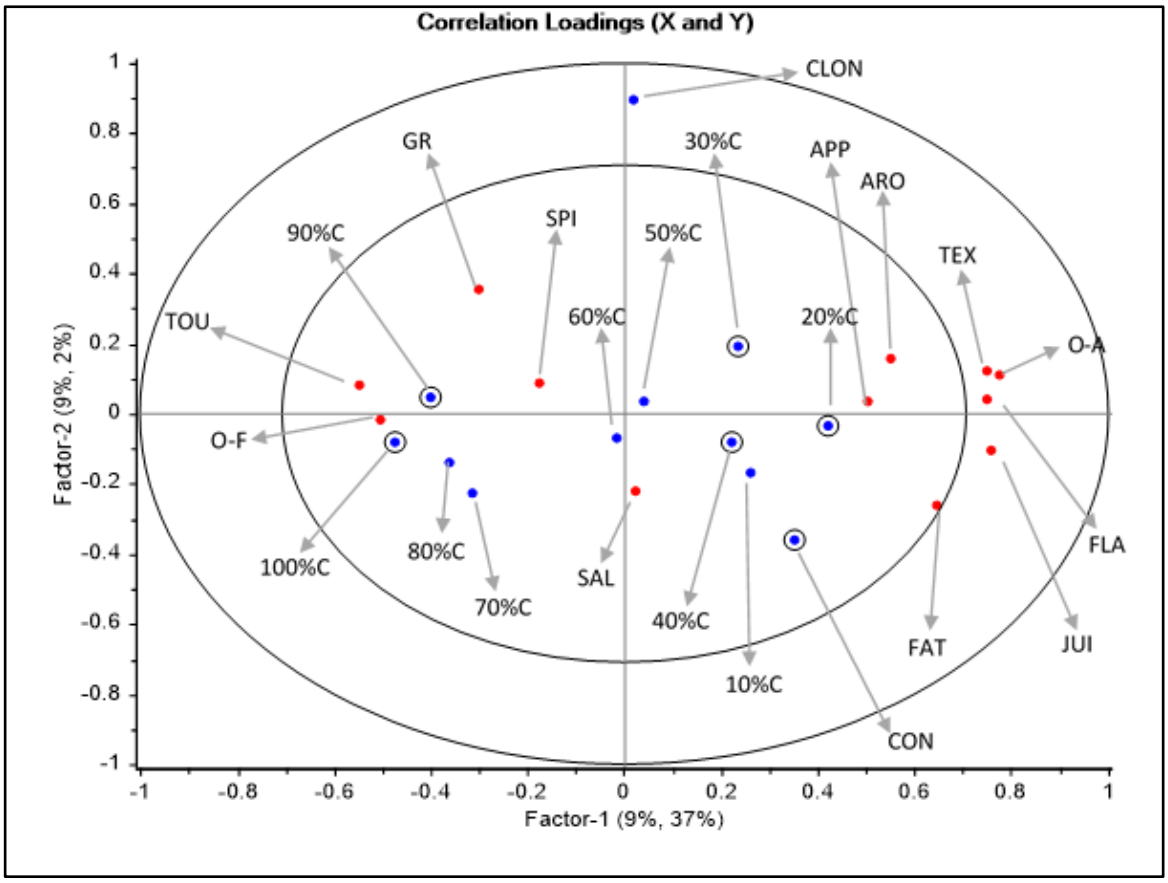
Table 2.7: CIELAB colour and pH results, red lentil treatment group. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05).

	L*	a*	b*	pH
Control	69.53 ± 2.25 [a]	1.31 ± 0.24 [a]	16.81 ± 0.95 [a]	6.04 ± 0.04 [d]
10% Red Lentil	69.70 ± 3.22 [a]	1.15 ± 0.51 [a]	16.56 ± 1.06 [a]	5.95 ± 0.04 [d]
20% Red Lentil	71.43 ± 2.76 [a]	0.88 ± 0.45 [a]	18.30 ± 1.24 [ab]	5.44 ± 0.06 [c]
30% Red Lentil	70.01 ± 5.12 [a]	1.37 ± 0.59 [a]	19.51 ± 1.19 [abc]	5.17 ± 0.04 [b]
40% Red Lentil	70.61 ± 5.56 [a]	1.43 ± 0.43 [a]	21.99 ± 1.99 [bc]	5.06 ± 0.04 [a]
50% Red Lentil	68.70 ± 4.58 [a]	2.05 ± 0.87 [a]	22.01 ± 1.08 [bc]	5.05 ± 0.03 [a]
60% Red Lentil	68.09 ± 3.94 [a]	2.33 ± 0.63 [a]	22.03 ± 1.39 [bc]	5.05 ± 0.01 [a]
70% Red Lentil	66.30 ± 5.78 [a]	2.99 ± 1.32 [a]	23.26 ± 1.53 [c]	5.03 ± 0.03 [a]
80% Red Lentil	66.28 ± 7.28 [a]	2.44 ± 1.11 [a]	23.69 ± 1.94 [c]	5.03 ± 0.01 [a]
90% Red Lentil	63.38 ± 6.58 [a]	2.64 ± 0.96 [a]	23.73 ± 2.15 [c]	5.02 ± 0.02 [a]
100% Red Lentil	64.23 ± 3.29 [a]	2.59 ± 0.98 [a]	23.26 ± 1.68 [c]	5.01 ± 0.01 [a]

2.3.3 Sensory analysis results

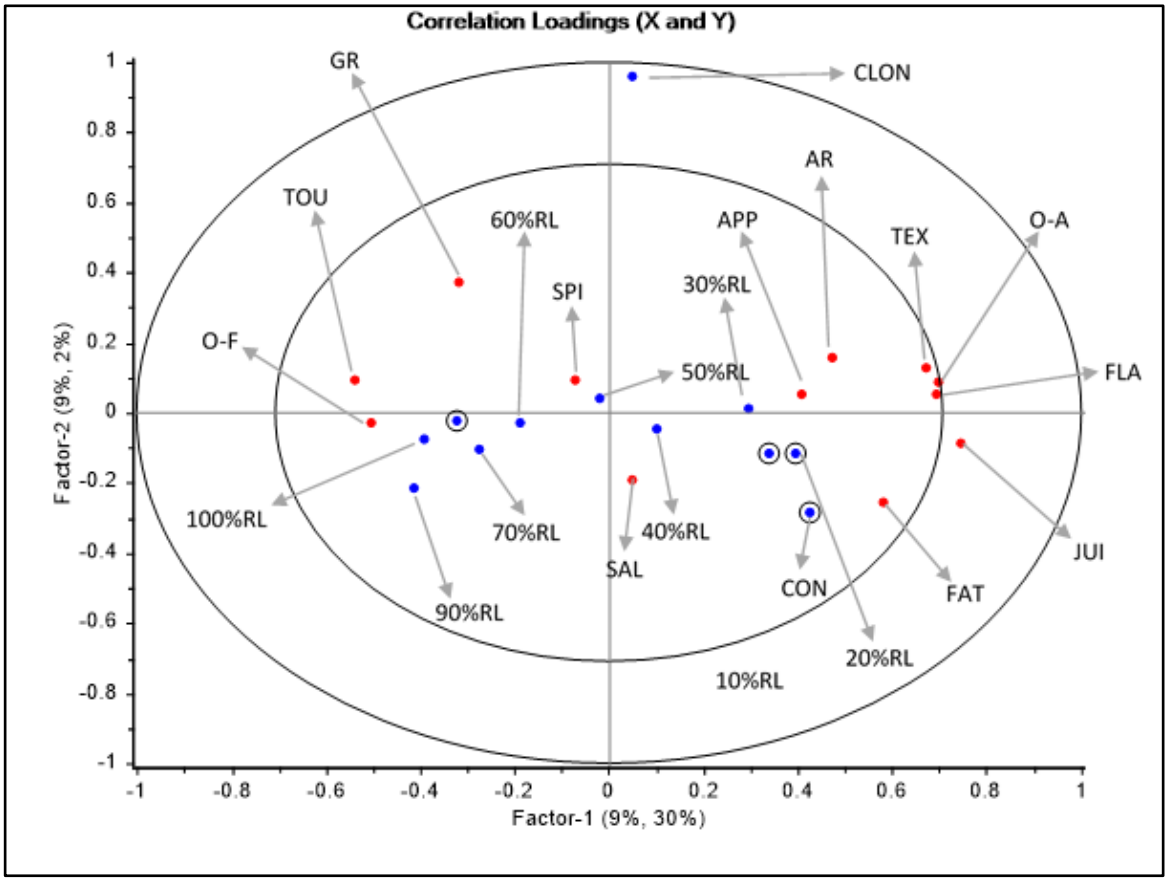
The sensory evaluation of the CP and RLP samples as well as the controls are presented in the APLSR plots in Figures 2.1 and 2.2, with the ANOVA values of regression coefficients shown in Table 2.8 and 2.9 for hedonic and sensory terms. The APLSR plots give an overview of the correlation between sensory attributes and samples. The x-axis of the plot is separated by the y-axis and a positive correlation between a sample and attribute is present when both a sample point and attribute point are located in close proximity and on the same side of the x-axis. A negative correlation exists if the opposite is the case. The corresponding ANOVA values for Figures 2.1 and 2.2 are presented in Table 2.8 and 2.9 respectively. A significant result exists if the P≤0.05 and the correlation can be negative or positive, as indicated by the algebraic signs. Significant positive and negative results are shaded in green and red respectively in Table 2.8 and 2.9.

Figure 2.1: APLSR graph for chickpea treatment group sensory results. Including control and commercially available control. Abbreviations: : **APP** = Appearance, **FLA** = Flavour, **TEX** = Texture, **ARO** = Aroma, **O-A** = Overall Acceptability, **GRA** = Grains, **FAT** = Fatness, **SPI** = Spiciness, **SAL** = Saltiness, **JUI** = Juiciness, **TOU** = Toughness, **O-F** = Off-Flavour, **CON** = Control, **CLON VEG** = Clonakilty Veggie Pudding **CP** = Chickpea protein, **RLP** = Red lentil protein.



From Figures 2.1 and 2.2 as shown in the upper right-hand quadrant, liking of appearance, aroma, texture, and flavour are strongly correlated with higher overall acceptability. Juiciness and fatness intensity, while not significantly correlated directly with overall acceptability as per Figures 2.1 and 2.2, were also attributes that scored high in samples that were well received and accepted. On the other hand, the intensity of spiciness, grains, toughness, and off-flavour were not well liked by assessors. The assessors appeared to be largely neutral in their in their liking or disliking of sample saltiness intensity. Spice and grains are two key components of white pudding products, yet here samples that were perceived to be more intense in spiciness and/or graininess did not yield samples with high overall acceptability. It is possible that the assessors mis-perceived the bitter, astringent flavour of the more acidic/lower pH (Tables 2.6 and 2.7) higher replacement samples as being more intensely spicy as well as he drier nature of these samples to be grainier, despite the fact that spice and grains remained consistent throughout the formulations.

Figure 2.2: APLSR graph for red lentil treatment group sensory results. Including control and commercially available control. Abbreviations: : **APP** = Appearance, **FLA** = Flavour, **TEX** = Texture, **ARO** = Aroma, **O-A** = Overall Acceptability, **GRA** = Grains, **FAT** = Fatness, **SPI** = Spiciness, **SAL** = Saltiness, **JUI** = Juiciness, **TOU** = Toughness, **O-F** = Off-Flavour, **CON** = Control, **CLON VEG** = Clonakilty Veggie Pudding **CP** = Chickpea protein, **RLP** = Red lentil protein.



However, it is also possible that the drier nature of these samples heightened the intensity of the already present grains. From Figure 2.1 it is clear that it is the lower replacement level samples that were most acceptable to the assessors, up to 40% for CP and from Figure 2.2, up to 30% for RLP. Toughness was the attribute that assessors disliked the most as is clear from Figures 2.1 and 2.2 and indeed, it is the samples that were significantly ($P<0.05$) higher in hardness values that correlated more with more intense toughness in the upper left-hand quadrants of Figures 2.1 and 2.2. Off-flavour was also an attribute that did not correlate with overall acceptability, with some assessors reporting the higher replacement >60% samples to be bitter, acidic and/or astringent, again this issue is most likely arising from the lower pH of the CP and RLP powders. From Table 2.8, the control and 10-40%C samples were all significantly positively ($P<0.05$) correlated with overall acceptability, as was the 100%C sample, however, this is almost certainly an anomaly given

this particular sample's place in the left-hand sector of Figure 2.1 and its other attributes not correlated with overall acceptability, illustrated in Table 2.8. Similarly, in Table 2.9 the 10-30% RL samples were all significantly positively (P<0.05) correlated with overall acceptability. In both groups the 60-100% treatments were significantly negatively (P<0.05) correlated with overall

Table 2.8: Beta coefficients, chickpea treatment group sensory results. Figures shaded in green and red are positive and negative significant correlations respectively [+/- (P<0.05)]. Abbreviations: : **APP** = Appearance, **FLA** = Flavour, **TEX** = Texture, **ARO** = Aroma, **O-A** = Overall Acceptability, **GRA** = Grains, **FAT** = Fatness, **SPI** = Spiciness, **SAL** = Saltiness, **JUI** = Juiciness, **TOU** = Toughness, **O-F** = Off-Flavour, **CON** = Control, **CLON VEG** = Clonakilty Veggie Pudding **CP** = Chickpea protein, **RLP** = Red lentil protein.

	APP	FLA	TEX	ARO	O-A	GRA	FAT	SPI	SAL	JUI	TOU	O-F
CON	0.0172	9.89E-06	3.12E-05	0.0023	1.65E-05	-4.51E-5	2.62E-06	-0.0498	0.0788	2.38E-07	-4.65E-06	-0.0001
10% C	0.0802	0.0001	0.0065	0.0134	0.001	-0.0157	0.0026	-0.1996	0.1369	0.0013	-0.1124	-0.0025
20% C	0.0001	2.38E-07	1.79E-06	6.68E-05	1.19E-06	-0.0134	2.44E-05	-0.0798	0.295	5.13E-06	-0.0005	-6.44E-6
30% C	0.0206	0.0026	0.0011	0.0292	0.0021	-0.9828	0.0081	-0.5137	-0.6068	0.0277	-0.1527	-0.0117
40% C	0.0168	0.0044	0.0019	0.1106	0.0034	-0.0707	0.003	-0.1038	0.6601	0.0041	-0.0219	-0.0102
50% C	0.8092	0.8045	0.2514	0.9295	0.4291	-0.9727	0.8965	-0.5994	-0.2011	0.5889	-0.1633	-0.7224
60% C	-0.2525	-0.5357	-0.6678	-0.859	-0.6915	-0.6367	0.7616	-0.8915	0.5285	-0.8172	0.4321	0.7838
70% C	-0.0792	-2.98E-06	-0.0002	-3.81E-06	-3.10E-06	0.7382	-0.0082	0.9218	0.4968	-3.58E-07	0.2794	0.0011
80% C	-0.2148	-1.14E-05	-3.65E-05	-3.61E-05	-1.91E-06	0.3203	-0.0341	0.6185	0.661	0	0.0014	0.0078
90% C	-0.0429	-3.10E-06	-1.19E-07	-0.0104	-1.19E-06	0.0571	-2.38E-07	0.225	-0.8727	-7.95E-05	0.0012	0.0028
100% C	-0.0003	-1.19E-07	0	-2.07E-05	0	0.0783	-1.59E-05	0.0829	0.1327	0	3.58E-07	0.0004
CLON	0.555	0.4284	0.0092	0.0191	0.0375	2.38E-07	-1.19E-07	0.2294	-0.0043	-0.1045	0.3651	-0.6859

acceptability, with the exception of 100% CP as already mentioned. In both groups samples that were significantly positively correlated with overall acceptability were generally correlated with significant positive liking of/intensity of appearance, flavour, texture, aroma, fattiness, and juiciness and significantly negatively correlated with intensity of graininess, toughness, and off-flavour. The inverse of these correlations was the case for samples that were significantly negatively correlated with overall acceptability. The Clonakilty Veggie Pudding, used here as a commercially available control, was also significantly positively (P<0.05) acceptable to assessors,

despite the fact that it possessed a significant positive correlation with graininess and significant negative correlations with fatness and saltiness, which was not the case for the other well accepted samples.

Table 2.9: Beta coefficients, red lentil treatment group sensory results. Figures shaded in green and red are positive and negative significant correlations respectively [+/- (P<0.05)]. Abbreviations: : **APP** = Appearance, **FLA** = Flavour, **TEX** = Texture, **ARO** = Aroma, **O-A** = Overall Acceptability, **GRA** = Grains, **FAT** = Fatness, **SPI** = Spiciness, **SAL** = Saltiness, **JUI** = Juiciness, **TOU** = Toughness, **O-F** = Off-Flavour, **CON** = Control, **CLON VEG** = Clonakilty Veggie Pudding **CP** = Chickpea protein, **RLP** = Red lentil protein.

	APP	FLA	TEX	ARO	O-A	GRA	FAT	SPI	SAL	JUI	TOU	O-F
CON	0.0011	5.01E-6	6.79E-6	0.0043	1.19E-6	-5.33E-5	9.54E-6	-0.0987	0.3074	7.63E-6	-5.96E-6	-0.0039
10% RL	0.0411	7.40E-5	0.0016	0.0222	0.0001	-0.0156	6.20E-6	-0.0592	0.7195	0.0003	-0.0105	-0.0193
20% RL	0.0067	2.36E-5	0.0001	0.0021	6.23E-5	-0.0006	5.23E-5	-0.8467	0.3404	7.15E-6	-0.0001	-0.0204
30% RL	0.1526	0.0006	0.0073	0.0758	0.0001	-0.1036	0.0711	-0.0311	0.2298	0.0011	-0.0003	-0.0140
40% RL	0.5188	0.2747	0.3402	0.4908	0.2458	-0.3008	0.3644	-0.5743	0.5863	0.1737	-0.1883	-0.4217
50% RL	-0.8385	-0.7906	-0.8592	-0.9390	-0.8450	0.6963	-0.5635	0.9275	-0.7578	-0.7099	0.7418	0.9968
60% RL	-0.3220	-0.0373	-0.0563	-0.2003	-0.0240	0.4593	-0.0913	0.9449	-0.8222	-0.0278	0.0753	0.5769
70% RL	-0.2145	-0.0062	-0.0128	-0.0591	-0.0003	0.5610	-0.0180	0.7338	0.9949	-0.0098	0.0199	0.4327
80% RL	-0.0319	-0.0008	-0.0008	-0.0074	-0.0005	0.1750	-0.0024	0.8992	-0.8548	-0.0032	0.0118	0.2249
90% RL	-0.0685	-9.30E-6	-0.0007	-0.0068	-4.77E-7	0.6970	-0.0194	0.8521	0.6773	-0.0002	0.0053	0.2270
100% RL	-0.4034	-0.0002	-0.0239	-0.1637	-2.26E-6	0.2303	-0.0002	0.8168	-0.6337	-0.0391	0.0297	0.0170
CLON VEG	0.5550	0.4284	0.0092	0.0191	0.0375	2.38E-7	-1.19E-7	0.2294	-0.0043	-0.1045	0.3651	-0.6859

The sensory results clearly show that samples that scored highly for juiciness and fattiness were those that were the most acceptable. Such a trend is well illustrated at this point with previous studies showing lower-fat meat sausages to be often rejected or considered less acceptable to their traditional fat level counterparts. Animal fat is a major contributor to the flavour, texture, juiciness and mouthfeel of meat products and the lack of that traditional meat flavour in meat analogues carried in said animal fat, that consumers have become so accustomed to, remains a drawback to greater acceptance of such products and replicating this flavour is one of the biggest challenges to overcome in the future progress and development of meat analogues (Tahmasebi, Labbafi et al. 2016), (Calkins and Hodgen 2007), (Graça, Godinho et al. 2019). Potential improvements in sensory quality and consumer acceptability could be achieved under the parameters of this study via use of fats and/or oils of plant origin in the formulation, such as

canola, avocado, coconut, sesame etc. to improve juiciness, mouthfeel, and texture at higher replacement levels. However, this would have to be tested as it is worth noting that many of the fat-soluble flavour volatiles present in meat products (aldehydes, hydrocarbons, alcohols) are deposited in animal fat and may not be replaceable or replicable in/by plant-sourced fats (Arshad, Sohaib et al. 2018). Another alternative to improve the sensory quality and mitigate issues arising in this study could be the use of starches, polysaccharides and/or hydrocolloids (in greater quantities than are present here see Table 2.1) to bind water and improve the juiciness and texture of the cooked product. For example, whole cowpea flour used as a substitute for ground beef at varying levels in frankfurter-type sausages significantly ($P<0.05$) increased moisture content and significantly reduced ($P<0.05$) cook loss, while hydrolysed collagen (not vegan) achieved a 50% fat reduction also in frankfurter-type sausages, both without affecting the sensory quality and acceptance of the sausages (Akwetey, Ellis et al. 2012), (Sousa, Fragoso et al. 2017). The saltiness intensity results are interesting as there was no significant increase or decrease in the perception of saltiness intensity for any sample from either group despite the fact that the salt content in both groups saw significant ($P<0.05$) increases. The most likely explanation for this, is that at all replacement levels the saltiness of the puddings is masked by other strongly flavoured ingredients mainly the seasoning, onion and the bitter, astringent flavours of the replacement proteins at the higher levels which itself is evidenced by the increase in off-flavour intensity as the levels of CP and RLP content increase. In terms of sample performance compared to the controls, the 20% replacement treatments in both the CP and RLP groups outperformed both the meat containing control and the vegetarian Clonakilty Veggie Pudding. The commercially available Clonakilty product was significantly ($P<0.05$) correlated with overall acceptability, however, some assessors deemed it to be too grainy and not what they would expect from a white/black pudding product. Indeed, the sample was correlated strongly with graininess intensity (Table 2.8). The sensory results suggest that if improvement can be made on the formulation used here to achieve a vegan product, this could lead to the creation of a 100% vegan or extremely low meat and fat white pudding product that would be at least on par with if not overtake an already commercially available product in terms of sensory acceptance.

2.4 Conclusion

Meat and fat replacement in white pudding with CP or RLP under the parameters of this study is possible up to a certain point, 40% for CP and 30% for RLP, without any significant decline in white pudding sensory or technological quality. Of all the samples tested here, 20% CP was the most acceptable to the assessors, followed by 20% RLP. The 100% vegan samples (100% CP and 100% RLP) were the worst performers for sensory acceptance as well as technological functionality. Fat

content, moisture content, pH and flavour are the major limiting factors in the use of these CP and RLP powders in their native states, to replace meat and fat at levels greater than 50% in white pudding. Both the CP and RLP treatment groups exhibited higher overall acceptance to a point (30% replacement) than the commercial control, however, from 50% replacement on the commercial control performed better in overall acceptability than both the CP and RLP treatment groups. Further research would be useful to determine whether CP and RLP can be use in creating 100% vegan meat analogues with the aid of additional plant-sourced fats and/or further processing techniques like extrusion, and whether these adjustments can improve the technological functionality and sensory acceptance of said 100% vegan meat analogues from the levels achieved in this study.

Chapter 3

Investigation of the technological, sensory and microbiological effects of a phosphate-free brine system containing Aquamin Soluble, citrus fibre and carrageenan, for injection in a processed poultry meat system.

3.1 Abstract

Sodium triphosphate was sequentially replaced (25%, 50%, 75%, 100%) with Aquamin soluble, citrus fibre and carrageenan in a brine system for injection into chicken breast fillets to produce restructured chicken hams and a phosphate-free brine. The effect of the phosphate replacement and the replacement agents on the technological, sensory and microbiological quality of the cooked restructured chicken hams was investigated. Phosphate replacement resulted in significantly ($P<0.05$) more acidic brines and more acidic cooked restructured chicken products and significantly ($P<0.05$) lower cook yields and sliceability of samples. Water holding capacity (WHC) was significantly ($P<0.05$) increased when the phosphate was replaced. The sensory quality of the products was not impacted by phosphate replacement and there were no significant differences in overall acceptability between samples with some assessors reporting not being able to differentiate between samples as they were so similar. The acidic nature of the replacement brines may have had a slight antibacterial effect however, these results were not statistically significant ($P>0.05$). Overall, the replacement of phosphate with Aquamin soluble, citrus fibre and carrageenan yielded cooked samples that were largely on par with the control samples (samples injected with just phosphate and salt) Further optimisation of the brine formulation should be performed in order to improve cook yield and protein solubilisation/gel stability of the 100% phosphate replaced sample.

3.2: Materials and methods

3.2.1: Experimental design

Preliminary trials were conducted (data not shown) to determine which ingredients and concentrations would be used for phosphate replacement in the brine formulations. Aquamin soluble, carrageenan and citrus fibre were selected as replacement ingredients on the basis of their performance in these trials, solubility and their performance in other studies found in the literature (citrus fibre and carrageenan only). Fresh chicken fillets were selected as the food system into which the brines would be injected as phosphate is commonly injected into poultry meats. A sequential replacement style trial (replacement in 25% increments) was selected to determine the effect of phosphate replacement on the chicken breast meat and the formation of restructured chicken hams. Fresh chicken breasts were obtained from Ballyburden Meats, Ballincollig, Co. Cork. These chicken breasts were divided into approximately 2Kg batches then vacuum packed and frozen until use in testing with the exception of shelf-life analysis where fresh chicken was obtained and cooked on the same day. Prior to injection the chicken breasts were allowed to defrost overnight in a 4°C chiller. The production of brine, injection of chicken breast

and cooking of same was repeated in triplicate for a total of 3 batches of 5 treatments (control, 25% replacement, 50% replacement, 75% replacement and 100% replacement) a total of 15 treatments were produced in total across the 3 batches.

Aquamin soluble was selected as one of the replacement ingredients despite its lower pH due to its solubility, other Aquamin products were tested in preliminary trials (most notably Aquamin F) but they were discounted as they were not soluble and therefore not suitable for brine production.

Table 3.1: Brine formulations for injection into approx. 2Kg chicken breast. Bracketed values are in g to make up 20,000g brine.

	H_2O %	**Salt %**	**Phosphate %**	**Aquamin %**	**Citrus Fiber %**	**Carrageenan %**
Control	94.5 (18,900)	3.5 (700)	2 (400)	0	0	0
25% Replacement	94.5 (18,900)	3.5 (700)	1.5 (300)	0.3 (60)	0.1 (20)	0.1 (20)
50% Replacement	94.5 (18,900)	3.5 (700)	1 (200)	0.6 (120)	0.2 (40)	0.2 (40)
75% Replacement	94.5 (18,900)	3.5 (700)	0.5 (100)	0.9 (180)	0.3 (60)	0.3 (60)
100% Replacement	94.5 (18,900)	3.5 (700)	0	1.2 (240)	0.4 (80)	0.4 (80)

3.2.2: Procedure for brine production

The water was weighed accurately into a large tub and the ingredients were weighed out as per the formulation. The phosphate was slowly added first as it takes time to dissolve (10 minutes). Next the carrageenan was added slowly and allowed to dissolve. A mixer (Silverson AXR, Waterside, Chesham Bucks. England) was used to vigorously agitate the solution at max speed. The salt was then slowly added at this stage. Any remaining ingredients (Aquamin soluble and citrus fibre) were slowly added and allowed to dissolve. The solution was then agitated for a further 5 minutes with the Silverson after all the ingredients had been added and dissolved.

Prior to injection a sample of the brine solution was removed in a vial for pH analysis.

3.2.3: Procedure for injection and tumbling

Defrosted chicken was drained of any drip loss then weighed and recorded. The chicken breasts were spread evenly on the conveyor belt of the injector (Inject Star BI18, Inject Star Maschinenbau

GmbH, Hagen-brunn, Austria) fitted with needles intended for use in poultry. The pump was inserted into the tub containing the brine solution and the injector was started. The chicken was passed through the injector flipped over and passed through a further 2 times. The chicken was left to briefly drain of any surface brine then reweighed and recorded. The injected chicken was then added to the mixer (Stephan UMC 5 electronic, Stephan Food Service Equipment GmbH, Hameln, Germany), which simulates an industrial scale tumbler. The paddle speed was set to 50 RPM and the chicken was tumbled for 30 minutes, checking for drying out of the chicken every couple of minutes. The weight of the chicken was accurately recorded following tumbling. The tumbled chicken was then vacuum packed into a cooking bag then tied in netting to help with forming the desired shape.

3.2.4: Cooking method

The chicken ham in its cook bag and netting was placed in the Zanussi convection oven (C. Batassi, Conegliano, Italy) and the temperature probe was inserted into the centre of the chicken ham. The oven was set to 85°C at 100% steam and the restructured chicken ham was cooked until it reached an internal temperature of 74°C (approximately 2.5-3hours). The chicken was then allowed to cool before being chilled overnight at 4°C. The chicken roll was then weighed and sliced the following day.

3.2.5: Brine uptake

Brine uptake by the chicken breasts was calculated using the following formula

[(Post Injection Wt. – Pre-Injection Wt.) ÷ Pre-Injection Wt.] x 100 = Uptake%

Prior to analysis samples were blended until homogeneity in a Buchi mixer, vacuum packed and chilled at 4°C until analysis.

3.2.6: pH analysis

The method used was adopted and modified from (Câmara, Okuro et al. 2020). 15g of cooked sample was blended with 30g of distilled water at 27-30°C until homogeneity is achieved. The pH meter (Seven Easy pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) was calibrated before any measurements were taken. The probe from the pH meter was inserted into the blended sample to measure the pH. The probe was rinsed with distilled water between measuring samples. Samples were measured in triplicate.

Brine samples were taken following brine makeup and pH was measured at room temperature using the same pH meter.

3.2.7: Colour analysis

Surface colour of the restructured chicken ham rolls were measured in triplicate using a Konica Minolta CR-400 Chroma-Meter (Minolta Camera Co., Osaka Japan). The Chroma-Meter featured a measuring head (CR-400) with an 8mm diameter measuring area, a 2° standard observer and a data processor (DP-400). Prior to testing, the Chroma-Meter was calibrated on the CIELAB colour space system using a white tile (Dc: L = 97.79, a =-0.11, b=2.69). The L* value represents lightness, a* represents redness and b* represents yellowness (ISO 1976). The measuring head of the colorimeter, which contains a xenon arc lamp, was applied to the sample surface 3 times for each triplicate sample giving a total of 9 readings per treatment. The light reflects from the sample surface and is sent to the microprocessor where the data was expressed in the tri-stimulus Lab values.

3.2.8: Cook yield

Cook yield was determined by dividing the cooked weight of the restructured chicken ham by the raw weight just before cooking, i.e. the post tumbling weight. Results were expressed as a %.

$$[\text{Cooked Wt. (g)} \div \text{Post Tumbling Wt. (g)}] \times 100 = \text{Cook Yield\%}$$

3.2.9: Slicing procedure

The chicken ham was set on the slicing machine with the blade set to 2mm. The chicken was then sliced 15 times, with the slices allowed to fall onto greaseproof paper. Intact slices were counted and recorded as was the overall sliceability of the chicken roll.

3.2.10: Water holding capacity analysis

Water holding capacity (WHC) was measured using a modified method of the one described by (Lianji M. and N. 1989). Approximately 10g of sample was wrapped in cheesecloth and placed in 30ml centrifuge tubes with cotton wool at the base of each tube. The samples were centrifuged (Beckman J2-21, Beckman Instruments Inc., CA, USA) at 5,000 rpm for 10min at 4°C. Following centrifugation, the cheesecloth was removed, and samples were reweighed. Measurements of the moisture content (M) of samples were carried out on the Smart Trac rapid moisture/fat analyser (CEM GmbH, Kamp-Lintfort, Germany) The percentage WHC was calculated using the following equation:

$$\% \text{ WHC} = [1 - [(B - A) / (B \times M)]] \times 100$$

Where B denotes the weight of the sample before centrifugation, A denotes the weight of the sample after centrifugation and M the % moisture of the sample as measured on the CEM.

3.2.11: Ash content

Approximately 5g of homogenized sample was weighed into pre-weighed crucibles and dried in an oven at 135°C for 2 hours and were then pre-ashed over a hotplate at 300°C for approximately one hour or until the samples had stopped smoking. This was done in duplicate for each sample.

The ash content of the duplicate chicken samples was determined using a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) per the AOAC method (Kolar 1992). The samples were placed into the muffle furnace at 550°C until a grey ash was produced (approximately 5.5 hours). Samples were removed from the furnace using tongs and placed in a desiccator to cool. Once cooled the samples were weighed and the ash content was calculated using the following method.

%Ash = [(Crucible and Ash wt. – Crucible wt.) ÷ Sample wt.] × 100

3.2.12: Salt (NaCl) content

The salt content was determined in duplicate by titration using a silver nitrate (Kirk and Sawyer 1991). Silver nitrate (0.1 N $AgNo_3$) solution was standardised against 0.1% sodium chloride (NaCl) solution. Samples were ashed in a muffle furnace as per the ash analysis method. The ash was washed into a conical flask with 20ml distilled water. 2ml of indicator (potassium chromate and potassium dichromate) was then added to the conical flask. Standardised silver nitrate was used to titrate the solution from a clear yellow colour to an opaque light orange colour and the titre level (ml) was recorded. A blank titration was conducted using 20ml distilled water. % Ash was then calculated using the following equation.

%Salt = V_1 - V_2 / M x Molarity of $AgNO_3$ x 5.844

Where:

V_1 = Titre for test sample (ml)

V_2 = Titre for blank (ml)

M = Mass of sample (g)

Molarity of $AgNO_3$ = 0.1M

3.2.13: Protein content

Protein content of the restructured chicken ham samples was determined in duplicate using a slightly modified Kjeldahl method (Suhre, Corrao et al. 1982). The digestion block (Foss Tecator™ Digestor, HillerØd, Denmark) was pre-heated to 410°C. 0.5g of sample was weighed into digestion

tubes along with two “kjeltabs” 15ml of sulphuric acid and 10ml of hydrogen peroxide. Two blank tubes (containing no sample) were prepared in the same way. The tubes were placed in the pre-heated digestion block for approximately 45min until the solution became colourless (completely digested) and the tubes were then left under the fume hood to cool. 50ml of distilled water was added to each tube before the tubes were transferred to the distillation unit (Foss Kjeltec 2100, HillerØd, Denmark) along with a receiver flask which contained 50ml of boric acid and indicator (Bromocresol green and methyl red). Once the distillation was complete, the receiver flask solution was titrated with 0.1 N hydrochloric acid until the green colour reverted back to the original red colour. The protein content was calculated using a nitrogen conversion factor of 6.25.

% Protein = titre wt. – blank wt. x 0.0014 x 100 / sample wt. = %N x 6.25

3.2.14: Fat and moisture content

A SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany) was used to measure the fat and moisture content of the samples. Moisture content was measured as follows. The SMART Trac instrument was tared using two CEM measuring pads. Approximately 5g of sample was weighed onto one of the measuring pads and then thinly spread across the pad before the second measuring pad was placed on top and gently squeezed together. The sample-containing pads were placed on the scale within the SMART Trac instrument, the lid closed, and the moisture percentage was generated. Following moisture analysis, the sample-containing pads were removed from the SMART Trac instrument, placed on a sheet of CEM film, folded and then rolled up within the CEM film, inserted and compacted into a SMART Trac tube and transferred to the fat analysis component of the instrument which generated the fat percentage of each sample (Bostian, Fish et al. 1985).

A correction factor had to be calculated as this SMART Trac instrument was undercalculating fat content. Standardised fat samples were obtained, and fat analysis was carried out using out using both the SMART Trac instrument and the Soxhlet method. The correction factor was calculated from the difference between the Soxhlet result and the SMART Trac result.

3.2.15: Sensory analysis

Sensory analysis was conducted in duplicate using a panel of untrained assessors (n=30). The sensory analysis was split over 2 separate sessions, with the panellists receiving the same treatments in both sessions (Stone, Bleibaum et al. 2012). Each sample was assigned a randomly generated 3-digit code, the samples were sliced and presented randomly to prevent first order and carryover effects, to the panellists on paper plates (Lynch, Macfie et al. 2007). Panellists were

presented with plastic cutlery for each sample and were also instructed to cleanse their palate between samples using the provided distilled water. The panellists were asked to quantify their assessment on 10cm line scales in the sensory questionnaire. This questionnaire was divided into 2 sections as follows: (1) hedonic, where liking of appearance, colour, aroma, flavour and texture were assessed and (2) intensity, where magnitude of residue/gel on the sample surface, saltiness, off-flavour and overall acceptability were assessed (Cheng, O'Sullivan et al. 2020). Panellists were reimbursed for their time with vouchers.

3.2.16: Packaging stability and microbiological analysis

Chicken breast fillets were obtained from Ballyburden meats (Ballincollig, Cork, Ireland) and were injected and cooked on the same day as delivery. On day 0 (Defined as the day after cooking) the cooled restructured chicken hams were sliced with the blade of the slicing machine set at 2mm and 50g of sliced sample was placed in polystyrene/ethylvinyl-alcohol/polyethylene trays (<1cm-^{3}/m^2/24h/at STP), (203 x 146 x 32mm), (Bachmann Forming AG, CH-6280, Hochdorf, Switzerland) and using modified atmosphere packaging (MAP) technology trays were flushed with 70% N_2 : 30% CO_2. Gas flushing was performed using a vacuum-sealing unit (VS 100, Gustav Müller and Co. KG, Bad Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH and Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3cm^3/m^2/24h/at STP) laminated barrier film with a polyolefin heat sealable layer and subsequently stored for up to 42 days at 4°C.

The gas atmosphere (%O_2 and %CO_2) in the packages was checked using a gas analyser (PBI Dansensor A/S, DK-4100, Ringsted, Denmark) where the instrument needle was inserted through a rubber septum attached to the polyolefin seal layer.

Microbiological analysis of the packaged sliced samples was carried out on days 0, 7, 14, 21, 28, 35 and 42 of storage. On analysis days 10g of sample was transferred into a stomacher bag, diluted with 90ml of sterilised maximum recovery diluent (Oxoid, Basingstoke, England) and stomached for 3 minutes (Steward Stomacher 400 Lab Blender, London UK) resulting in a 10^1 dilution used for analysis. Serial dilutions were prepared and 1ml aliquots from each dilution were plated onto Compact Dry TC (total count) and Compact Dry EC (E. coli) plates (Nissui Pharmaceutical Co., Ltd., Japan). The plates were incubated at 37°C for 48hrs (TC) and 34hrs (EC) to determine mesophilic counts. Results were expressed as $\log_{10}$ CFU (colony forming units)/g sample. On days 0, 14, 28 and 42, 25g of sample was transferred into a stomacher bags, diluted with 225ml of sterilised buffered peptone water and stomached for 1 minute. The bags were then incubated at 37°C for 24hrs. following incubation, 0.1ml of each diluted sample and 1ml of sterilised distilled water were

plated onto Compact Dry SL (salmonella) plates (Nissui Pharmaceutical Co., Ltd., Japan) and then incubated at 44°C for 24hrs. As this was a confirmatory test, results were presented as detected (D) or not detected (ND).

3.2.17: Statistical analysis

Statistical analysis was carried out using SPSS version 22 for Windows (SPSS, Chicago, Illinois, USA) and Unscrambler software version 10.5 (CAMO ASA, Trondheim, Norway). One-way ANOVA was used to analyse the technological and compositional data, Tukey's post-hoc test was used to perform multiple comparisons between the treatment group's means, with significance defined as ($P<0.05$).

Sensory analysis data was analysed using Unscrambler software. The x-matrix was designated as 0/1 for treatment and the y-matrix designated as sensory variables. To identify significance for the relationships determined in the quantitative APLSR, regression coefficients were analysed by jack-knifing which is based on cross-validation and stability plots (Martens and Martens 2001).

3.3: Results and discussion

3.3.1: Compositional results

Table 3.2: Compositional results. Mean values (±SD) in the same column bearing different superscripts are significantly different ($P<0.05$). Rep = Replacement.

	Ash %	Salt %	Fat %	Moisture %	Protein %
Con	2.09 ± 0.29^{a}	0.64 ± 0.26^{a}	2.38 ± 0.28^{a}	73.08 ± 1.35^{a}	22.80 ± 0.88^{ab}
25% Rep	2.06 ± 0.10^{a}	0.77 ± 0.06^{a}	2.37 ± 0.47^{a}	73.13 ± 0.78^{a}	21.54 ± 0.98^{a}
50% Rep	1.88 ± 0.17^{a}	0.66 ± 0.12^{a}	2.24 ± 0.21^{a}	71.87 ± 0.89^{a}	23.89 ± 0.87^{ab}
75% Rep	1.91 ± 0.17^{a}	0.83 ± 0.16^{a}	2.06 ± 0.61^{a}	71.96 ± 1.87^{a}	22.92 ± 1.21^{ab}
100% Rep	1.70 ± 0.05^{a}	0.73 ± 0.10^{a}	2.84 ± 0.11^{a}	70.41 ± 1.15^{a}	24.85 ± 0.97^{b}

Composition results from the injected cooked chicken deli rolls are presented in Table 3.2. The replacement of phosphate in the brine formulation had no significant ($P>0.05$) impact on the ash content, salt content, fat content or moisture content of the samples. The protein analysis did yield a significant ($P<0.05$) change amongst the samples, where the 100% replacement sample had a significantly higher protein content than the 25% sample, note that none of the samples were significantly different in protein content from the control. Ash content followed a decreasing trend as phosphate replacement increased, though this was not significant ($P>0.05$). Salt content remained largely unchanged across the samples, this was to be expected as the salt content of the

brines was controlled and therefore consistent across all treatments. Any fluctuations up or down in salt and ash content can therefore be explained as variances in mineral uptake and mineral content of the raw chicken breast fillets, where a small amount of variability is to be expected. Fat content remained relatively consistent during the trial with only slight variability across the treatments, though no of these differences were significant ($P>0.05$), with these variances explained as the variability in fat content of the chicken breast fillets themselves, which can also explain any fluctuations in protein content, given that no fat or protein sources were used in formulating the brines. The moisture content does trend downwards as phosphate replacement increases, though this is also not at a significant ($P>0.05$) level. Given that the injection of poultry meat with brine is to impart technological improvements to the meat and not influence the composition of the meat per se, large changes in composition results were not expected and these results were in line with those from a recent study which investigated the composition of both conventionally and organically produced chicken (Capan and Bagdatli 2021).

3.3.2: Technological results

CIELAB colour results are shown in Table 3.3. L* values rise as the phosphate in the brine is replaced and the 100% replacement sample is significantly ($P<0.05$) lighter than the control. The a* values did not significantly ($P>0.05$) change with phosphate replacement. The b* values decreased as the phosphate was replaced. Each sample, except for the 50% replacement sample had significantly ($P<0.05$) lower b* values than the control and thus less yellow in colour. This is interesting given the fact that the control brine was a pale grey translucent colour and as phosphate replacement level increased this colour became more yellow/orange in colour, influenced by the colour of the citrus fibre predominantly. Visually the samples themselves were not different in colour, all possessing the characteristic off-white colour of cooked chicken breast.

Table 3.3: CIELAB colour results. Mean values (±SD) in the same column bearing different superscripts are significantly different ($P<0.05$). Rep = Replacement.

	L*	a*	b*
Con	81.30 ± 2.01^{a}	1.95 ± 0.40^{a}	13.40 ± 0.48^{a}
25% Rep	83.27 ± 1.10^{ab}	2.39 ± 0.17^{a}	10.37 ± 0.39^{c}
50% Rep	82.31 ± 0.96^{ab}	1.87 ± 0.69^{a}	12.39 ± 0.15^{ab}
75% Rep	83.36 ± 0.51^{ab}	1.82 ± 0.82^{a}	10.96 ± 0.83^{bc}
100% Rep	84.51 ± 0.38^{b}	1.59 ± 0.34^{a}	11.18 ± 0.62^{bc}

Technological results are shown in Table 3.4. Brine uptake showed no significant (P>0.05) differences between treatments, suggesting that any slight variations seen in uptake can be explained as slight differences in the chicken breast themselves (fat content, muscle fibre orientation etc.) and not due to the impact of the various brine formulations. Cook yield reduced significantly (P<0.05) from the control at 50-100% replacement and a linear drop in cook yield is seen in parallel with phosphate replacement. The 25% replacement sample was not significantly lower than the control. Cook yield is one of the most commonly measured technological attributes for meat products given its influence on product yield and profitability as well as the relationship between cook yield and WHC, texture and sensory quality of cooked meat products (Toscas, Shaw et al. 1998), (Zhuang, Bowker et al. 2014).

Table 3.4: Technological analysis results. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05). Rep = Replacement.

SAMPLE	UPTAKE (%)	COOK YIELD (%)	BRINE pH	MEAT pH	SLICES (X/15)	WHC (%)
Control	26.14 ± 4.40^{a}	75.62 ± 3.03^{a}	8.16 ± 0.02^{a}	6.53 ± 0.06^{a}	13.67 ± 1.53^{a}	73.45 ± 1.08^{a}
25% Rep	27.56 ± 5.62^{a}	72.36 ± 2.65^{ab}	7 ± 0.01^{b}	6.41 ± 0.09^{ab}	14.33 ± 1.16^{a}	78.75 ± 1.04^{b}
50% Rep	26.03 ± 4.41^{a}	68.97 ± 2.80^{b}	5.65 ± 0.04^{c}	6.21 ± 0.06^{bc}	10.33 ± 3.06^{ab}	78.88 ± 1.65^{b}
75% Rep	29.69 ± 2.17^{a}	67.76 ± 1.42^{b}	4.67 ± 0.09^{d}	6.08 ± 0.13^{cd}	6 ± 1.00^{bc}	77.33 ± 1.18^{b}
100% Rep	24.47 ± 3.25^{a}	66.22 ± 0.82^{b}	4.18 ± 0.02^{e}	5.89 ± 0.06^{d}	3.33 ± 3.51^{c}	78.04 ± 1.30^{b}

From Table 3.4, cook yield decreases by approximately 10% from the control to the 100% replacement sample. Previous work has shown the same effect, whereby replacing phosphate with other ingredients resulted in decreased cook yield in cooked hams, ground and intact turkey breast and chicken breast (Resconi, Keenan et al. 2015), (Sammel and Claus 2007), (Smith and Young 2007). Brine pH dropped in parallel with phosphate replacement, with each treatment significantly (P<0.05) lower in pH than its predecessor in the replacement sequence. Given that Aquamin soluble is treated with organic acids during production to render it soluble and has a pH of 4 in 1% aqueous solution, and the citrus fibre is also low in pH, a drop in brine pH is to be expected (Marigot Ltd. 2020), (FiberStar 2021). Typically a brine that is low in pH is undesirable given that one of the reasons phosphate is used in brines is to increase the pH of said brine and thus the meat, away from the meat's isoelectric point, thus increasing the WHC, protein solubilisation and so on (Offer and Trinick 1983), (Smith and Young 2007). Cooked sample pH decreased alongside phosphate replacement. From 50% replacement on the samples were

significantly (P<0.05) lower in pH than the control. As the brine pH dropped approximately twofold from the control to the 100% replacement treatment, a drop in cooked sample pH was expected. While statistically significant, the drop in cooked sample pH from control to 100% replacement was less than 1 and the 100% replacement sample had a pH close to the expected range of cooked chicken breast (pH 6.09 – 6.21) and is above the approximate isoelectric point of pH 5.5 of meat proteins (Dykes, Coorey et al. 2019), (Fletcher, Qiao et al. 2000). This modest drop in pH is a reflection of the high buffering capacity of chicken breast, as the white muscle and the presence of certain dipeptides in chicken breast impart greater pH buffering capacity in the meat (Lykkeboe and Johansen 1975), (Castellini and Somero 1981). A further point to note with pH is the production method of the chicken used, free-range and organic chicken has been consistently found to be higher in pH than conventionally produced chicken and slaughter conditions both pre and post is also known to impact on meat pH, so careful selection of chicken breast could yield a product with a higher pH than even when injected with the 100% phosphate replaced brine seen in this study (Debut, Berri et al. 2005), (Schneider, Renema et al. 2012), (Funaro, Cardenia et al. 2014). The number of intact slices obtained from each cooked chicken roll gives an indication of the level of myofibrillar protein extraction and gelling as well as the stability of this protein gel structure. Table 3.4 shows that the 25% replacement treatment yielded the highest number of intact slices, and the 100% treatment yielded the lowest. The 75% and 100% replacement treatments yielded significantly (P<0.05) fewer intact slices than the control. These results suggest that a higher to moderate level of phosphate in combination with Aquamin soluble, citrus fibre and carrageenan offered the best myofibrillar protein extraction and gelling properties of the different brine formulations investigated in this study. This improved protein extraction and gelling has been shown in previous work with STPP in poultry meat, to offer the most optimum results in WHC and cook yield (Xiong and Kupski 2007), (Smith and Young 2007). WHC results show that replacing phosphate with Aquamin soluble, citrus fibre and carrageenan increased the WHC of the samples significantly (P<0.05). This increase was not linear in parallel with replacement, rather an approximate 5% increase is observed once replacement of phosphate begins and the WHC does not increase as replacement level increases, with none of the replacement treatments significantly (P>0.05) different from each other. This increase in WHC is observed despite the fact that the pH of the phosphate replaced brines and the chicken samples themselves is lower than would typically be considered to be optimal. However, the results shown here are in accordance with other studies where both citrus fibre and carrageenan (used independently of each other) increased the WHC of a variety of processed meat products including low-fat frankfurters, ground beef meatballs and restructured chicken breast hams (Polásek, Salek et al. 2021), (Gedikoğlu and

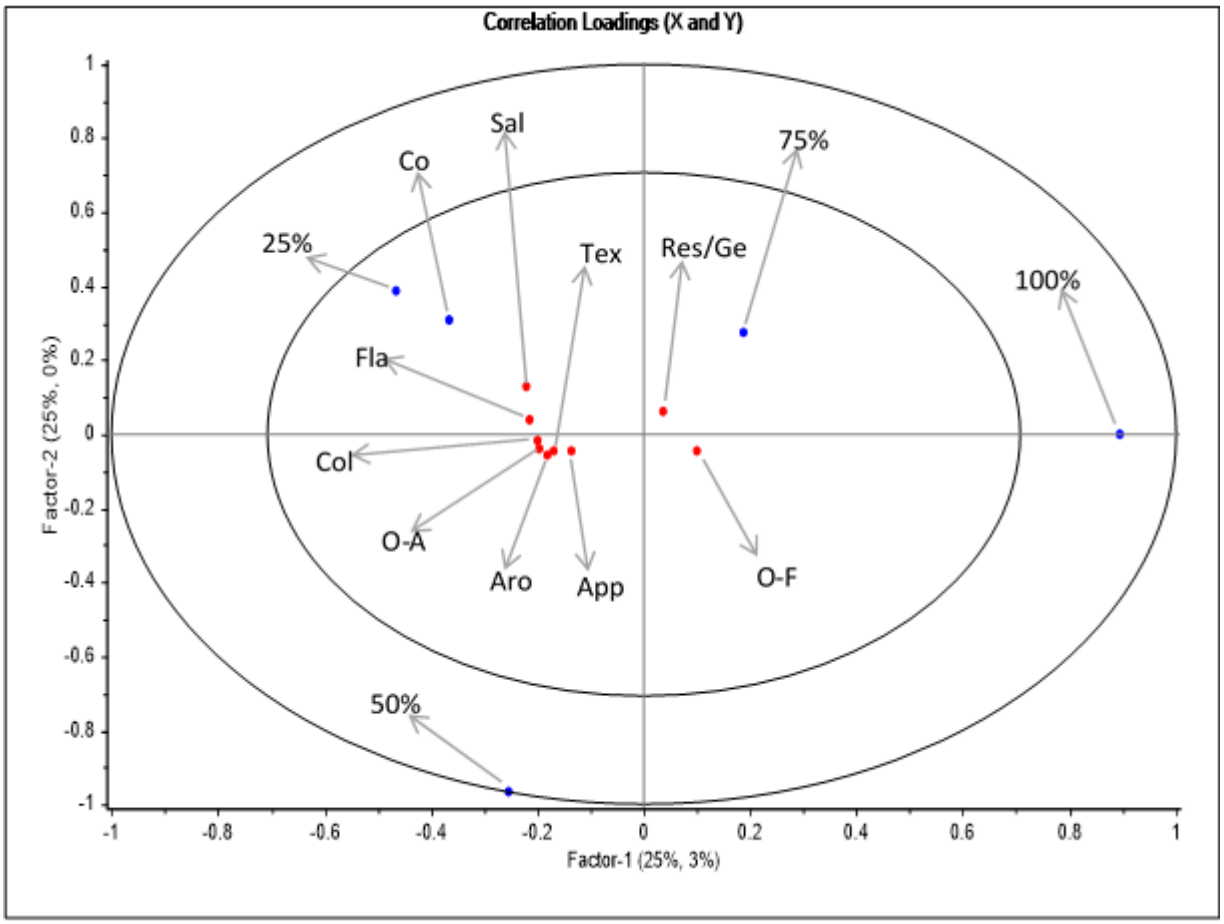
Clarke 2019), (Song, Pan et al. 2016). The results shown in this study and in previous works suggest that the replacement ingredients used have a meaningful impact on WHC, capable of replacing the WHC effect of phosphate. However, as evidenced by the results in Table 3.4, there appears to be a ceiling to this effect.

3.3.3: Sensory Results

The sensory evaluation of the cooked restructured chicken hams is presented in the ANOVA partial least squares regression (APLSR) plot in Figure 3.1, with the ANOVA values of regression coefficients shown in Table 3.5 for hedonic and sensory attributes. The APLSR plot gives an overview of the correlation between sensory attributes and samples. The plot is divided by an x and y-axis and a positive correlation exists between a sample and a sensory attribute exists when both points are in close proximity and on the same side of the x-axis. A negative correlation exists if the opposite is the case. The corresponding ANOVA values are presented in Table 3.5. A significant result exists if $P \leq 0.05$ and this significance can be negative or positive as indicated by the algebraic signs.

From Figure 3.1, in the lower left quadrant, liking of colour, aroma, appearance and texture are significantly correlated with overall acceptability. Liking of flavour and the intensity of saltiness, while not significantly correlated with overall acceptability, were also attributes that scored high in samples that were well accepted. On the other hand, samples that had higher levels of residue/gel on the sample surface and had high levels of off-flavour were not well liked by assessors and were not correlated with overall acceptability. While the off-flavour point is on the same side of the x-axis as overall acceptability they are not in close proximity and given the low levels of off-flavour that was reported during the trial, it is safe to say that any link that exists between off-flavour and overall acceptability is a reflection of these low levels and/or absence of off-flavour. From Figure 3.1 none of the sample points are in close proximity to any of the sensory attributes, suggesting no correlations are present, however, to gain a better understanding of these relationships the ANOVA values of regression coefficients shown in Table 3.5 must be examined.

Figure 3.1: APLSR graph for sensory results of cooked restructured chicken hams and control. Abbreviations as follows: APP = appearance, COL = colour, ARO = aroma, FLA = flavour, TEX = texture, RES/GEL = residue/gel, SAL = saltiness, O-F = off-flavour, O-A = overall acceptability.



From Table 3.5 the only statistically significant results that were identified were in the 100% replacement treatment, which was negatively correlated with liking of aroma and flavour and negatively correlated with saltiness intensity (higher saltiness intensity). However, while these correlations were significantly negative, the overall acceptability of the 100% replacement samples was not significantly different to any of the other samples including the control. As can be seen in Table 3.5 no other significant results were present, with this being an indication of the similarity of the treatment samples and the control to each other. Assessors were also asked to rank the samples in order of preference from 1 to 5 (from most to least) during the trial with the 50% and 75% replacement samples selected equally as the most preferred (Data not shown). However, it is worth noting that panellists reported not being able to differentiate between samples or that the samples were very similar to each other, and the only differences were in magnitude of intensity of certain attributes. Therefore, from these sensory results it can be concluded that replacing phosphate in a brining system such as used here, with Aquamin soluble,

citrus fibre and carrageenan will have no significant impact on the sensory quality and overall acceptability of the cooked restructured chicken hams injected with said brine.

Table 3.5: Beta coefficients, phosphate replacement trial sensory results. Figures shaded in green, and red are positive and negative significant correlations respectively [+/- (P<0.05)]. Abbreviations as follows: APP = appearance, COL = colour, ARO = aroma, FLA = flavour, TEX = texture, RES/GEL = residue/gel, SAL = saltiness, O-F = off-flavour, O-A = overall acceptability Rep = replacement.

	APP	COL	ARO	FLA	TEX	RES/GEL	SAL	O-F	O-A
Control	0.7792	0.6211	0.6471	0.2606	0.3037	-0.5236	0.0862	-0.3819	0.5804
25% Rep	0.4256	0.1112	0.5826	0.1768	0.6636	-0.7532	0.0916	-0.5355	0.2273
50% Rep	0.2533	0.3625	0.1861	0.8174	0.2976	-0.3976	0.3715	-0.797	0.2457
75% Rep	-0.5093	-0.5769	-0.993	-0.9878	-0.671	-0.3676	-0.5934	0.8409	-0.5632
100% Rep	-0.2532	-0.0787	-0.0327	-0.0234	-0.0646	0.9217	-0.0244	0.3494	-0.0529

Overall, from a sensory point of view it is both reasonable and viable to replace the phosphate with the aforementioned ingredients without affecting sensory quality. Previous attempts to replace phosphates in meats and processed meat products using various ingredients have yielded positive results, however, the complete replacement of phosphate has been difficult to achieve, with the absence of phosphate and/or the replacement ingredients cited as contributing to negative effects on texture and sensory quality (Long, Gál et al. 2011), (Resconi, Keenan et al. 2016). However, other studies have shown that complete replacement of phosphate in meat products is possible without affecting sensory quality, with winter mushroom powder use in sausages and a proprietary blend, containing citrus fibre, in chicken and citrus fibre in pork sausage yielding positive results (Casco, Veluz et al. 2013), (Choe, Lee et al. 2018), (Powell, Sebranek et al. 2019). The main issues seen in studies where full replacement of phosphate was deemed non-viable, were regression in texture and juiciness from a sensory quality perspective. However, from the results of this study, the improvements in WHC (Table 3.4) aid in offsetting at the least or perhaps improving the sensory quality of the restructured chicken hams. While the same level of protein solubilization is not seen from the 100% replacement treatment as is seen in the control, which in theory would affect the texture of the chicken from both a mouth feel and visual perspective, the sensory panel did not significantly prefer any sample with regards to texture. It should be noted that the reduction in protein solubilization seen in the 100% replacement sample may pose an issue if the chicken was to be marketed as “sliced”, because the 100% samples yielded significantly fewer slices than the control and tended to break up more during the slicing process. While this was not an issue here, at a larger scale consumers may prefer

the appearance of more uniform slices, however, if the product was marketed as “chicken pieces” or as “shredded chicken” as is commonly seen on supermarket shelves, this appearance issue is not relevant. Ultimately under the parameters of this particular study the full replacement of phosphate did not detrimentally affect the sensory quality of the samples and the 100% replaced samples were on par with the phosphate-containing control.

3.3.4: Microbiological results

Microbiological results are shown in Tables 3.6 and 3.7 for total viable count (TC) and E. coli (EC) and in Figures 3.2 and 3.3 for microbial growth over time from Day 0 to Day 42 for TC and EC respectively. For TC on day 0, there was no recorded growth, which was to be expected given that all samples were cooked <24hrs before plating. On day 42 TC numbers for all treatments were as follows, control 7.05 ± 1.60 cfu/g, 25% replacement 6.89 ± 1.07 cfu/g, 50% replacement 4.27 ± 3.95 cfu/g, 75% replacement 5.32 ± 1.78 cfu/g, 100% replacement 4.75 ± 4.13 cfu/g (Table 3.6). As is shown in Figure 3.2, throughout the 42 days of storage the TC count is lower in the replacement treatments than in the control, with the 75% replacement and 100% replacement treatments yielding the lowest TC numbers. However, despite the trends on show in Figure 3.2, no statistical significance ($P>0.05$) was found between TC growth in any of the treatments.

Table 3.6: Total viable count (TC) over time. Average Log_{10} CFU/g ± standard deviation. Mean values with different superscripts in the same column are significantly different ($P<0.05$). Rep = Replacement.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	0^a	2.44^a	4.46^a	5.94^a	6.20^a	7.10^a	7.05^a
25% Rep	0^a	2.18^a	3.08^a	4.33^a	6.60^a	4.92^a	6.89^a
50% Rep	0^a	1.06^a	4.12^a	5.77^a	4.63^a	6.01^a	4.27^a
75% Rep	0^a	1.27^a	2.38^a	4.04^a	6.12^a	3.81^a	5.32^a
100% Rep	0^a	0^a	3.21^a	4.09^a	4.28^a	3.52^a	4.75^a

EC growth over time is presented in Table 3.7 and Figure 3.3. From Figure 3.3 the control treatment has noticeably more EC growth from day 21/28 onwards and no EC growth was detected in the 75% and 100% replacement treatments. EC growth levels (cfu/g) were significantly ($P<0.05$) higher in the control treatment on Day 35 than in the 25%, 75% and 100% replacement treatments, however, no significant differences were found on any of the other treatment days.

Figure 3.2: Average total viable count (TC) over time. Log_{10} CFU/g vs Days.

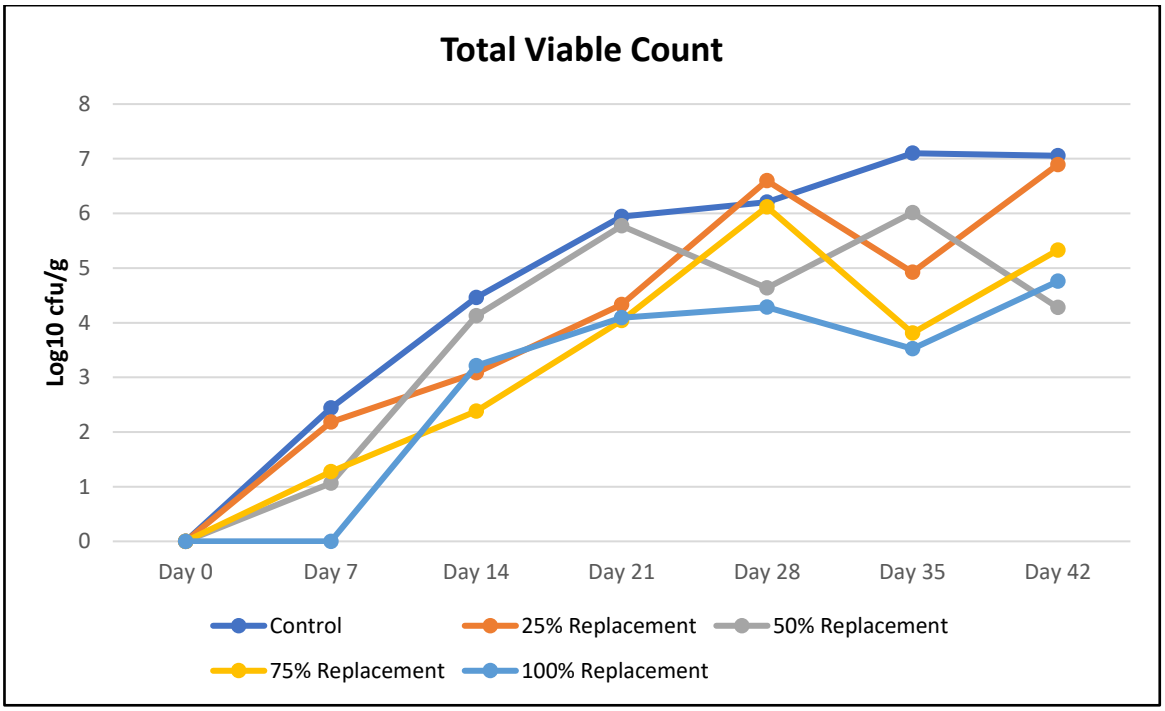
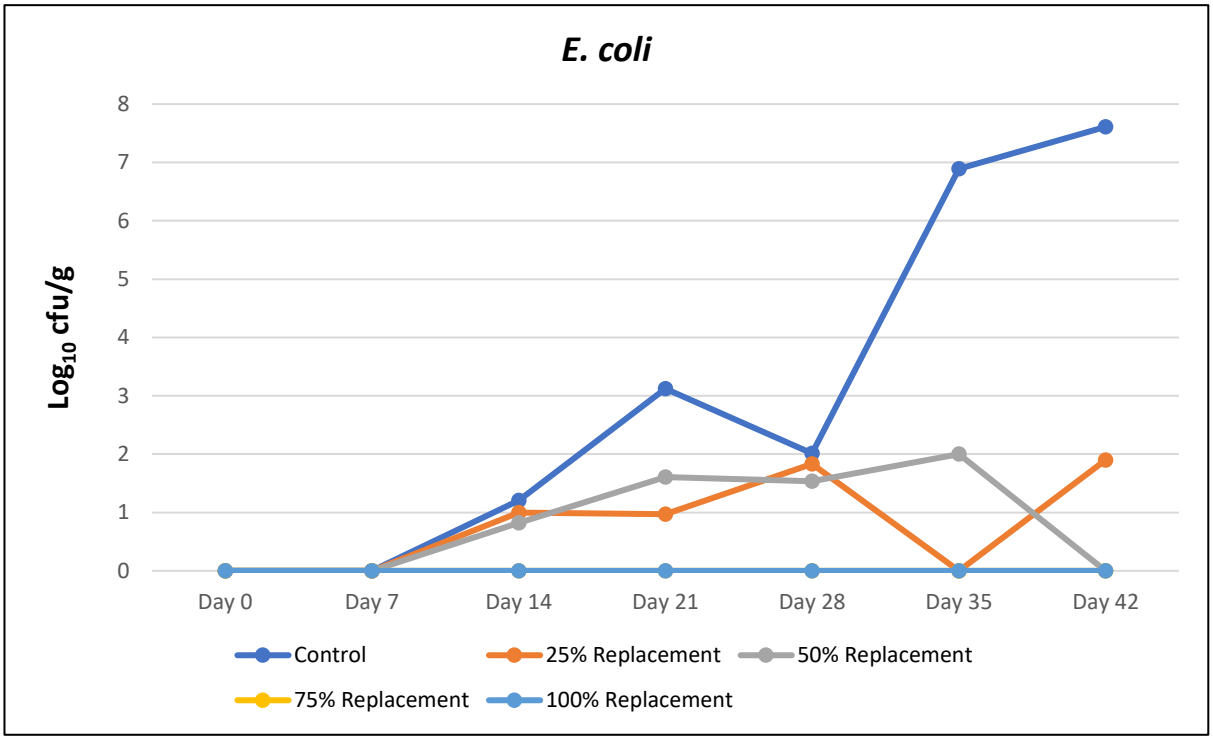


Table 3.7: E. coli (EC) growth over time. Average Log_{10} CFU/g ± standard deviation. Mean values with different superscripts in the same column are significantly different (P<0.05).

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	0^{a}	0^{a}	1.20^{a}	3.12^{a}	2.01^{a}	6.89^{a}	7.61^{a}
25% Rep	0^{a}	0^{a}	0.99^{a}	0.97^{a}	1.83^{a}	0^{b}	1.90^{a}
50% Rep	0^{a}	0^{a}	0.82^{a}	1.61^{a}	1.53^{a}	2^{ab}	0^{a}
75% Rep	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{b}	0^{a}
100% Rep	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{b}	0^{a}

Figure 3.3: Average E. coli count (EC) over time. Log_{10} CFU/g vs Days.



Salmonella is a gram-negative bacterium and the cause of cases of gastroenteritis, with poultry meat and related products prominent transmission vectors for Salmonella spp. as the bacterium is naturally present in the intestinal tract of many warm-blooded animals and its occurrence in poultry carcasses is widely reported (Milkievicz, Badia et al. 2021), (Chen, Godwin et al. 2018). Salmonella was not found in any of the samples at any stage during storage (Table 3.8). This was an expected result because, barring any potential cross-contamination after the cooking step, none of the bacterium should've been present as cooking to an internal temperature of 74°C is known to cause a 7-log reduction in Salmonella (NACMCF 2007). MAP is considered an effective strategy in extending the shelf life of various products including cooked and raw meats (Zhang, Wang et al. 2015).

Table 3.8: Salmonella confirmatory test results. D = Detected, ND = Not Detected.

	Day 0	Day 14	Day 28	Day 42
Control	ND	ND	ND	ND
25% Rep	ND	ND	ND	ND
50% Rep	ND	ND	ND	ND
75% Rep	ND	ND	ND	ND
100% Rep	ND	ND	ND	ND

Under MAP conditions the high presence of CO_2 is the primary limiting factor for microbial growth, while the N2 is to prevent package collapse (Zhang, Wang et al. 2015), (Economou, Pournis et al. 2009). Thus, the combination of cooking samples to an internal temperature of 74°C and the MAP conditions used in packaging the samples, a longer shelf life or reduction in microbial growth can be achieved as is evidenced from microbiological results here. Facultative anaerobes such as lactic acid bacteria (LAB) are less susceptible to MAP conditions, which is also true, though to a lesser extent, for Enterobacteriaceae which includes E.coli and Salmonella (Säde, Murros et al. 2013). For this reason, LAB tend to be the dominant species seen in cooked poultry products packaged under MAP and in cooked packaged meat products in general (Chenoll, Macián et al. 2007). LAB were not tested for in this study, however, pH was measured at specific time points during storage as a drop in pH is one specific outcome of food spoilage caused by LAB in cooked meat products (Egan 1983), (Martins, Longhi et al. 2020). pH change within treatments over storage is shown in Table 3.9, with measurements taken on days 0, 14, 28 and 42.

Table 3.9: pH change within treatments over time. Mean values (±SD) in the same column bearing different superscripts are significantly different (P<0.05). Rep = Replacement.

	Control	**25% Rep**	**50% Rep**	**75% Rep**	**100% Rep**
Day 0	6.53 ± 0.06^{a}	6.40 ± 0.09^{a}	6.21 ± 0.05^{a}	6.08 ± 0.12^{a}	5.88 ± 0.06^{a}
Day 14	6.51 ± 0.03^{a}	6.42 ± 0.04^{a}	6.17 ± 0.05^{a}	6.09 ± 0.03^{a}	5.88 ± 0.10^{a}
Day 21	6.44 ± 0.03^{ab}	6.27 ± 0.03^{a}	6.16 ± 0.04^{a}	6.01 ± 0.02^{a}	5.78 ± 0.03^{a}
Day 28	6.40 ± 0.03^{b}	6.30 ± 0.05^{a}	6.11 ± 0.03^{a}	5.97 ± 0.03^{a}	5.77 ± 0.06^{a}

From Table 3.9, within all treatments the pH drops over time during storage, pH dropped significantly (P<0.05) in the control treatment from day 0 (pH 6.53 ± 0.06) to day 42 (pH 6.40 ± 0.03), however, no other significant results were found in any of the other treatments, with only a slight drop in pH recorded in the samples. The drop in pH seen here can be potentially explained as the growth and proliferation of LAB, which given that LAB are the dominant species seen in low O_2 atmosphere packaging is almost certain to contribute to the pH drop. A second possible reason is the MAP conditions themselves whereby the high CO_2 levels in the packaging decrease the pH of the sample. This is due to the CO_2 dissolving/diffusing into the sample and carbonic acid is produced thus dropping the pH (Al-Nehlawi, Saldo et al. 2013), (Bruce, Wolfe et al. 1996). This effect has been shown in other studies where a drop in pH of fresh pork, fresh turkey sausages and chicken frankfurters was observed in samples under MAP of varying gas concentrations, including the same 70% N_2, 30% CO_2 used here (Luong, Jeuge et al. 2020), (Tovunac, Galic et al. 2011). From the microbiological results, the replacement of phosphate does not have a negative

effect on the microbiological quality or shelf life of the samples. In fact, the higher replacement levels, 50%, 75% and 100%, may have had more of an antimicrobial effect than the control as can be seen from Figures 3.2 and 3.3, though this effect was only statistically significant on day 35 for E. coli growth. However, when all the E. coli results are examined, no growth was recorded in the 75% and 100% replacements, suggesting that the Aquamin soluble, citrus fibre and carrageenan may have had a greater antimicrobial effect than the control, or more likely the drop in pH of the cooked samples due to the more acidic nature of the higher replacement brines (Table 3.4) combined with the MAP conditions inhibited the growth of E. coli in the 75% and 100% replacement samples. In terms of the effect of replacement of phosphate on the shelf life of the samples, given that only total viable count, E. coli and Salmonella were tested for in this study and no other potentially pathogenic and/or food spoilage microorganisms like Campylobacter or lactic acid bacteria were screened for, a comprehensive shelf life for the samples is difficult to define. However, for cooked sliced meats stored in MAP according to the Food Safety Authority of Ireland, the TC results were satisfactory for cfu/g up to day 28 for the control and 25% replacement samples and up to day 35 for the 50%, 75% and 100% replacement samples, after which point they were borderline results but not unsatisfactory. For the E. coli results the 75% and 100% replacement samples were satisfactory for the duration of storage given no E. coli growth was recorded, the control and 50% replacement samples were satisfactory up to day 14 and unsatisfactory from day 21 and the 25% replacement sample was satisfactory up to day 14 and unsatisfactory from day 28 (FSAI 2020). From these results the 75% and 100% replacement samples would appear to have a considerably longer shelf life than the rest of the samples, but that is not a definitive result and more microbiological analysis is warranted to determine an acceptable shelf life for the samples. There hasn't been much previous research done into the antimicrobial effect of phosphate on the microbial quality of poultry meats and one study reported mixed findings regarding phosphate's antibacterial effects in processed cheeses (Buňková, Pleva et al. 2008). However, given that phosphates are used first and foremost to increase tenderness, juiciness and cook yield as opposed to any antimicrobial effect, this lack of research is understandable. Therefore, in brining in its most basic form, the main preservative agent is salt (Keeton 2001). Salt content of the brines is controlled and remains consistent in this study (Table 3.1) so any antimicrobial behaviour observed in the treatments are highly likely to be due to the progressively acidic nature of the replacement brines and the MAP which leads to the replacement of certain bacterial strains with LAB in the samples, with certain LAB strains themselves having antimicrobial effects (Saucier, Gendron et al. 2000). This is reflected in the growing popularity of utilising acidic marinades and/or brines in poultry processing as

antimicrobial agents to extend shelf life, even at the expense of certain technological parameters associated with a lower pH (Carroll, Alvarado et al. 2007), (Alvarado and McKee 2007).

3.4: Conclusion

The 100% replacement of phosphate in brine with Aquamin soluble, citrus fibre and carrageenan can be achieved, albeit with a negative impact on the cook yield and protein solubilisation/gel stability of the cooked restructured chicken hams. However, these issues may not be as relevant depending on how a cooked chicken product injected with the 100% replacement brine is marketed or presented, for example consumers may be willing to compromise for a "clean label" phosphate-free product or if the chicken is presented as shredded or as pieces the protein gel structure is not as important. However, it is worth noting that the formulation used in this research is not strictly clean label as it contains carrageenan, which despite it being sourced from red seaweeds does not qualify for clean label status. It is also worth noting that many consumers do not know where carrageenan comes from, so careful labelling on the packaging could overcome this issue. The sensory, microbiological and WHC results point to the replacement agents as being both promising and viable alternatives to sodium triphosphate for poultry injection. Optimisation of the 100% replacement brine should be performed to attempt to address the quality issues previously mentioned. Sodium bicarbonate is one other potential replacement agent that warrants investigation for inclusion, following promising results as a complete replacement for phosphate in chicken meat batters (Lu, Kang et al. 2021). Overall, the 100% replacement, phosphate-free brine largely on par with or better than the control in many of the quality parameters tested, with the obvious notable exceptions of cook yield and protein gel stability.

Chapter 4

General conclusion

General conclusion

Meat has held a highly valued and important status in the diet of peoples and cultures all over the globe for centuries throughout human history. Indeed, meat is still a highly valued component of people's diets due to its high protein content, nutritional value, palatability and its unique and diverse sensory profiles. However, as the global population continues to rise, meat supply is struggling to keep up with demand, which inevitably leads to strategies to try match this demand, some of which will have negative consequences from unsustainable or unethical farming and production practices. Meat processing is a necessary practice to extend the shelf life of meats and meat products but also to minimise waste by utilising less premium cuts of meat and connective tissues to render them more attractive to customers i.e. value-added meat products. However, meat processing typically results in increased fat, salt and additive contents in processed meat products which opens up a myriad of potential health problems and concerns. Given these health concerns and the environmental and other reasons outlined in chapter 1, the consumer demand for plant-based meat alternatives has grown dramatically this century. This demand is reflected both in the ever-expanding research ongoing in the area and the range of options currently available on the market as meat and processed meat product alternatives.

As the growing popularity of vegetarian, vegan or a more plant-based approach grows, continued research into more options in the plant-based alternative sphere is both warranted and valuable. Therefore, the research work presented in chapter 2 of this thesis, where the use of CP and RLP as alternative protein sources in a processed meat product, could prove to be a valuable scientific base for future research using these proteins in this or in a similar application. From the sensory results in chapter 2 it is clear that while replacement was acceptable up to a certain level the sensory quality and acceptance declines dramatically once 50% replacement is reached and exceeded. This is where the challenge surrounding direct and complete meat and fat replacement lies, recreating the unique sensory and technological qualities that meat and fat bring to foods. Fat is an important component of mouthfeel, texture, juiciness and flavour of meat products and if it is not suitably replaced, a deterioration of all these factors is inevitable. The CP and RLP used in this research were extremely low in fat content and therefore, were unable to offset any of the sensory and technological issues arising from the reduction in the animal fat component at moderate to complete replacement levels. Dryness was also major limiting factor in the higher replacement samples, which impacted on texture, mouthfeel and succulence or juiciness of the samples. However, the samples were well liked, accepted and even performed better than the control both for sensory and technological parameters at the 20-30% replacement levels,

indicating that CP and RLP could be viable options for partial meat and fat replacement, but further research and optimisation is needed to achieve higher and complete replacement.

There are a number of potential avenues to expand on the work done in the completion of this thesis. The first would be adding fat to the formulation in the form of plant-based lipids such as coconut oil or vegetable oils, in order to try and improve the textural and sensory issues seen here that arose from what was effectively non-replacement of the fat component of the formulation. It should be noted that carrageenan and waxy maize starch were employed in an attempt to overcome this issue but at the concentrations used that was clearly not a success at the higher replacement levels. Particular care should be exercised in the selection of fat sources, given that processed meat products tend to be quite high in saturated fats, from both a health and marketing perspective creating a more favourable fat profile in a plant-based alternative is attractive. Efforts should be made to reduce the saturated fat content of the final product, which may rule out coconut oil due to its fatty acid profile. Therefore, I feel that chia oil or whole chia seeds themselves with their high polyunsaturated fat and omega-3 content could be a potential fat source that offers both a possible answer to sensory issues as well as increased water binding due to their technological nature and finally as a source of added dietary fibre. The second potential avenue of research would be the use of hydrocolloids in greater quantities than were used in the formulation (Table 2.1). Citrus fibre, locust bean gum, enzyme modified guar, various starches or even seaweeds could all be examined as potential replacement agents that would raise the WHC (as was seen in chapter 3 with citrus fibre, used in a different setting) of the samples, which in turn will improve the texture, juiciness and mouthfeel of the samples. The final avenue of research is further and alternative processing techniques for the CP and RLP themselves. HPP and/or extrusion could be utilised in order to impart a certain texture on the proteins as well as elicit more technological function such as a potential increase in water binding capacity, in order to facilitate their use in creating a product that more closely resembles the texture and technological attributes of a white pudding style product.

As was outlined in detail in section 2 of chapter 1, phosphates are used in meat processing due to their unique ability to influence the pH, dissociate the actomyosin complex, increase water binding and their preservative properties. Alongside these functions, various phosphates play important biological roles in most living organisms and are a necessary component of a healthy individual's diet. For these reasons phosphates are important compounds in our lives, however, as with many compounds, often it is the dose that makes the poison. There is new but growing evidence that phosphates may be linked to numerous health issues including bone health problems, kidney disease, CVD and micronutrient deficiencies. The data supporting these links to

health problems is new and at times conflicting or transient, nonetheless this emerging data should be a point of note for most and a potential point of concern or at least warrants attention for those with chronic renal disease, especially given how ubiquitous phosphates have become in food production with the average person consuming in excess of the ADI. These health concerns, coupled with the rise in consumer demand for clean label products which are free-from phosphates and other additives, has led to a drop in phosphate usage in certain countries and a growing interest in industry in finding suitable phosphate replacement agents that don't compromise on product quality. The research outlined in chapter 4 of this thesis attempted to do just that, replace phosphate in a given brine solution for injection into a specific meat system, chicken breast fillets in this case, and determine the viability of the replacing agents from technological, sensory and microbiological perspectives.

The technological results described in chapter 3, for the use of Aquamin soluble, citrus fibre and carrageenan to replace STPP in the control brine solution, suggest that 100% replacement of STPP is indeed possible. The WHC results in particular show that the 100% replacement sample performed much better than the control despite the substantial drop in brine pH and modest drop in meat pH when compared to the control. Issues with using this particular formulation to completely replace phosphate centre around the pH. Ideally the brine should raise the meat pH in order to facilitate the dissociation of actin and myosin thus improving cook yield and the protein structure stability in the cooked product, evidenced here by poor cook yield performance and poor sliceability compared to the control. These technological deficits could be potentially mitigated with some optimisation of the brine formulation and the production method. Sodium bicarbonate has shown potential as a possible phosphate replacement agent in previous studies and should warrant investigation for inclusion in the current 100% replacement brine solution. Sodium bicarbonate also has a higher pH (~8.5) than Aquamin soluble, carrageenan and citrus fibre and could therefore help raise the pH of the brine and thus the meat and aid in solubilising the actin and myosin, improving protein structure and cook yield (National Center for Biotechnology Information 2021). However, one thing to note with sodium bicarbonate is that it would remove the clean label status of a brine and subsequent injected product if it was used in a brine formulation. Optimisation of the production method itself may yield improved cook yield and protein structure results. Scope for optimisation is probably greatest at the tumbling stage, where post injection the chicken could be left to tumble for longer, potentially under vacuum, thus creating more physical damage to the meat allowing for more surface area for the NaCl component of the brine to solubilise the salt soluble actin and myosin filaments, thus increasing

the quantity of salt solubilised myofibrillar proteins which would then gel together during the cooking stage.

In terms of sensory results, the control and all the various replacement treatments were largely on par with each other, with the sensory assessors even reporting difficulty in discerning between samples. These results suggest that notwithstanding the decreases seen in cook yield and sliceability of the replacement samples when compared to the control, all treatments performed up to or above the standards set by the control, with a few minor exceptions for specific sensory attributes as outlined in section 3.3.3 of chapter 3. The observed decrease in sliceability may impact on the sensory results depending on the context of how the samples are presented. Slices from the control and 25% replacement treatment held up much better than the other samples when sliced, its entirely conceivable that assessors may prefer the appearance of a uniform intact slice than one that is not intact. However, full slices were not presented to the assessors for the purpose of standardising sample presentation, so the sliceability issues/weaker protein gel structure would not have impacted on the sensory results of this study. In terms of flavour the main brine component that would contribute to the flavour of the cooked product was salt, which was controlled for and remained consistent across all treatments. While not relevant to the research work presented in chapter 3, the phosphate-replaced brine could be further optimised from a sensory perspective via addition of additional seasonings and/or spices to the brine formulation which will further enhance the sensory profile of the cooked final product. A sensory profile that exceeds that of a product produced using a traditional phosphate brine offers an attractive prospect to producers and consumers alike. This may even offset some of the technological issues namely the integrity of slices with customers conceivably willing to compromise on certain attributes such as the sliceability if other attributes are optimised and/or more attractive than the alternative, such as phosphate-free clean label status and better sensory quality overall.

The impact of phosphate replacement on microbiological quality of the samples was not affected to any significant ($P>0.05$) degree by the replacement of phosphate. This can be viewed as either a positive or a neutral, positive in that the change in phosphate level in the brines had no deleterious impact on microbiological quality and neutral in that microbiological quality was not improved above the levels seen in the control. Further microbiological analysis may be appropriate to conduct going forward as part of a next steps protocol, yet as is shown in chapter 3 microbiological quality was consistent across all experimental treatments including the control.

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