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Innovative Processing Strategies for the Development of Highly-Dispersible, Protein-Enriched Dairy Powders

Thesis presented by

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Declaration

Innovative processing strategies for the development of highly-dispersible, protein-enriched dairy powders

This is to certify that the work I 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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Abstract

The application of milk protein concentrate (MPC) powder ingredients, in the formulation of nutritional products (e.g., follow-on infant formula, protein bars, clinical and sports performance beverages) continues to drive research interest among scientists to better understand, predict and control the physical and functional properties of such powders. They represent a rich source of versatile milk proteins and are prepared from the ultrafiltration and diafiltration of skim milk, followed by evaporation and spray drying. However, the primary technological hurdle limiting their application is suboptimal rehydration performance in water, specifically slow and/or incomplete dispersion and solubility. This presents a significant challenge for the food and beverage industry, particularly in relation to creating more sustainable manufacturing conditions and optimising the quality of final products. The objective of this thesis was to investigate the impact of composition (e.g., protein content), processing parameters (e.g., heat treatment) and novel technologies (e.g., gas injection) on the physical and functional properties of MPC powders. Initial studies on MPC ingredients demonstrated that high-protein content (>65%, w/w) negatively influenced bulk powder (e.g., density and flowability) and rehydration properties, with results generally improving as protein content decreased to 40-55% (w/w). Heat treatment of liquid MPC prior to spray drying demonstrated that thermal processing (≥100 °C for 30 s) significantly increases concentrate viscosity and impairs powder rehydration performance, likely due to protein aggregation, but can provide a pH-dependent improvement in heat stability. Research involving the injection of nitrogen gas (N_2) into liquid MPC prior to spray drying generated regular and agglomerated (i.e., fines returned to the top of the spray dryer) powders with distinct physical and bulk handling properties and significantly improved dispersion and dissolution.

Regular MPC powders produced using N_2 injection (NI) had lower density, poorer flowability, increased specific surface area, and altered surface composition. However, these powder particles underwent significant breakdown during reconstitution in both ambient and warm water, demonstrating that NI directly prior to spray drying can enhance the dispersion and solubilisation of micellar casein-dominant dairy powders. These powders were further processed downstream of drying using milling to alter powder properties and yielded samples with higher density, lower air content and altered surface composition, and while they did not disperse and solubilise to the same extent, their rehydration properties remained better than those produced without NI. This thesis provides new insights into the relationship between processing modifications and the physicochemical properties of milk protein concentrate, and will support the development of techno-functional, protein-enriched dairy ingredients for incorporation into nutritional food and beverage products.

List of publications and conference contributions

Peer-reviewed papers:

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McSweeney, D. J., Maidannyk, V., O'Mahony, J. A., & McCarthy, N. A. (2021). Influence of nitrogen gas injection and agglomeration during spray drying on the physical and bulk handling properties of milk protein concentrate powders. *Journal of Food Engineering, 293*, 110399.

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*Cenini, V. L., Gallagher, L., McKerr, G., McCarthy, N. A., McSweeney, D. J., Auty, M. A. E., & O'Hagan, B. M. G. (2020). A novel approach for dynamic *in-situ* surface characterisation of milk protein concentrate hydration and reconstitution using an environmental scanning electron microscope. *Food Hydrocolloids*, 105881.

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Chapter 1

Manufacture and physical properties of micellar casein-dominant dairy powders

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Contents

1.1. Introduction

Bovine milk is a complex fluid, consisting predominantly of water (87%), while the remaining fraction is composed of protein, fat, carbohydrate, minerals and vitamins. The two main classes of protein are the casein and whey proteins, the carbohydrate is lactose, while the prominent minerals include calcium, sodium and potassium (Deeth and Hartanto, 2009). Milk serves as a raw material for many different food products and due to its short shelf life and high-water content, dehydration to a more stable form, through evaporation and spray drying, is commonly performed to create dried dairy ingredients which can be readily stored and transported globally (Schuck, 2002). Furthermore, the development of, and improvements in, membrane filtration technologies have facilitated the fractionation of liquid milk to produce dairy products with specific nutritional and functional properties and to meet the demands of health-conscious consumers. For example, ultrafiltration (UF) and diafiltration (DF) are often used for the concentration of the proteins present in skim milk, with such retentates spray dried to produce high-protein (>80%, w/w) dairy powders such as milk protein concentrate (MPC). However, the unit operations involved in producing these ingredients can induce several physicochemical changes to the product (e.g., protein aggregation during heat treatment), which can impact subsequent functionality. The physical and bulk handling properties (e.g., density and flowability) of powders can also be modified by the processing conditions applied and are an important consideration for producers of dairy ingredients.

Micellar casein-dominant dairy powders, such as MPC and milk protein isolate (MPI), have valuable nutritional (e.g., low lactose and high calcium content) and functional (e.g., heat stability and gelation) properties due to the high concentration of casein proteins and are often incorporated into beverage formulations for adult,

clinical and sports nutrition applications. However, these powders have poor physical and bulk handling characteristics (e.g., low bulk density, poor flowability and high air content), which presents challenges for producers of such powders. A comprehensive overview of the relevant aspects of dairy proteins, the processing steps involved in the manufacture of high-protein, micellar casein-dominant powders, their applications industrially and the physical properties of these powders is provided herein.

1.2. Dairy proteins

The two main types of protein in bovine milk are caseins and whey (~3.4 g/100 mL total). They differ by the precipitation of the caseins from solution at 30 °C and pH 4.6 (isoelectric point), with the whey or serum proteins remaining soluble under these conditions (Fox and McSweeney, 1998). The ratio of casein to whey proteins in bovine milk is approximately 80:20 and it contains all of the essential amino acids required by humans; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Chatterjee et al., 2014), making it an excellent source of high-quality dietary protein. As a result, protein-enriched dairy ingredients have considerable economic value due to their nutritional attributes, in addition to their functional properties (Fox, 2001).

1.2.1. Casein

The casein proteins in milk are present in the form of casein micelles, colloidal particles with a mean diameter of approximately 150 nm (size range from 50-600 nm) and are largely responsible for milk's white colour (Fox and Brodkorb, 2008). Due to their large size, 90-95% of the casein micelles can be sedimented by ultracentrifugation at 100,000*g* for 1 h (Fox, 2003). The biological role of casein

micelles is to facilitate the transport of a high quantity of nutrients (e.g., calcium, phosphorous and amino acids) to the mammalian neonate to ensure their nutritional requirements are met (Horne, 2011). Casein represents a group of phosphoproteins, containing four different molecules: α_{s1}-, α_{s2}-, β- and κ-casein. One way in which they differ from each other is the degree of phosphorylation; for example, α_{s2}-casein contains between 10 and 13 phosphate residues, while κ-, β-, and α_{s1}-caseins have approximately 1, 5 and 8 phosphate groups, respectfully, usually at serine residues. The presence of phosphate groups has a major influence on the caseins as it can affect the binding of metals (e.g., calcium, zinc and inorganic phosphate) and molecular charge (O'Mahony and Fox, 2013). Some casein proteins (e.g., α_{s2}- and κ-casein) contain cysteine residues, which allow them to form disulphide linkages. The caseins are very heat stable (e.g., can tolerate heating at 140 °C for 20 min) and are good emulsifying agents due to their hydrophobic and hydrophilic regions along the polypeptide chain (Fox and McSweeney, 1998). They exhibit a tendency to self-associate, and this is attributed to hydrophobic interactions, while the extent of polymerisation is governed by electrostatic repulsion and ionic strength (Horne, 1998, 2002). Three of the caseins, α_{s1}-, α_{s2}- and β-casein, are sensitive to calcium, while κ-casein is not, due to differences in the quantity of calcium-binding phosphoserines, i.e., the ability to bind calcium ions decreases as the number of phosphate groups in these phosphoserine residues decreases (Gaucheron, 2005).

Caseins account for 94% of the molecular weight of the micelle, with the remaining fraction consisting of low molecular mass species such as calcium, phosphate, citrate and magnesium and is collectively known as colloidal calcium phosphate (CCP; O'Mahony and Fox, 2013). The CCP plays a fundamental role in the stability and integrity of casein micelle structure (Deeth and Hartanto, 2009), and

removal of this component will result in dissociation of the colloid, e.g., acidification results in the formation of a curd. The casein micelle is quite hydrated, with an estimated 3.4 g of water chemically and physically associated with each g of protein (Morris et al., 2000).

1.2.1.1. Characteristics of casein proteins

Caseins are considered rheomorphic proteins (i.e., they have an open structure or conformation), which is related to the high number of proline residues present in the amino acid chain that can interrupt secondary structure (Holt and Sawyer, 1993). Casein proteins demonstrate micro-heterogeneity due to post translational modifications (e.g., glycosylation of κ-casein and phosphorylation of α_{s1}-, α_{s2}- and β-casein), and these changes play an important role in casein micelle formation and stability (Holland and Boland, 2014).

The concentration of α_{s1}-casein in bovine milk is 12-15 g/L, representing ~40% of total casein (Huppertz, 2013). The reference protein for this family of caseins is α_{s1}-casein B-8P, whereby B represents the genetic variant, and 8P refers to the number of phosphorylated amino acids in the polypeptide chain. This protein contains 199 amino acids, eight of which are phosphoserines, and it has a molecular weight of 24.6 kDa. The protein can self-associate to form larger structures (i.e., dimers and tetramers), the size of which are influenced by pH and ionic strength. For example, at pH 6.6, monomers of α_{s1}-casein occur when the ionic strength is 0.003, but dimers and tetramers form when it increases to 0.2 (Swaisgood, 2003).

The concentration of α_{s2}-casein in bovine milk is 3-4 g/L (Huppertz, 2013). α_{s2}-Casein A-11P is regarded as the reference protein for the α_{s2}-casein family. It contains 207 amino acids in its primary structure and has a molecular weight of 25.2

kDa. It is the most hydrophilic of the caseins due to the presence of 33 positively charged and 39 negatively charged amino acid residues (Huppertz, 2013; Swaisgood. 1993). α_{s2}-Casein contains cysteine residues in its amino acid sequence at positions 36 and 40 (Farrell et al., 2009), which enable disulphide bond formation. It is the most calcium sensitive protein of the casein family, with near complete precipitation taking place in 2 mM of $CaCl_2$ (Aoki et al., 1985), caused by the high number of negatively charged phosphate groups which can bind the positively charged calcium ions, resulting in the neutralisation of electrostatic charge (Swaisgood, 2003).

The β-casein content of bovine milk is 9-11 g/L and it represents 35% of the casein fraction in bovine milk (Huppertz, 2013). The reference protein is β-casein A^2-5P, which consists of 209 amino acid residues and has a molecular weight of 24 kDa. It is considered the most hydrophobic of the caseins (Swaisgood, 2003) due to the C-terminal region of the protein, from residue 136-209, which contains many hydrophobic amino acids and does not have a net charge. However, the N-terminus of the protein (residues 1-40) is far more hydrophilic (Huppertz, 2013) as it contains five phosphorylated serine residues (Darewicz et al., 2000). Therefore, β-casein is considered amphipathic (i.e., it consists of hydrophobic and hydrophilic domains) and consequently has good surface activity, enabling it to be used for emulsification and foam stabilisation (Dickinson, 2003). At low temperatures (0-4 °C), β-casein exists as a monomer, but as the temperature is increased to 15-30 °C, the protein self-associates and micelles form (De Kruif and Grinberg, 2002). This is attributed to the temperature dependent hydrophobic interactions (O'Connell, 2003) and has facilitated the manufacture of β-casein dairy ingredients designed for infant formula applications (McCarthy et al., 2013; Atamer et al., 2017).

The concentration of κ-casein in bovine milk is 2-4 g/L (Huppertz, 2013).

κ-Casein A-1P, the reference protein for the κ-casein family, contains 169 amino acid residues in its primary amino acid sequence and has a molecular weight of 19 kDa. This protein is less sensitive to calcium in comparison to the other caseins, due to its low number of phosphate groups. It is the only casein that is capable of being glycosylated, and this primarily occurs at threonine residues. Similar to α_{s2}-casein, it also contains two cysteine residues and is able to participate in disulphide bonding.

1.2.1.2. Casein micelle structure

The definitive structure of the casein micelle remains an inconclusive and complex subject of great debate and many reviews regarding this have been published (de Kruif and Holt, 2003; Horne, 2006; Fox and Brodkorb, 2008; Dalgleish and Corredig, 2012; Lucey and Horne, 2018). The three models proposed to describe the casein micelle structure are the dual binding, submicellar and nanocluster models. The dual binding model suggests that (i) cross-linking of hydrophilic regions containing phosphoserine *via* CCP and (ii) hydrophobic bonding, controls the micelle structure (Horne, 1998, 2002). The submicelle model, as modified by Wasltra (1999), proposes that the casein micelle is a spherical particle with a core composed of small units called submicelles that contain casein proteins. The submicelles have a diameter of approximately 14 nm, with calcium phosphate positioned within and between them and κ-casein located on the surface of the micelle (i.e., the "hairy layer") to provide stability. The nanocluster model (Fig. 1.1) was developed by Holt (1992). In this model, small clusters of calcium phosphate have a molecular mass of 61 kDa, a radius of 2.3 nm and are enclosed by a protein shell of approximately 49 phosphopeptide chains (Holt et al., 1998b). Crosslinking between the calcium phosphate nanoclusters and the phosphorylated α_{s1}-, α_{s2}- and β-casein facilitates the formation of the casein

micelle structure (De Kruif and Holt, 2003). Furthermore, the casein protein tails emitting from the nanoclusters interact with other proteins (i.e., self-associate) *via* weak interactions (i.e., hydrogen bonding, hydrophobic interactions and ionic bonding) to create a protein matrix (De Kruif et al., 2012). The κ-casein is positioned on the surface of the casein micelle, acting as a polyelectrolyte brush and this protein contributes significantly to maintaining the micelle structure *via* polymeric or steric stabilization (de Kruif and Zhulina, 1996).

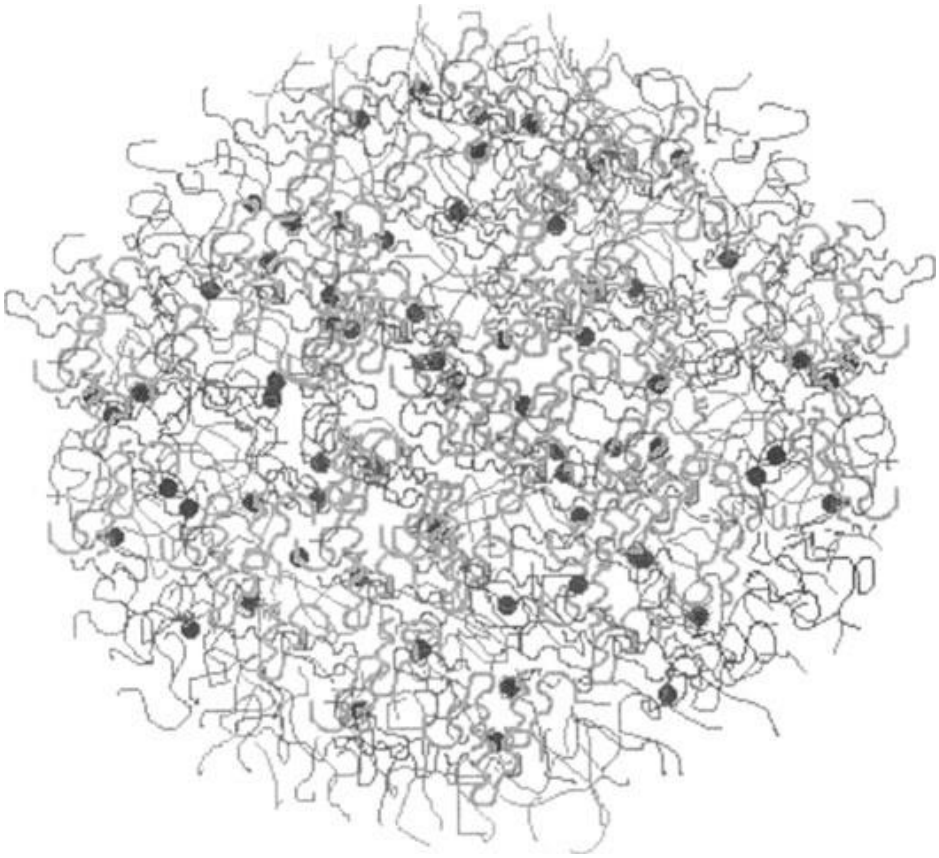

Fig. 1.1. Casein micelle structure according to the Holt or nanocluster model (de Kruif and Holt, 2003). Dark spheres represent calcium phosphate nanoclusters.

Huppertz et al. (2017) recently suggested that non-spherical primary casein particles (PCPs), linked together by calcium phosphate nanoclusters, are the building blocks of

casein micelles and they associate to form a porous network stabilised by κ-casein. Holt (2021) recently updated the nanocluster model and suggested that polar (charged or neutral) interactions are more significant than non-polar interactions in the association of caseins, referred to as intrinsically disordered proteins. Furthermore, the core of the micelle consists of free caseins along with caseins bound directly to calcium phosphate nanoclusters, while the coat of the micelle consists of free caseins (Holt and Carver, 2022). It is important to mention that the micelle structure can be altered by a number of environmental and compositional factors, including changes in pH (Vaia, 2006), heating in the presence of ethanol (O'Connell, 2001), cooling (Rose, 1968), the addition of chemicals to milk (e.g., urea; Holt, 1998a) and unit processing operations (e.g., high-pressure homogenisation; Sandra and Dalgleish, 2005).

1.2.2. Whey proteins

Whey proteins, also referred to as serum proteins, represent 20% of the protein fraction of bovine milk (Fox and McSweeney, 1998). The main whey proteins present in bovine milk are β-lactoglobulin, α-lactalbumin and bovine serum albumin, representing 50, 20 and 5-10% of total whey protein, respectively. Other whey proteins which occur in milk at lower concentrations include lactoferrin and immunoglobulins. A distinguishing feature of this family of proteins is that they remain soluble at pH 4.6, unlike the caseins which precipitate from solution. Furthermore, they have a globular shape in their native form, are not sensitive to calcium ions, and do not contain phosphate groups (Fox, 2001). However, due to their globular, quaternary structure, they are less heat stable during thermal processing compared to the caseins (e.g., denatured by heating at 90 °C for 10 min). Whey protein ingredients, such as whey protein concentrate, can be isolated and prepared commercially from liquid

whey using membrane filtration technology (i.e., UF and DF), evaporation and ion-exchange chromatography or electrodialysis (O'Mahony and Fox, 2013). They are incorporated into a range of value-added, nutritional food and beverage products given their high biological value due to the abundance of branched chain (i.e., valine, leucine and isoleucine) and essential (e.g., methionine, tryptophan) amino acids (Walzem and German, 2002).

β-Lactoglobulin, the most abundant whey protein in bovine milk (i.e., 50% of total whey protein and 12% of total protein), contains 162 amino acids in its primary structure and has a molecular weight of 18 kDa (Fox and McSweeney, 1998). The protein has two intramolecular disulphide bonds, due to the five cysteine residues located along the polypeptide chain. It has a number of sulphur containing amino acids (e.g., methionine), which play a nutritional role and give the protein a high biological value (O'Mahony and Fox, 2013). The free sulphydryl or thiol group is exposed when β-lactoglobulin is subjected to temperatures greater than 65 °C and can subsequently form a disulphide linkage with other proteins such as α-lactalbumin and κ-casein (Deeth and Hartanto, 2009). β-Lactoglobulin contains a hydrophobic cavity which enables the protein to bind hydrophobic ligands, e.g., retinol and fatty acids (Kontopidis et al., 2004; Jameson et al., 2002).

α-Lactalbumin is the second most abundant whey protein in bovine milk (i.e., 20% of total whey protein and 3.5% of total protein), has a molecular weight of 14 kDa and an isoelectric point of 4.8 (Fox and McSweeney, 1998). It has 123 residues in its amino acid sequence, many of which are tryptophan and sulphur. Protein structure is stabilised by four intramolecular disulphide bonds, and unlike β-lactoglobulin, it has no free thiol group. α-Lactalbumin can act as a metalloprotein and bind calcium ions *via* its asparagine residues, which makes it the most heat stable of

the whey proteins (Fox, 2003).

The concentration of serum albumin in milk is low (0.1-0.4 g/L) but it is a relatively large protein with a molecular mass of approximately 66 kDa. Immunoglobulins are complex proteins that provide immunity, and three of the 5 classes are present in milk (IgA, IgG and IgM). The level of immunoglobulins present in milk can vary from 0.6-1 g/L in mature milk, to 100 g/L in colostrum (Fox and McSweeney, 1998).

1.3. Manufacture of micellar casein-dominant dairy powders

MPC, MPI and micellar casein concentrate (MCC) are some of the powdered dairy ingredients produced industrially which contain a high concentration of micellar casein proteins. The membrane filtration technology applied to the skim milk determines the profile of the product. For MPC manufacture, UF membranes are used for protein concentration, with the final product containing the same ratio of casein:whey as present in the initial skim milk. However, in the case of MCC, microfiltration (MF) membranes, which have a larger average pore size, are used to selectively concentrate the casein proteins, with the majority of whey proteins passing into the permeate, along with lactose and minerals. The high-protein retentate generated by membrane filtration processes is usually evaporated to remove water and increase the total solids content prior to spray drying (Singh, 2007). A summary of the processing steps involved in producing MPC powder is displayed in Fig. 1.2.

1.3.1. Heat treatment (Option A)

In a typical dairy processing plant, the first heat treatment milk undergoes is pasteurisation or high temperature short time treatment, whereby it is held at 72-75 °C

for 15-20 s and then cooled, to eliminate pathogenic and spoilage microorganisms and increase product shelf life. Following the removal of fat, the skim milk may undergo further heat treatments to alter the functionality of the final product. For example, skim milk may undergo high-heat treatment (e.g., 90 °C for 5 min, 120 °C for 1 min or 135 °C for 30 s) to alter heat stability and water absorption properties for use in recombined evaporated milk (Kelly and Fox, 2016). Bovine milk is very heat stable

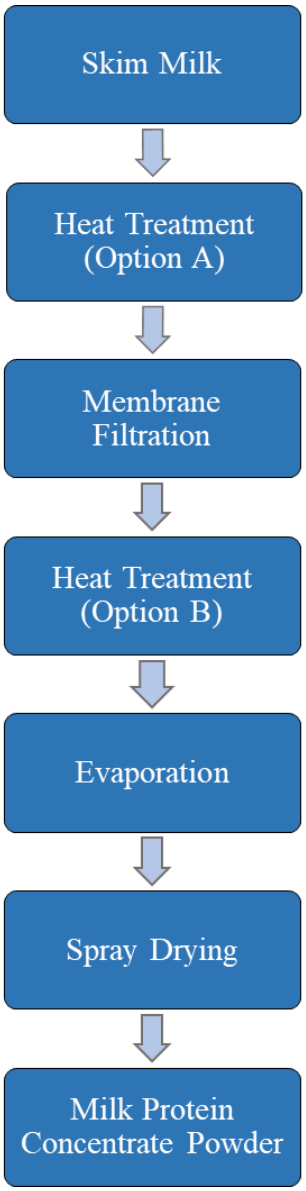

Fig. 1.2. Flow diagram of milk protein concentrate powder manufacture.

due to its casein content and can withstand heating at 140 °C for 20 mins before coagulating (Fox and Morrissey, 1977). A range of heat treatments have been applied to the skim milk feed before UF and subsequent manufacture of MPC, including 85 ºC for 30 s (Lin et al., 2018), 95 °C for 45 s (Gazi and Huppertz, 2015) and 72-130 °C for 30 s (Carr, 1999), and can usually be achieved using indirect (e.g., plate heat exchanger) or direct (e.g., steam injection) methods (Kelly and Fox, 2016). The heat treatment parameters applied at this stage have a significant effect on the unfolding of whey proteins and their subsequent interaction with other whey proteins and the casein micelles *via* hydrophobic and disulphide bonding (Smits and van Brouwershaven, 1980). A temperature of 65 °C can initiate denaturation of minor whey proteins (e.g., serum albumin), while heat treatment at a temperature greater than 70-75 °C can lead to denaturation of the major whey proteins (Oldfield et al, 1998). Heat stability is influenced by a range of compositional factors including pH, protein concentration and mineral content (Fox and Morrissey, 1977). Anema and Li (2003) reported a greater increase in casein micelle size upon heating at pH 6.5 than at pH 6.7 due to association of whey proteins with casein micelles. Oldfield et al. (2005) reported that of all the heating steps involved during the manufacture of dairy powders (i.e., pre-heating, evaporation and drying), it is the pre-heat stage that imparts the greatest extent of protein denaturation.

The influence of pre-heat treatment on MPC powder solubility has been reported by Gazi and Huppertz (2015); low (72 °C for 15 s) and medium (95 °C for 45 s) heat treatment of skim milk prior to membrane filtration did not result in significantly different solubility initially and after storage at 20 °C, despite higher α-lactalbumin and β-lactoglobulin denaturation (25 and 65%, respectively) in the medium-heat treated sample, however, when powders were stored at elevated

temperatures (i.e., 37 and 50 °C), whey protein solubility was lower in the medium-heat treated sample. This information can be used to optimise storage conditions and prevent decreases in powder quality when such ingredients are transported globally. Lin et al. (2018) did not report a difference in solubility between MPC powders produced from pasteurised (72 °C for 15 s) and medium-heat (85 °C for 30 s) treated skim milk. However, Carr (1999) did report a decrease in MPC powder solubility as the heat treatment temperature applied to skim milk before membrane filtration was increased from 72 to 130 °C.

1.3.2. Membrane filtration

In the production of MPC, pasteurised skim milk is first passed through a UF system. The molecular weight cut off is approximately 10,000 Da, which enables materials with a lower molecular weight (e.g., lactose and soluble salts) to pass through into the permeate stream (Carr and Golding, 2016), while protein, fat and the mineral salts associated with the casein micelle are retained. MF is used for the production of micellar casein powders depleted in whey proteins, e.g., MCC or native phosphocaseinate (NPC), and has a pore size of >0.1 µm compared to 0.001-0.1 µm for a UF membrane (Kelly, 2011). Membrane processes such as UF and MF are characterised by crossflow membrane filtration, whereby the feed entering the system is parallel to the membrane surface and is subjected to a pressure to promote fractionation. In the case of UF, the process typically involves pressures ranging from 0.01 to 0.06 MPa (Caric et al., 2009). During the manufacture of MPC, UF usually takes place at <20 °C which helps prevent changes to protein structure (Kelly, 2011). DF is an additional membrane filtration step whereby water is passed through the UF retentate to further remove lactose and soluble salts. It is utilised when a protein

concentration of greater than 70% is desired in the final powder (Singh, 2007). DF operates on the basis that the addition of water lowers both the viscosity and osmotic pressure, enabling more of these soluble components to pass through the membrane into the permeate fraction (Smith, 2013).

1.3.3. Heat treatment (Option B)

Heat treatment can also be applied to the liquid milk protein concentrate that is produced by UF, to modify the functional properties of the powder. For example, McCarthy et al. (2017) reported that industrial MPC used in their study had been heated at 120 °C for 3 s prior to evaporation and spray drying. Recent studies by Ho et al. (2018, 2019) have investigated the effect of heat treatment on heat stability, protein denaturation and viscosity of liquid MPC (19.8%, w/w, total solids). Tari et al. (2021) also reported the effect of heat treatment (85 °C for 5 min and 125 °C for 15 s) on the physicochemical and acid gelation properties of liquid MPC. However, the effect of heat treatment at this stage of the process on the subsequent functional properties of spray-dried MPC powders has not been reported in the literature.

1.3.4. Evaporation

The primary role of evaporation in dairy processing is to lower the water content of the feed prior to spray drying. As the total solids content of dairy streams increases, there is a corresponding increase in viscosity (Singh, 2007). Therefore, for protein-based streams such as MPC, the total solids is only brought up to ~30% (w/w), compared to ~50% total solids (w/w) for lower protein concentrates such as skim milk. Evaporation is usually conducted in a multiple-effect, falling film evaporator, which can be tubular or plate-type, and occurs as the milk flows down a vertical surface

(Caric et al., 2009). In a plate-type evaporation system, a spray nozzle distributes the product as a thin film onto the plate, while in a tubular system, a horizontal spreader plate is used, and once it reaches the end of the evaporator, a separator is used to divide the vapour from the concentrate (Tetra Pak, 2015). The vapour produced in one effect can be used for heating in the subsequent effect, resulting in greater thermal efficiency and less steam consumption. This design is possible as evaporation is performed at low pressure (i.e., under vacuum), which reduces the possibility of protein denaturation occurring by lowering the boiling temperature (Pisecky, 2012c). The rate of water removal during evaporation is influenced by a number of factors, including the rate at which heat is transferred from the heating surface to the liquid, the surface area of the liquid and the rate at which vapour is removed from the liquid surface. For highly viscous liquids such as MPC, forced circulation evaporation is often used, whereby the product is moved through the calandria by a circulation pump and evaporated when the pressure drops as it enters the separator (GEA, 2021). This approach minimises fouling as evaporation does not occur on the heating surface. Evaporation during the production of dairy powders is generally conducted at a temperature between 40 and 70 °C (Birchal et al., 2005), but has been reported to induce some physicochemical changes upon milk proteins. For example, Martin et al. (2007) showed that casein micelles increased in size during evaporation, possibly due to further attachment of denatured whey proteins to the casein micelle surface. It is also important to consider that not all dairy processors may evaporate concentrates prior to drying as the viscosity of the feed may be too high, particularly in the case of protein-enriched products.

1.3.5. Spray drying

Spray drying involves the dehydration of a liquid concentrate to powder, whereby the feed from the evaporator is pumped to the atomizer and is converted to a fine dispersion (Kelly and Fox, 2016). The exposure of these droplets to hot air results in the formation of dry powder particles, which usually have a moisture content of 3-5% (Deeth and Hartanto, 2009).

The function of the atomisation device in spray drying is to transform the concentrated milk into numerous small droplets with a large surface area, thus facilitating a high rate of evaporation when exposed to the hot drying air (Schuck, 2011). The three main types of atomisers used in the dairy industry are pneumatic (i.e., two-fluid), pressure nozzle and rotary wheel (O'Sullivan et al., 2019). Pressure nozzle atomisers consist of either a grooved core or swirl chamber in the head of the nozzle which provides turbulence, and an orifice through which the liquid leaves. This atomisation device converts the pressure energy generated by a high-pressure pump into kinetic energy to spray the liquid into the drying chamber as thin sheets which resemble a hollow cone (Pisecky, 2012b). The size of the nozzle components and the pressure applied (150-250 bar), along with the properties of the feed material, control the spray pattern and droplet size; for example, viscous liquids reduce the spray angle, while increasing the atomisation pressure will reduce droplet size. Rotary or wheel atomizers consist of horizontal discs with radial vanes (straight or curved) or bushings. The liquid enters the device through the centre and moves out to the edge of the wheel where the droplets are quickly formed and removed, achieving atomisation by centrifugal force and peripheral speeds of 100-200 m/s. The two-fluid or pneumatic nozzle combines the feed material with compressed air, either internally or externally, and these are more commonly utilised in pilot-scale settings (Pisecky, 2012b). The type of atomising device used offers different advantages and disadvantages for

product functionality and spray drying operations. For example, pressure nozzles produce powders with high bulk density and low occluded air content, but nozzle blockage is more likely, while rotary devices can atomise viscous concentrates and are less susceptible to blocking but are more expensive to operate (Kelly and Fox, 2016; O'Sullivan et al., 2019).

There are three principal types of spray dryers used in dairy processing: single-stage, two-stage and three-stage. In a single-stage spray dryer, the entire dehydration process takes place in the drying chamber (Kelly and Fox, 2016). As a result, a high inlet air temperature is required to sufficiently lower the moisture content, and this can have a subsequent impact on powder quality and functional properties. For example, a study by Fang et al. (2012) investigated the influence of inlet (77-178 °C) and outlet air temperature (54-103 °C) on the functionality of MPC and reported that solubility of the powder decreased with a rise in processing temperature. In two-stage spray drying, the powder leaving the drying chamber has a moisture content of 10-15% (Kelly and Fox, 2016), and the product then undergoes a second drying step in an internal or external fluidised bed dryer to reduce its moisture content further (Pisecky, 2012d). Approximately 10% less heat is required for two-stage compared to single-stage drying and a greater inlet air temperature and feed concentration can be used which is economically favourable. A two-stage dryer can be used to produce both agglomerated and non-agglomerated powders. A three-stage spray dryer is characterised by the use of an internal fluidised bed as the second dehydration stage, and an external vibrating fluid bed as the final or third drying step (Schuck, 2011).

Agglomeration is a size enlargement unit operation often performed during dairy powder manufacture. It involves joining primary powder particles together to create larger powder clusters, in which the original particles can still be identified. The

two methods of agglomeration performed during spray drying are primary and secondary, while each of these can be either forced or spontaneous. For example, primary spontaneous agglomeration involves the random collision of atomised droplets, while forced secondary agglomeration occurs when fines (i.e., small powder particles removed from the cyclone or bag house filters during drying) are returned to the top of the chamber where they come into contact with the spray cloud from the nozzle and form larger particles (Pisecky, 2012a). Strategies which can be applied to improve agglomeration include spray drying concentrates with high total solids, introducing more fines closer to the atomising device, producing larger primary powder particles, and having a powder with a higher moisture content following the primary drying stage (Skanderby et al., 2009). Wet agglomeration can also be performed during processing and involves combining the powder with a binding solution to adhere particles to each other, e.g., pneumatically mixing powder with water in a fluidised bed (Cuq et al., 2013). Agglomeration can alter the bulk handling and physical properties of dairy powders, particularly by decreasing bulk density but increasing powder flowability, particle size and porosity (Turchiuli et al., 2013). Moreover, agglomeration is commonly performed for certain dairy ingredients to improve their wetting and dispersion properties, e.g., skim milk (Skanderby et al., 2009). However, agglomeration reduces the rehydration performance of high-protein, casein-dominant dairy powders (Gaiani et al., 2007). Ji et al. (2016) reported that agglomerated MPI powders displayed improved wetting behaviour compared to standard MPI but had poorer dissolution.

1.3.6. Applications

Following spray drying, micellar casein-dominant dairy powders are

incorporated into a range of food and beverage products, including performance and clinical nutrition beverages, follow-on infant formula, high-protein bars, cheese, ice cream and yogurt (Agarwal et al., 2015). Therefore, the functionality of these powders in different systems is of relevance to dairy ingredient researchers. Modifications to the processing conditions used to produce micellar casein-dominant powders enables ingredient companies to produce tailor-made products for specific customer applications (e.g., MPC with a lower calcium content).

The behaviour of reconstituted casein-dominant powders during commercial heat processes (e.g., ultra-high temperature treatment or retort sterilisation during the production of shelf-stable beverages) is an important consideration to reduce fouling of industrial pipelines and maintain product quality (Gandhi et al., 2017). This requires the product developer to have knowledge of the optimal processing conditions for their ingredients (e.g., temperature and pH). The stability of MPC powders (37-90%, w/w, protein) to heat treatment (140 °C) following reconstitution (3.5%, protein, w/w) and adjustment to a pH range of 6.3-7.3 has been reported by Crowley et al. (2014b). At pH <6.8, heat stability decreased with increasing protein content of the powder, likely due to the reduced net-negative charge on the casein micelles resulting from high calcium ion activity. At pH >6.8, heat stability was generally higher for MPCs higher in protein (i.e., 60-80%, w/w) due to lower calcium ion activity as pH increased and less heat-induced dissociation of κ-casein from the casein micelle to the serum phase. A subsequent study by Crowley et al. (2015) reported the stability of these MPC powders to retort sterilisation (120 °C) over the pH range 6.3-7.3 after reconstitution to 8.5% protein (w/w). Heat stability of MPC increased with increasing protein content of the powders from 35 to 70% (w/w) due to reduced gelation of serum proteins and decreased dissociation of κ-casein from the micelle, while MPC80 had poor heat

stability at pH 6.3-6.8 due to high calcium ion activity. This study also involved a medium-heat treated MPC (95 °C for 45 s), and it was reported to have higher heat stability at pH 6.3-7.1 due to prior whey protein denaturation. The stability of reconstituted MCC powders (8%, w/v, protein), over the pH range 6.5-7.3, to heating at 110-150 °C, has been reported by Sauer and Moreau (2012), with heat treatment and pH having large effects on particle size and aggregation. For example, samples were coagulated at pH <6.7 and temperatures >130 °C, the particle size increased with increasing temperature at pH 6.9, while temperature did not have a considerable effect on particle size at pH >6.9. The dissociation of caseins, particularly κ- and β-casein, increased with increasing pH at all temperatures. Therefore, to improve the heat stability of MCC dispersions, it was suggested that pH should be increased, or treatment temperature decreased, in order to reduce protein aggregation and coagulation.

High-protein (≥80% protein, w/w) MPC powder is a suitable ingredient for nutritional beverage applications and has demonstrated better performance during ultra-high temperature treatment (145 °C for 5 s) than low-heat skim milk powder (Singh et al., 2019). In this study, MPC reconstituted to 14% protein (w/w) was more heat stable than skim milk powder at 7.5% protein (w/w; 2.54 and 1.77 min, respectively), despite higher viscosity and ionic calcium levels, with the authors attributing this to differences in mineral composition due to the membrane processing conditions used to produce MPC. However, when the protein content of this MPC was reduced to 7.5% protein by the addition of minerals and lactose, it had slightly lower heat stability (1.51 min). Pandalaneni et al. (2018) formulated dairy-based beverages (8%, w/w, protein) using both regular and calcium-reduced (20 and 30% calcium depletion) MPC powders (85%, w/w, protein). Heat stability (140 °C and pH 7) was

higher for calcium-reduced (20%) MPC compared to regular MPC, to which sodium hexametaphosphate was added (0.15 and 0.25%). A further study by Pandalaneni et al. (2019) reported that the beverages containing MPC with 20% calcium depletion had better storage stability than MPC with 30% calcium depletion due to lower viscosity and less casein micelle dissociation. Sunkesula et al. (2021) also investigated the effect of pH on the heat stability of calcium-reduced (20, 30 and 40%) MPC powders reconstituted to 10% protein (w/w). MPC with 30% of the calcium removed had higher heat stability at pH 6.7 (25.3 min) and pH 6.9 (27.8 min) compared to the control sample at these pH values (13.0 and 20.3 min, respectively), while heat stability was highest for the powder depleted in calcium by 40% at pH 7.1 (30.9 min).

MPC is commonly used to enhance the protein content of yogurts (Jorgenson et al., 2019). Mistry and Hassan (1992a) reported that skim milk could be fortified with MPC to a maximum protein content of 5.6% without any deleterious changes in rheological and sensory properties. The additional protein provided the yogurt with a firmer body and decreased whey protein separation, therefore reducing the requirement for stabilisers.

Protein bars are another product category in which MPC has been utilised. The physicochemical changes in protein bars, formulated using MPC, during storage at 20 °C, has been reported by Loveday et al. (2009). Bar hardness increased over time, with the authors suggesting phase separation due to the migration of water from protein to glucose and glycerol as a possible cause. Banach et al. (2014) investigated the use of extruded or toasted MPC on the textural properties of high-protein bars. The bars created from MPC extruded at 65 °C had lower hardness and fracturability values (i.e., they were softer) compared to the unmodified and toasted MPC. Banach et al. (2016) reported that the use of transglutaminase-crosslinked or calcium-reduced MPC in

protein bar formulations did not significantly improve textural stability during storage compared to unmodified MPC. However, in a further study by Banach et al. (2017), the textural properties of protein bars were improved when a fine jet-milled MPC, having a lower powder particle size, was used instead of a standard MPC powder, with this research highlighting the impact of processing conditions on powder functionality and end-user applications.

The application of casein-dominant powders in milk protein-based, oil-in-water emulsions has also been reported. This is of relevance to infant formula manufacturers in particular as the MPC would likely undergo wet-blending and heat treatment processes by the end-user. Ye (2011) investigated the ability of MPC powders, with a range of calcium contents (0.3-2.23 g/100 g), to form stable emulsions at different protein concentrations (0.3-5%, w/w). The stability of emulsions increased as the calcium content of the MPC decreased, at protein concentrations of 0.3-2%, due to the presence of dissociated casein and smaller particles, while at higher protein levels, stability decreased with decreasing calcium content as a result of depletion flocculation caused by unadsorbed protein. This demonstrates that processing conditions used during MPC manufacture can play a significant role in downstream functionality.

1.4. Physical and bulk handling properties of micellar casein-dominant powders

The main physical and bulk handling properties of dairy powders are shown in Fig. 1.3, with many of these inter-related. Processing parameters influencing particle and bulk powder properties, and the techniques to measure them will be discussed, with an emphasis on micellar casein-dominant powders.

1.4.1. Powder particle size

Particle size is an important indicator of powder quality, bulk handling (e.g., flowability and compressibility) and rehydration properties (e.g., wettability). It can be determined using sieves, microscopy analysis, or most commonly, using laser diffraction equipment (e.g., Malvern Mastersizer) due to the speed of measurement.

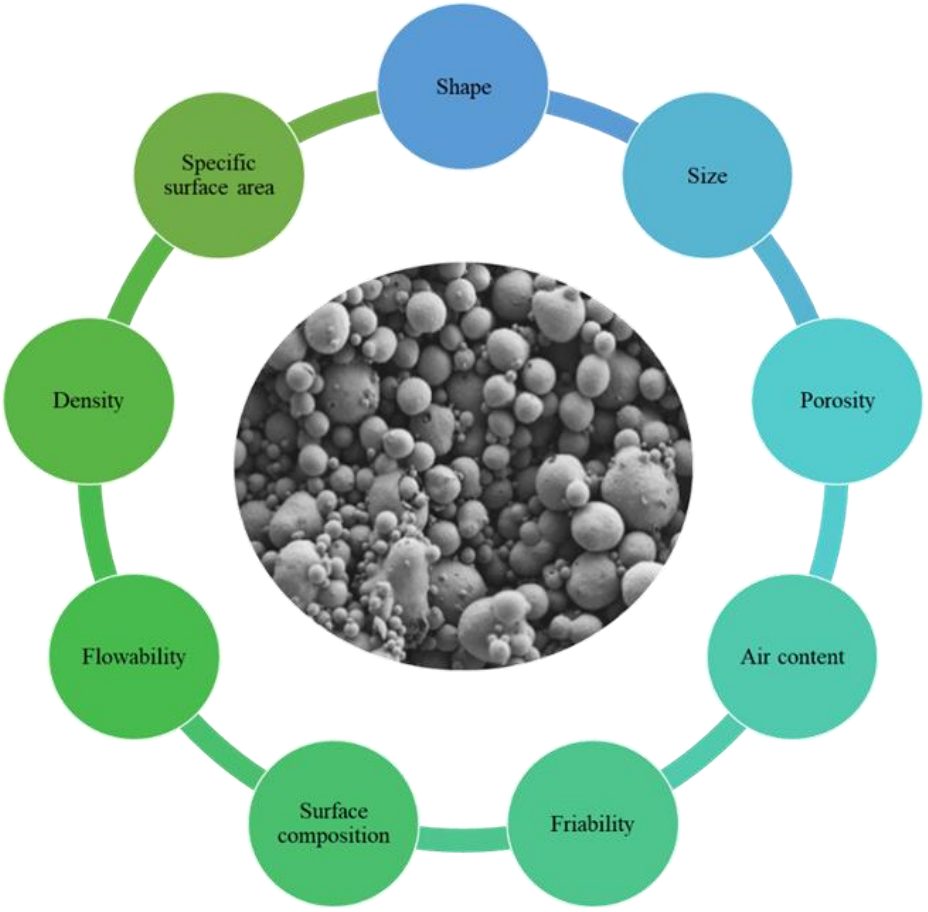

Fig. 1.3. Summary of the main physical and bulk-handling properties of dairy powders.

The laser diffraction data, which relies on light scattering calculations (e.g., Mie theory), is usually presented as a volume-based distribution in a frequency graph but can also be presented in terms of mass or surface area in either a histogram or cumulative distribution graph (Fitzpatrick, 2013). Numerical values for particle size

are often reported as D_{10}, D_{50} and D_{90}, which represent the average size of particles below which 10, 50 and 90% of the particle volume exists, while the $D_{[4,3]}$ or volume-weighted mean particle diameter is another measurement used. Powder particle size during spray drying is influenced largely by the compositional and physicochemical properties of the liquid concentrate (e.g., total solids content and viscosity), atomisation device and agglomeration (Walstra et al., 2006). Concentrate viscosity is the most important factor controlling droplet size and is also influenced by prior heat treatment, residence time, temperature, and homogenisation (Schuck, 2009). Crowley et al. (2014a) reported a D_{90} value of 58 µm for an MPC80 powder produced at pilot-scale from a concentrate of 15.7% total solids (w/w), while Pathania et al. (2018) reported a D_{90} value of 72 µm for a commercial MPC80 product which was spray dried at a higher total solids content (~30%). The composition of the starting material can also influence powder particle size, with Rupp et al. (2018) reporting an increase in MPC powder particle size with increasing protein content of the concentrate before spray drying.

1.4.2. Particle shape and structure

Particle shape and structure play a role in bulk powder properties such as density and flowability as it can influence how closely particles pack together (Fitzpatrick, 2013). These particle properties can be observed visually using imaging techniques such as scanning electron microscopy. The spray drying operation (e.g., laboratory-scale or two-stage pilot-scale), composition of the feed material entering the spray dryer, the air inlet and outlet drying temperatures, and the type of atomisation nozzle used, can all affect particle shape and structure. Kalab et al. (1989) showed that powders produced from UF of whole milk and single-stage, laboratory-scale spray

drying had smooth particle surfaces, with dimples and venation. Mistry et al. (1992b) produced powder particles from the UF and DF of skim milk, followed by pilot-scale spray drying using a rotary atomiser, with air inlet and outlet temperatures set at 120-125 and 75-80 °C, respectively. The high-protein powders (76-82%, w/w) had large indentations with smooth surfaces, while a skim milk powder had a more wrinkled surface due to its higher lactose content. Sadek et al. (2014) and Lanotte et al. (2018) used both single droplet and monodisperse drying techniques to investigate the influence of protein type (i.e., casein and whey) on the shape and structure of powder particles. Casein particles, produced from NPC, were collapsed and wrinkled, while the whey protein particles, generated from whey protein isolate, had a more spherical shape. The shape and structure of dairy powder particles generated from spray drying may influence powder bulk properties such as flowability. Fu et al. (2012) reported that lactose powders with more spherical particles had better flowability. However, Murphy et al. (2020) did not find a relationship between particle sphericity and flowability of infant milk formula powders, possibly due to differences in surface composition.

1.4.3. Surface composition

The surface composition of dairy powders refers to the distribution of milk components (e.g., proteins, fat and lactose) at the powder particle surface and can be analysed using microscopy (e.g., confocal laser scanning, transmission electron and atomic force microscopy), and spectroscopy (e.g., x-ray photoelectron spectroscopy) techniques (Gaiani et al., 2013). Powder surface composition is influenced by the characteristics of the feed material being dehydrated, processing parameters and subsequent powder handling and storage. Kim et al. (2009) reported that increasing

total solids content of the concentrate and inlet air temperature resulted in powder with decreasing fat, but increasing levels of protein and lactose, on the surface of skim milk powder particles. The authors suggested this was caused by higher concentrate viscosity, which restricted the movement of droplet components and accelerated particle crust formation. Gaiani et al. (2010) reported that as outlet air drying temperature increased from 70 to 150 °C on a laboratory-scale spray dryer, less fat and more protein were present on the surface of native micellar casein (NMC) powder particles. Gaiani et al. (2009) stored NPC powders following manufacture and observed an increase in surface fat content from 6% at day 0 to 12 and 16% after 30 and 60 d of storage (20 °C), respectively. However, Fyfe et al. (2011) stored commercial MPC powder at 25 and 40 °C for a total of 90 d and did not observe a significant change in the proportion of fat (~30% at day 0) at the powder surface and it decreased instead. Surface composition can directly impact powder functionality, with Kim et al. (2005) reporting a relationship between surface fat and poor powder flowability. Moreover, Gaiani et al. (2010) reported that higher surface fat contents resulted in longer wetting times for NMC powders (e.g., wetting times of 932 and 631 s for powders with surface fat values of 5.3 and 0%, respectively).

1.4.4. Particle strength and friability

Friability refers to the ability of powder particles to fragment into smaller particles, while attrition is the unwanted breakdown of powder material (Barbosa Canovas et al., 2005). Friability can be measured by determining the average particle size when the powder is subjected to different air pressures (Schuck et al., 2012c). This is important as the fracture or breakage of powder particles during bagging, transport or storage, particularly of agglomerated products, could lead to undesirable

changes in density, particle size, flowability and subsequent reconstitution properties. Powders are often pneumatically conveyed in powder manufacturing plants to transport them from the dryer to silos and packaging facilities. However, it is known that air velocity can cause powder breakage in these systems (Taylor, 1998). Hazlett et al. (2021b) reported that pneumatic conveying altered the physical and rehydration properties of agglomerated powders (e.g., whey protein concentrate, infant formula and fat-filled milk powder), whereby particle size, flowability and wettability were reduced. Hanley et al. (2011) investigated the influence of pneumatic conveying on the physical and bulk handling of infant formula powders and reported that mode of conveying, number of passes and air velocity all had an effect on powder bulk density, while mode of conveying also reduced the particle size. Therefore, it is possible that agglomerated, micellar-casein dominant powders could experience similar changes in powder properties during such processes.

1.4.5. Density, air content and porosity

Bulk density refers to the mass of powder in a unit volume. It is an important characteristic of dairy powders in terms of economy and functionality. High powder bulk density will keep international shipping costs lower as a larger mass of powder will fill a product bag of fixed dimensions, leading to further savings with packaging material. The loose bulk density is typically measured by recording the weight of powder in a graduated cylinder, while a tapped bulk density measurement is performed by applying a defined number of taps (e.g., 100) using a stamping volumeter (Gea Niro, 2006). Furthermore, the density of the particles within a milk powder sample can be obtained using a gas pycnometer. Powder bulk density and air content are closely related (i.e., a powder with a high bulk density will have a low air content, and

vice versa). The air content of a powder is presented in terms of occluded (air within particles) and interstitial (air between particles) and both can be derived from equations involving density and compositional values (Schuck et al., 2012a). Two of the main factors affecting powder density are the incorporation of air into the feed before spray drying and the density of the ingredient's constituents, e.g., low-protein powders have higher density than high-protein powders due to higher lactose and lower air content (Kelly and Fox, 2016). As mentioned in Section 1.3.5., the type of atomisation device used can influence bulk density and air content (e.g., pressure nozzles produce powders with a low air content). Concentrates with a high protein content, particularly when the whey proteins are un-denatured, generate substantial foam, which results in high volumes of occluded air within powders (Pisecky, 2012a). MPC powders have been reported to contain high amounts of air. For example, Crowley et al. (2014a) reported that MPC80 had an occluded air value of 53.7 mL/100 g, compared to 18.1 mL/100 g for an MPC35, while the interstitial air values were 206 and 98 mL/100 g, respectively.

Powder porosity is the ratio of the void volume (i.e., space between and within particles) to the total volume of the bulk powder (Schulze, 2008). It can be calculated using values derived from particle and bulk density analysis (Barbosa Canovas et al., 2005). Powder porosity plays a central role in the rehydration behaviour of dairy powders, with Bouvier et al. (2013) reporting that increasing porosity of MPC powder using extrusion-porosification enhanced powder particle dispersion in water.

1.4.6. Flowability and compressibility

Flowability is a measure of a powder's resistance to movement and good flowability is crucial to ensure smooth operation of powder production facilities (e.g.,

consistent discharge of the correct quantity of powder into a subsequent processing step such as dry blending of ingredients). However, high-protein dairy powders generally have poor flowability due to several factors, including bulk composition (e.g., protein and fat content), particle and surface properties (e.g., size and density), interparticle interactions (e.g., van der Waals, electrostatic and liquid bridging) and environmental conditions (Hazlett et al., 2021a). The two flow patterns which occur in industrial powder storage containers are mass- and core-flow. Mass-flow is the preferred option for cohesive bulk particulates with poor flowability and therefore requires careful design of equipment (e.g., minimum outlet dimension) to achieve reliable movement of powder (Farnish and Berry, 2013). Flowability is influenced by many compositional and physicochemical properties of a powder, including fat and moisture content, powder particle size and storage conditions (Fitzpatrick et al., 2005, 2007; Rennie et al., 1999). Determination of milk powder flowability has been reported using a variety of techniques including the Jenike powder shear tester (Jenike, 1964), annular or ring shear cell tester (Teunou et al., 1999; McGlinchey, 2005) and measurement of the angle of repose (Geldart et al., 2006; Schuck et al., 2012b). Crowley et al. (2014a) recently used a Brookfield powder flow tester to evaluate the flow behaviours of MPC powders. The flow index values for MPC80, 85 and 90 were 3.9, 3.5 and 3.6, respectively, and were therefore classified as cohesive according to the Jenike classification system. On the contrary, MPC60 had a flow index value of 9.9 and was regarded as easy flowing. This difference in flowability was attributed to the smaller powder particle size and higher specific surface area of the high-protein MPC powders. Fitzpatrick et al. (2007) reported flow index values of 2.1 and 2.0 for high-protein rennet casein and sodium caseinate powders, respectively, but attributed this to their high moisture contents (10.1 and 7.1%, respectively).

Wall friction angle refers to the adhesion that occurs between a powder and the wall of a powder storage hopper, while the effective angle of internal friction is a measure of the friction between powder particles themselves (Barbosa Canovas et al., 2005). In addition to flowability, an annular shear tester can also be used to obtain these values, which inform the process of silo design to ensure reliable powder flow. If wall friction is not adequately considered during this process, challenges associated with powder build-up and formation of stagnant zones (i.e., rat-holing and cohesive arching) can occur in the silo (Schulze, 2008). Crowley et al. (2014a) reported that wall friction angle values for MPC80 and MPC60 powders (21.7 and 15.9°, respectively) were significantly different due to increased specific surface area of the particles with increasing protein content. However, the effective angle of internal friction was similar between these powders.

Compressibility is another functional property of powders which can provide valuable information about how powders will behave during packaging, transport and storage (Bhandari, 2013). It can be measured by calculating the change in density of a powder sample following compression (Schuck et al., 2012b). Bulk density, particle size, moisture content, air content and cohesiveness can all contribute to how much a powder compacts under stress. Crowley et al. (2014a) reported compressibility values of 79.6% for an MPC80 powder due to a high interstitial air content, compared to 46.7% for MPC60.

1.4.7. Relationship between physical properties of powders and rehydration performance

The main physical properties of powders which can influence rehydration are shown in Fig. 1.4. How much water is absorbed by the powder particles and the rate

at which the particles sink below the surface of the water can be affected by surface composition (e.g., high surface fat may impair wetting) and the density of the powder (e.g., increasing bulk and particle density will promote powder sinking). Powder agglomeration is associated with improvements in powder wettability due to the generation of larger powder clusters with numerous pores and capillaries that facilitate water transfer. Similarly, in relation to dissolution, the primary physical properties which can improve rehydration performance are powder porosity and air content. Once water has been transferred into the powder particles, those with higher porosity and air contents would be expected to breakdown and disperse more readily due to improved water transfer, greater interstitial space, and less protein-protein interactions. Conversely, powder agglomeration has been reported to impair the dissolution of micellar-casein dominant powders (Gaiani et al., 2007) as it compounds the challenge of slow dispersion.

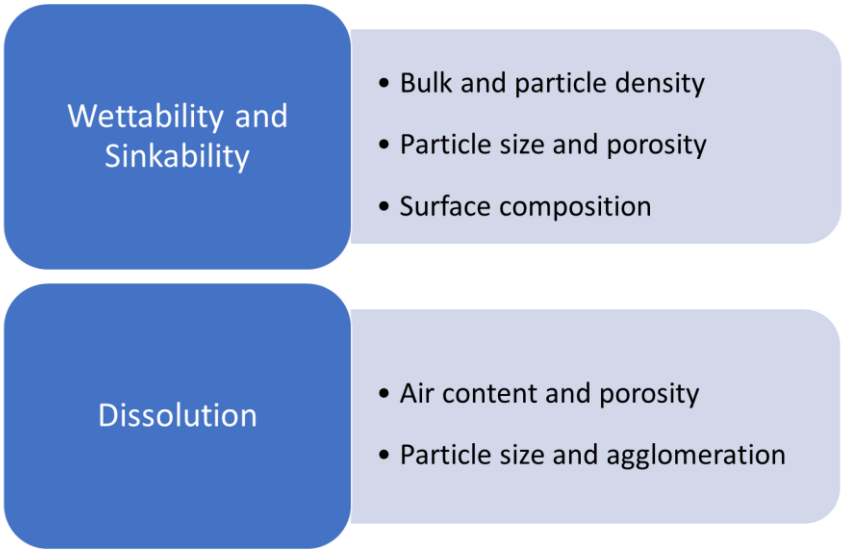

Fig. 1.4. Summary of the main physical properties of dairy powders which can influence rehydration performance

1.5. Conclusion

The processes by which dairy powders are produced greatly influences compositional, physical, structural and bulk handling properties. Heat treatment and membrane filtration parameters can alter protein denaturation levels and mineral composition, which will determine end-user applications, while spray drying can be used to directly modify powder particle size, air content and flowability, which have implications for powder quality, packaging and transport. The physical properties of powders can have a significant influence on their rehydration performance in water, and this will be discussed further in the next chapter.

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Chapter 2

Strategies to enhance the rehydration performance of micellar casein-dominant dairy powders

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Abstract

Due to their excellent nutritional (e.g., high calcium and low lactose content) and functional (e.g., heat stability and gelation) properties, the use of protein-enriched, micellar casein-dominant dairy powders, including milk protein concentrate/isolate and micellar casein concentrate, has increased considerably among food and beverage manufacturers. However, the poor, and often, inconsistent rehydration properties of these powders in water, specifically their low dispersibility and solubility, which has been attributed to protein-protein interactions related to the high proportion of micellar casein, remains a significant challenge. This review provides a detailed analysis of the main physical (e.g., injection of gas and ultrasonication) and chemical (e.g., ion exchange and pH adjustment) processing strategies that have been applied, at both laboratory and pilot-scale, to enhance the rehydration performance of high-protein, micellar casein-dominant dairy powders. The information provided will support the advancement of dairy ingredient research and the technological development of high-quality nutritional powders that can be used across several industrial applications.

Contents

2.1. Introduction

Milk protein concentrate (MPC), milk protein isolate (MPI), micellar casein concentrate (MCC) and sodium caseinate (NaCas) are some of the many casein-dominant powders currently available from the dairy industry. Two of the largest global producers of casein ingredients are the New Zealand and Irish dairy industries, producing 57,000 and 55,000 tonnes in 2019, respectively (Bord Bia, 2020). MPC and MPI powders are produced by ultrafiltration (UF) and diafiltration (DF) of skim milk, followed by evaporation and spray drying, while microfiltration (MF) is used in the production of MCC, by partially removing whey proteins. The final products normally contain at least 80% protein (w/w) and are extensively depleted in lactose and mineral salts. Applications of such micellar casein-dominant powders include medical nutritional beverages for individuals with disease-related malnutrition, performance nutrition bars for athletes, follow-on infant formulas, as well as cheese, yogurt and ice cream (Agarwal et al., 2015).

For many applications, rehydration of a powder in water or an aqueous medium is required for complete expression of protein functionality (Fang et al., 2011); therefore, achieving efficient dissolution of high-protein powders is normally essential for ingredient users (Freudig et al., 1999). For example, Karam et al. (2016) reported that the rehydration state of MCC powder influenced the textural and rheological properties of acid milk gels, whereby graininess decreased, and gel firmness increased, as the MCC ingredient became more soluble with rehydration time. Furthermore, for the consumer, complete rehydration of powered ingredients is a key quality indicator.

Rehydration of micellar casein-dominant powder is a complex process influenced by several factors (e.g., powder composition, powder density and structure, solvent composition and temperature) but generally constitutes five stages: (i) wetting,

(ii) sinking, (iii) swelling, (iv) dispersion, and (v) solubility or dissolution, as described by Crowley et al. (2016). The most commonly reported techniques in the literature to characterise these stages of rehydration include, but are not limited to, wetting behaviour using contact angle (Crowley et al., 2015, 2018), capillary rise and immersional wetting (Ji et al., 2015; Selomulya and Fang, 2013); dispersion by particle size analysis (static light scattering) following stirring (Gaiani et al., 2005; Jeantet et al., 2010) and solubility by determining changes in total solids or protein content of a powder dispersion before and after centrifugation (Bansal et al., 2017; Eshpari et al., 2014). However, it is evident that substantial variation exists with respect to the experimental parameters used for many of these techniques (e.g., for solubility determination, there are differences in the concentration of the dispersions, temperature of powder reconstitution and centrifugation conditions), which can make the comparison of results challenging. Furthermore, the authors are aware that in industrial settings, a glass slide is often used as an indicator of rehydration state by submerging it in a reconstituted product to observe the presence of insoluble material or flecks. Although this is a rapid method, it is highly subjective, and further demonstrates the uncertainty and discrepancy in how the rehydration properties of high-protein dairy powders are assessed. Furthermore, off-line techniques such as particle size analysis may not always be available to dairy processors with limited resources.

Previous reviews by Crowley et al. (2016) and Felix da Silva et al. (2018) have mainly focused on the manufacture, characteristics and stages involved in the rehydration of high-protein dairy powders, as well as advanced analytical techniques (e.g., nuclear magnetic resonance relaxometry, focused beam reflectance measurement) used for monitoring rehydration. However, the objective of this review

is to specifically provide an overview of the main processing and formulation strategies that have been investigated to modify the rehydration properties of high-protein, micellar casein-dominant dairy powders.

2.2. Scientific basis for poor and inconsistent rehydration properties

Research investigating why high-protein, micellar casein-dominant dairy powders express poor rehydration performance, both after spray drying and during storage, has presented several mechanisms responsible for the development of insolubility (Fig. 2.1). Anema et al. (2006) suggested that a network of casein micelles at the powder particle surface, formed by non-covalent bonding (e.g., hydrophobic interactions and/or hydrogen bonds), was responsible for the low solubility of MPC, with increasing storage time and temperature accelerating this deterioration in solubility. The low lactose content of MPC also facilitates protein-protein interactions as lactose would provide spatial separation of the casein micelles. This was supported by Havea (2006) who reported that the constituents of the insoluble material in MPC were linked together by non-covalent interactions.

Le et al. (2011) reported a correlation between the development of Maillard reaction products during MPC powder storage and a decrease in solubility. A subsequent study by Le et al. (2013) identified α_{s1}-casein as the predominant component of the insoluble fraction in MPC following storage and reported that methylglyoxal, formed in the advanced stages of the Maillard reaction, was capable of inducing non-disulphide, covalent cross-linking of the proteins. However, Nasser et al. (2018) reported that lactose, expected to be a key reactant in the Maillard reaction, did not play a significant role in the loss of solubility of MPC powder during

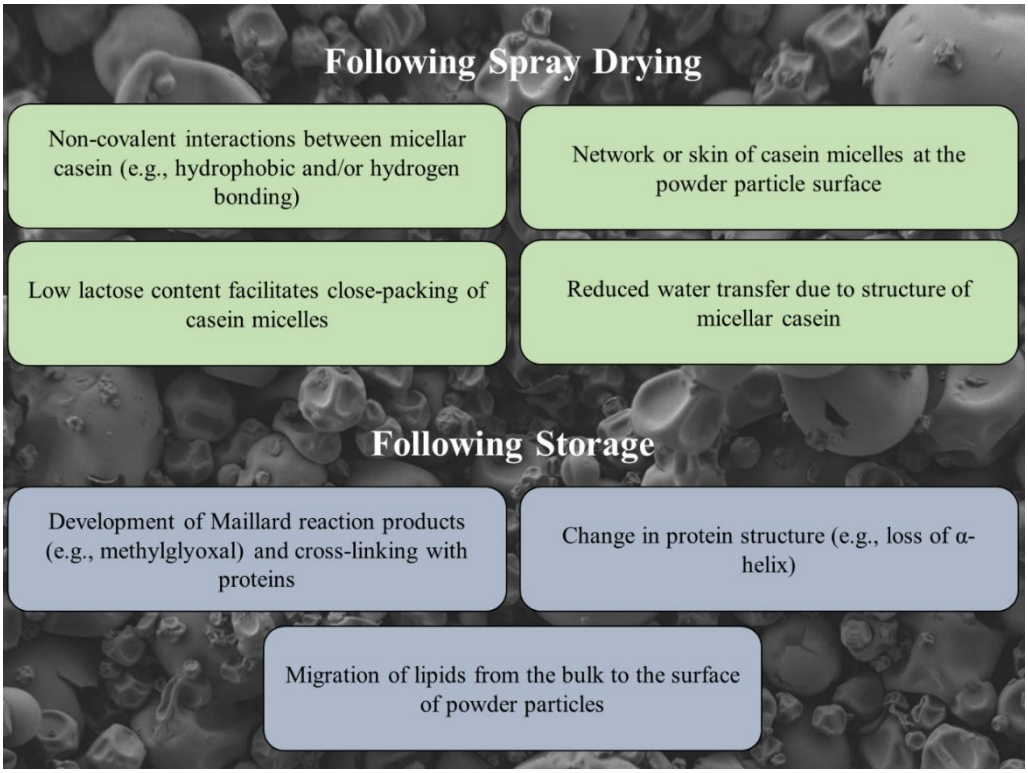

Fig. 2.1. Summary of research regarding the reasons for impaired rehydration of micellar casein-dominant powders following spray drying and on subsequent storage of spray-dried powders.

storage. Indeed, Nasser et al. (2017) established a relationship between loss of α-helix protein structure and a decrease in solubility of MCC powder during storage. Mimouni et al. (2009) reported that structural collapse and fragmentation of MPC powder particles during rehydration was restricted by the presence of a network of micellar casein at the surface of powder particles. Mimouni et al. (2010a) suggested that the loss of solubility of MPC powder during storage was caused by altered rehydration kinetics (i.e., impaired dispersion), due to the persistence of a closely-packed skin of casein micelles at the powder particle surface, while a study by Mimouni et al. (2010b) demonstrated that rehydration of MPC was characterised by distinct populations of slow (casein and colloidal mineral) and fast (whey protein and lactose) dissolving components, and that incomplete dispersion was not directly due to the formation of insoluble material during storage or reduced water penetration. Research by Schuck et

al. (1998, 2002) has suggested that the high micellar casein content of NPC reduces the transfer of water and subsequent rehydration of powder particles. Finally, despite high-protein dairy powders containing a low quantity of fat, this component is often over-represented at the surface of spray-dried powder particles and Gaiani et al. (2009) reported that lipids also migrated from the bulk to the surface of native phosphocaseinate (NPC) powder particles during storage, thereby increasing wetting times.

Several physical and chemical processing strategies have been investigated in an effort to resolve the aforementioned challenges. An overview of these approaches is given in Tables 2.1 and 2.2, while a schematic representation of the stages in the manufacturing process where some of these strategies may be implemented is given in Fig. 2.2. It is important to consider that many of the approaches discussed are applied for the purpose of creating a spray-dried powder with enhanced rehydration properties, while other strategies are examined in the context of aiding powder solubilisation after spray drying.

2.3. Physical processing strategies to enhance powder rehydration

2.3.1. Addition of gas to the concentrate before spray drying

The addition of gases to dairy concentrates prior to spray drying has been investigated as an approach for modifying the physical and rehydration properties of powders. Marella et al. (2015) injected carbon dioxide (CO_2) into skim milk before and throughout UF to modify the subsequent rehydration properties of MPC powder, with an improvement in powder solubility attributed to the solubilisation of calcium phosphate, caused by a reduction in pH due to the formation of carbonic acid (the effect of decreasing concentrate pH on subsequent powder rehydration is further

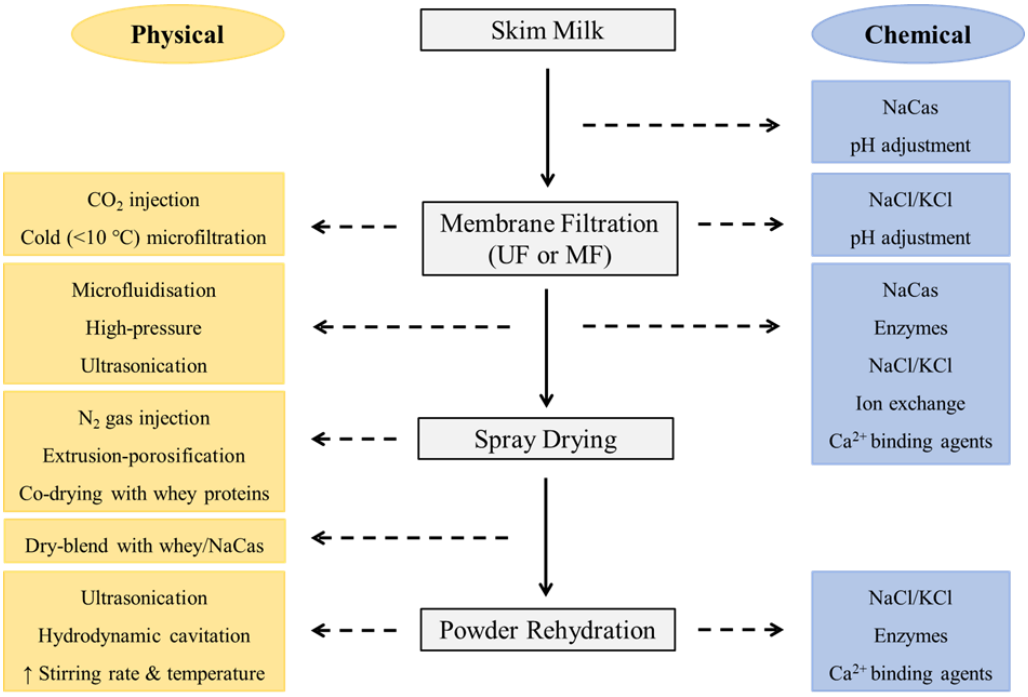

Fig 2.2. Schematic representation of the stages during processing where physical and chemical modifications may be implemented to alter powder rehydration properties.

discussed in Section 2.4.1). Aside from altering the chemical composition of the powder (i.e., lower calcium content), gas injection has been used to improve rehydration performance by modifying the structure of powder particles. Bell et al. (1963) produced skim milk powder with higher dispersibility by injecting compressed air into the product feed line of the spray dryer, between the high-pressure pump and atomisation nozzle. Recent studies by McSweeney et al. (2021a, b) demonstrated that nitrogen gas (N_2) injection prior to spray drying (i.e., between the high-pressure pump and atomisation nozzles) can improve the rehydration characteristics, particularly the dispersion and solubility, of MPC80 (i.e., 80% protein, w/w). This improvement in water transfer was attributed to higher powder porosity and interstitial space, combined with lower powder density. Particle size distribution (PSD) analysis showed that the mean D_{90} value (i.e., the size of particles below which 90% of the sample lies), following reconstitution in ultrapure water (50 °C), was significantly lower for MPC

powder produced using N_2 injection (0.4 µm) compared to the control (66 µm). Bouvier et al. (2013) used a novel technology called extrusion-porosification to produce MPC powders with a high dispersibility index (96%) compared to a conventionally spray-dried MPC powder (38%). This process involved the incorporation of CO_2 into a high-total solids (38%, w/w) concentrate using a twin-screw extrusion-aeration system, followed by spray drying of a high-solids foam; after 2 h of rehydration, only sub-micron sized particles were present in the sample produced using extrusion-porosification, indicating complete dissolution. The formation of numerous pores within the powder particles and the partial dissociation of casein micelles were responsible for the improvements in water transfer and rehydration. It is evident that using gases such as N_2 and CO_2 during dairy processing can enhance the dispersion of dairy protein powders via changes in composition (e.g., reduced calcium content following the incorporation of CO_2 into the liquid concentrate), micellar casein structure and/or powder particle structure, depending on where in the process it is applied. However, an important consideration is the altered physical and bulk handling properties of such ingredients produced using gas injection (McSweeney et al., 2021a); for example, the injection of N_2 directly prior to spray drying can lower the particle and bulk density and produce cohesive powders that do not flow easily, thereby potentially presenting challenges in industrial powder handling processes.

2.3.2. High shear: Homogenisation, microfluidisation and hydrodynamic cavitation

High-shear treatments, including homogenisation, microfluidisation and hydrodynamic cavitation (HC), have been investigated as processing technologies that could be used to improve powder rehydration, without altering the ingredients

Table 2.1. Overview of literature regarding physical processing strategies to enhance powder rehydration.

Strategy	Powder	Measurement techniques	Results	Reference
Addition of gases				
CO_2 injection during membrane filtration	MPC80	Dispersion: Particle size distribution (PSD) Solubility: Total solids (TS) before & after centrifugation (700*g* for 10 min)	↑ dispersion ↑ solubility	Marella et al. (2015)
N_2 injection before drying	SMP	Solubility: TS before & after filtration (100 and 150 mesh funnel)	↑ dispersion	Bell et al. (1963)
	MPC80	Dispersion: PSD Solubility: TS before & after centrifugation (3000*g* for 10 min)	↑ dispersion ↑ solubility	McSweeney et al. (2021b)
Extrusion-porosification	MPC80	Dispersion: PSD and dispersibility index Solubility: TS before & after centrifugation (160*g* for 5 min)	↑ dispersion ↑ solubility	Bouvier et al. (2013)
High-shear treatment				
Microfluidisation before drying	MPC80 & 90	Solubility: Protein content before & after centrifugation (3000 g for 10 min) Insolubility index: Sediment height after centrifugation (160*g* for 10 min)	↑ solubility MPC80 ↔ solubility MPC90	Augustin et al. (2012)
Homogenisation before drying	MPC80		↑ solubility	Augustin et al. (2012)
Hydrodynamic cavitation before drying	MPC80	Solubility: TS before & after centrifugation (700*g* for 10 min)	↔ solubility	Li et al. (2018)
Homogenisation after drying	MPC80	Solubility: TS before & after centrifugation (700*g* for 10 min)	↑ solubility	Sikand et al. (2012)

	MPC55 & 80	Dispersion: PSD	↑ dispersion	Warncke & Kulozik (2020)
	MPC80 & MCC	Dispersion: PSD Solubility: TS before & after centrifugation (4400 rpm for 5 min)	↑ dispersion ↑ solubility	Chandrapala et al. (2014a)
Hydrodynamic cavitation after drying	MPC80	Dispersion: PSD & analytical centrifugation (670*g* for 3 h)	↑ dispersion	Pathania et al. (2018)
High-pressure processing				
Before drying	MPC85	Solubility: Protein content before & after centrifugation (3000*g* for 10 min)	↑ solubility	Udabage et al. (2012)
Ultrasonication				
Before drying	MPC80		↑ solubility	Augustin et al. (2012)
	MPC80 & CaCas	Dispersion: PSD Solubility: TS before & after centrifugation (2125*g* for 5 min)	↑ MPC solubility ↔ CaCas solubility	Chandrapala et al. (2014b)
	MPC80	Dispersion: PSD Solubility: TS before & after centrifugation (4400*g* for 10 min)	↑ dispersion ↑ solubility	Yanjun et al. (2014)
After drying	MPC80 & MCC		↑ dispersion ↑ solubility	Chandrapala et al. (2014a)
	MPC80	Dispersion: PSD Solubility: TS before & after centrifugation (700*g* for 10 min)	↑ dispersion ↑ solubility	McCarthy et al. (2014)
Membrane filtration				
Cold (4 °C) microfiltration	MCC75	Wettability: Contact angle Dispersion: PSD	↔ wettability ↑ dispersion	Crowley et al. (2018)
Microfiltration and acidification	MCC85	Insolubility index: Sediment height after centrifugation (700*g* for 10 min)	↑ solubility	Schäfer et al. (2021)

Feed concentration using nanofiltration	MPC60	Insolubility index: Sediment height after centrifugation (900*g* for 5 min)	↑ solubility	Cao et al. (2015, 2016)
Agglomeration and granulation				
Fluidised bed granulation with binders (lactose, sucrose or water)	MPI	Wettability: Washburn method Dispersion: PSD	↑ wettability ↔ dispersion	Ji et al. (2015)
Addition of lecithin or tween 80 during fluidised bed granulation	MPI	Wettability: Wetting time & contact angle Dispersion: PSD Solubility: Analytical centrifugation	↑ wettability ↔ dispersion & solubility	Wu et al. (2020)
Agglomeration using fines return during co-drying	NPC & WPI	Turbidity sensor	↑ wettability ↓ rehydration time	Gaiani et al. (2007)
Agglomeration using fines return	MPC80	Wettability: Capillary rise Dispersion: PSD Solubility: TS content before & after centrifugation (3000*g* for 10 min)	↑ wettability ↓ dispersion ↓ solubility	McSweeney et al. (2021b)
Rehydration conditions				
Influence of temperature, stirring speed & solid concentration	MCI	Dispersion: PSD	↓ rehydration time with ↑ in temperature	Jeantet et al. (2010)
Influence of temperature, agitator & stirring speed	NPC	Dispersion: PSD	↓ rehydration time with ↑ in stirring rate	Richard et al. (2013)

Milk protein concentrate (MPC); skim milk powder (SMP); calcium caseinate (CaCas); micellar casein concentrate (MCC); milk protein isolate (MPI); native phosphocaseinate (NPC); micellar casein isolate (MCI). The number following the powder abbreviation denotes the approximate protein content (%, w/w).

chemical composition. Microfluidisation is a form of homogenisation which operates on the principle that the liquid is divided into two or more microstreams which are directed towards each other using a high-pressure pump (McCrae, 1994), whereby a combination of turbulent flow, cavitation and shear reduce droplet size (Maa and Hsu, 1999). Augustin et al. (2012) reported the effect of homogenisation or microfluidisation of the liquid concentrate before spray drying on the solubility of high-protein MPC powders after production and subsequent storage at 22 °C for eight months. The solubility of the MPC powder produced following microfluidisation of the concentrate (800 bar) was 89.5% after manufacture and 68.7% after eight months of storage, while in comparison, concentrates homogenised at first- and second-stage pressures of 350 and 100 bar had solubility values of 74.5 and 58.7% after production and eight months of storage, respectively. The solubility of the control powder (i.e., no treatment) was 70.1 and 51.1% at these respective time points, but statistical significance was not provided. In a separate investigation within this study, microfluidisation was applied at three different pressures (400, 800 and 1200 bar) to liquid MPC before spray drying and it was reported that solubility of the MPC powders was not significantly different from the non-microfluidised powders after manufacture and 2 months of storage, suggesting its use before spray drying may not be worthwhile. Another study involving high-shear treatment of dairy concentrate, performed by Li et al. (2018), investigated the use of HC prior to spray drying and reported that concentrate viscosity decreased but powder solubility was not noticeably changed by the HC process. This technology involves the generation and collapse of bubbles due to changes in pressure, with the accompanying release of energy, causing a powerful mixing effect, which reduces particle size (Gogate, 2011).

An alternative option of using high shear to enhance powder solubilisation

after a standard spray drying process has also been reported by Sikand et al. (2012), whereby powder reconstitution in 37 °C water, followed by homogenisation (138 bar), improved the solubility of MPC powder. The mean solubility index, which represented the quantity of sedimented material present following centrifugation, was significantly lower (1.02 mL) when homogenisation was applied compared to non-homogenised MPC (1.79 mL). Similarly, Warncke and Kulozik (2020) investigated the effect of high-pressure homogenisation (HPH; 100-500 bar) on the solubility of reconstituted (45 min at 50 °C) MPC55, MPC80 and MCC powders. MPC55 already had a monomodal PSD in the casein micelle size range (i.e., 150-200 nm) after stirring and further treatment using HPH did not alter solubility. However, for MPC85, a monomodal PSD in this range was obtained after HPH at 200 bar, while a pressure of 500 bar was required to dissolve the MCC powder. Furthermore, HC has also been investigated as a physical processing strategy for accelerating rehydration of spray-dried powders. Pathania et al. (2018) demonstrated that HC was more effective in rapidly rehydrating MPC powders in comparison to conventional high-shear treatment. The volume-weighted mean particle diameter ($D_{[4,3]}$) value was significantly lower for the HC dispersion (0.19 µm) compared to the sample prepared using conventional high-shear mixing (5.62 µm).

It has been suggested by Augustin et al. (2012) that when high-shear treatments are applied to the concentrate prior to spray drying, these technologies may decrease viscosity and/or alter protein structure, thereby improving solubility of the subsequent powder. However, the exact mechanism by which this occurs has not been elucidated and some studies have found no beneficial effect on powder solubility using this specific approach (Li et al., 2018). Alternatively, when these physical processing strategies are used to reconstitute spray-dried powders, enhancement of solubility is

generally attributed to energy input, which accelerates the breakdown of large powder particles and disrupts protein-protein interactions; however, their use may incur high capital and operating costs. Overall, these technologies do not address the challenge encountered by ingredient manufacturers in creating high-quality, soluble powders for customers but would be useful for end-users who need to quickly reconstitute spray-dried dairy powders for use in various applications.

2.3.3. High-pressure processing

The use of high-pressure (HP) treatment in dairy processing has been reviewed by Huppertz et al. (2002, 2006), with some of the reported effects including whey protein denaturation and a change in casein micelle size, and the magnitude of these effects dependent on factors such as pressure and temperature. The potential use of HP treatment to enhance the rehydration characteristics of high-protein, micellar casein-dominant powders has been investigated by Udabage et al. (2012). A range of pressures (100-400 MPa) and temperatures (10-60 °C) were applied to liquid MPC and the subsequent solubility of the MPC powder investigated after spray drying. The most significant improvement in solubility of the MPC was obtained when a pressure and temperature of 200 MPa and 40 °C, respectively, were applied to the concentrate, with the powder solubility value after this treatment being 85% compared to 66% for the MPC which received no HP treatment at 40 °C, and this was attributed to the partial dissociation of casein micelles to their non-micellar form. The authors also found that a high-protein powder produced by dry blending NaCas and whey protein isolate (WPI) had higher solubility than MPC, showing that micellar casein hinders the reconstitution process of these powders. Furthermore, it is important to note that MPC powders could not be produced when the concentrates were subjected to 200 MPa at

10 and 25 °C, or 400 MPa at 25, 40 and 60 °C, due to gelation caused by whey protein denaturation and dissociation of casein micelles. Cadesky et al. (2017) also reported that HP processing (150-450 MPa) altered the physicochemical properties of liquid MPC and MCC, prepared at 2.5 and 10% protein (w/v). Dissociation of the casein micelles took place after the concentrates were subjected to a pressure of 150 MPa, while a gel formed after treatment at 450 MPa due to destabilisation and aggregation of casein micelles, with the denaturation of serum proteins also likely contributing. Therefore, gelation of concentrates would be an important factor to consider if HP were to be applied industrially for improving solubility of casein-dominant dairy powders. HP processing may be a useful strategy for partially dissociating casein micelles without altering the composition of the product or requiring the addition of other chemicals or ingredients; however, similar to high-shear treatments such as microfluidisation and HC, it may not be an economically feasible approach in terms of capital and operating costs.

2.3.4. Ultrasonication

There are two forms of ultrasonication (US) generally used in food processing: (i) low-frequency (16-100 kHz), high-intensity (10-1000 W/cm^{-2}) and (ii) high-frequency (100 kHz-1 MHz), low-intensity (<1 W/cm^{-2}) ultrasound (O'Sullivan et al., 2017). Ultrasonic waves of high intensity induce changes to food systems through cavitation, capable of generating large increases in temperature and shear (O'Brien, 2007; O'Donnell et al., 2010). Chandrapala et al. (2014b) performed US (frequency of 20 kHz, power of 31 W and amplitude of 50%) on reconstituted (i.e., stirred for 1 h at 22 °C followed by overnight storage at 4 °C) MPC and calcium caseinate (CaCas) dispersions prior to spray drying, and measured solubility initially and after storage

(30 and 60 d at 25 ºC) at a relative humidity (RH) of 23 and 75%. Powders had similar solubility values after manufacture; however, following 30 d of storage at 23% RH, US-MPC samples displayed higher solubility (~97%) than the MPC control (83%). After 60 d of storage, this trend persisted, with solubility values of ~88 and 63% for US and control MPC powders, respectively; in contrast, US did not alter the solubility of CaCas, remaining at ~90% throughout the study. The higher solubility of MPC powders after storage was attributed to the breakdown of whey protein-casein micelle aggregates during US. It is possible that the dispersions prepared for spray drying were not completely solubilised beforehand given the short reconstitution time, which may have contributed to the presence of large particles in the powder. Similarly, Augustin et al. (2012) performed US (24 kHz, 160 mL/min at 600 W) on UF retentate prior to spray drying and reported that the solubility of the MPC powder was only slightly improved, with the measured solubility for US and control MPC powders after manufacture being 74.7 and 70.1%, respectively, while after eight months of storage, solubility remained marginally higher (55.1%) for US-MPC compared to the control (51.1%). It appears that the application of US prior to spray drying does not significantly alter powder solubility initially but provides some protection against storage-induced loss of solubility. However, Yanjun et al. (2014) also investigated the relationship between the application of US (20 kHz, 12.5 W and 50% amplitude) to UF concentrates before spray drying and the solubility of the MPC powder. Solubility was significantly higher for the MPC which received 5 min of US pre-treatment (88.3%) compared to the control (35.8%). The authors attributed the increase in solubility to a change in protein structure and an increase in the presence of charged groups (e.g., COO^-), although this was not specifically measured. Similar to the results involving high-shear treatment of concentrates in Section 2.3.2., it is apparent that the

exact mechanism by which US prior to spray drying could confer enhanced solubility to powders remains unclear.

US has also been investigated for its potential in accelerating powder solubilisation after the spray drying process. Chandrapala et al. (2014a) compared the solubilisation of spray-dried MPC and MCC powders using US (20 kHz, 31 W, amplitude of 50%), HPH (single stage at 80 or 200 bar) or high-shear rotor-stator mixing (HSRSM; 17500 rpm). The $D_{[4,3]}$ values for MPC and MCC were considerably lower after US for 5 min (1.1 and 0.8 µm, respectively) compared to 5 min of HSRSM (25 and 52 µm, respectively). HPH performed similarly to US in reducing particle size as the $D_{[4,3]}$ was 1.2 and 0.3 µm for MPC and MCC, respectively. Each of these three approaches provided an improvement in solubilisation of micellar casein-dominant powders as they accelerated the structural collapse of powder particles and the release of their constituents (e.g., caseins, minerals). McCarthy et al. (2014) investigated the effect of US (20 kHz, 70.2 W and amplitude of 100%) and overhead stirring (450 rpm) on rehydration of MPC powder. PSD analysis showed that after 10 min of overhead stirring, the D_{90} of the MPC dispersion was 76.6 µm, compared to 0.41 µm after US for 1 min. Furthermore, the solubility of MPC dispersions after 10 min of overhead stirring in water at 25 and 50 °C was 45.8 and 89.7%, respectively, while solubility was 99.6% following US for 1 min. Similar to high-shear treatments described previously, US appears to be a useful technology in facilitating the rehydration of spray-dried, high-protein dairy powders, but it could also present several challenges with implementation at an industrial scale. For example, the installation of an US system would involve significant capital costs, be difficult to incorporate into a continuous industrial process, it generally provides a localised effect, and the probe could erode over time and contaminate the product with metal fragments.

2.3.5. Membrane filtration: Micro-, ultra- and nanofiltration

As membrane filtration is the technological enabler in the production of high-protein products, it seems logical that interventions offering potential to improve subsequent powder solubility would be considered at this stage in the process, with several recent studies reporting the impact of membrane filtration unit operations and processing conditions on the physicochemical properties of the derived streams and subsequent spray-dried powders. Crowley et al. (2018) produced MCC powders using MF and DF of skim milk at both cold (<10 °C) and warm (50 °C) temperatures, followed by spray drying. No differences were recorded between powders in their wetting behaviour or contact angle, as measured using optical tensiometry. PSD analysis demonstrated that MCC powders produced using cold MF had higher dispersibility than powders produced using warm MF; for example, after rehydration in water (50 °C) for 90 min, 48% of the particles had diameters <1 μm for MCC powders produced using cold MF, compared to 7.5% for powders produced using warm MF. This suggests that a higher proportion of casein micelles were present in solution (i.e., released from dissolved powder particles) following reconstitution of the cold MF powders. The superior dispersibility of MCC powder produced using cold MF was likely a result of several factors, including lower calcium, lower β-casein and higher whey protein content in such powders.

Schäfer et al. (2021) used membrane filtration and pH adjustment to produce calcium-reduced MCC powders. This was achieved by concentrating the skim milk at pH 6.2 using MF, followed by acidification of the MF retentate to pH 5.6 and performing both MF and DF prior to spray drying. Powders depleted in calcium by approximately 50% had significantly higher solubility compared to the control powder, as they formed 3.1 and 4.7 mL of insoluble material, respectively. France et

al. (2021) recently investigated the impact of temperature (4, 8, 12, 16 and 20 °C) and transmembrane pressure (0.05 or 0.30 bar) on membrane filtration performance and the physicochemical properties of the streams produced from the MF of skim milk. Concentrate viscosity was higher and membrane flux was lower when MF was performed at 4 °C, while protein retention by the membrane increased as the temperature and transmembrane pressure were increased. The effect of temperature (5, 20 and 50 °C) during UF of skim milk, the initial step in MPC manufacture, has been reported by Puri et al. (2020). Similar to the previous study, permeate flux was lower at lower temperature, most likely due to increased viscosity, resulting in membrane fouling and the blockage of pores. The retentates produced at 5 and 20 °C had a significantly lower content of total calcium and phosphorus compared to that produced at 50 °C, suggesting that some colloidal calcium phosphate (CCP) was solubilised at the lower processing temperature. The effect of cold UF on the rehydration properties of MPC powders has not been established in the literature but would likely generate improvements in powder dispersion due to a lower total calcium content. The industrial application of cold membrane filtration to manufacture high-protein, micellar casein-dominant powders would possibly be limited by the operating costs to maintain a low processing temperature, higher pressures to pass components of the viscous feed through the membrane and longer operating times to achieve the desired protein content in the retentate.

Cao et al. (2015) compared the use of nanofiltration (NF) or evaporation (EP) for concentration of UF retentate before spray drying on the physicochemical properties of MPC powders. The insolubility index (ISI) was significantly lower for NF-MPC (0.32 mL) compared to EP-MPC (0.90 mL), while the free sulfyhdryl group content of NF-MPC powder was significantly higher than that of EP-MPC. It is

possible that the heat treatment received by the concentrate during EP may have caused the formation of protein aggregates which subsequently sedimented during centrifugation. A follow-up study by Cao et al. (2016) investigated the influence of storage on these powders over 24 weeks at 25, 35 and 45 °C. NF-MPC had better solubility compared to EP-MPC after storage; for example, after 24 weeks at 25 °C, the ISI was approximately 2.4 and 4.8 mL for NF- and EP-MPC, respectively. It is apparent that membrane filtration conditions and concentration processes applied prior to spray drying play a crucial role in manipulating the rehydration properties of micellar casein-dominant powders.

2.3.6. Agglomeration during spray drying and fluidised bed granulation

Agglomeration is generally used to improve the physical (e.g., flowability) and rehydration (e.g., wettability) characteristics of low-protein dairy powders such as whole milk and fat-filled powders (Pisecky, 2012), but has recently been investigated as a strategy to modify the functionality of high-protein powders. Gaiani et al. (2007) spray dried WPI, NPC and NPC plus WPI concentrates, and produced agglomerated and non-agglomerated variants of the powders to investigate the influence of protein type and agglomeration on powder rehydration, with agglomeration performed by returning fine particles to the top of the drying chamber and bringing them into contact with the atomised feed. The wetting behaviour of agglomerated, casein-dominant powders was improved compared to the non-agglomerated powders, but dissolution was impaired. McSweeney et al. (2021b) produced agglomerated MPC powders using fines return during spray drying and reported greater capillary rise wetting and water diffusion, but impaired dispersion and solubility, for the agglomerated powders compared to non-agglomerated MPC.

When agglomeration is performed in a fluidised bed towards the end of the spray drying process, the term granulation is often used to describe this process of joining powder particles together using binding agents. Ji et al. (2015) granulated MPI powders in a fluidised bed system using water or binders (i.e., sucrose or lactose solutions). Wettability was higher for MPI agglomerated using lactose, while it was lowest for the non-agglomerated MPI. The quantity of water absorbed increased with increasing powder particle size for all samples. However, PSD analysis demonstrated that granulation and the use of hydrophilic binders did not result in any improvement in the dispersion and solubilisation of the MPI powders. Wu et al. (2020) sprayed surfactants (Tween 80 and lecithin) onto MPI powder during granulation in a fluidised bed and reported that wetting times were lower for Tween 80 and lecithin coated powders in comparison to the MPI powder with no added surfactant (e.g., 15-50 s for MPI coated with Tween 80 compared to 36 min for the MPI control), most likely due to reduced surface tension on inclusion of surfactant. However, dispersion and solubility were not significantly improved by the use of these surfactants. Therefore, agglomeration during spray drying and the use of surfactants or binders in fluidised bed granulation can improve the instant properties of micellar casein-dominant powders but are generally ineffective in improving the key subsequent stages of rehydration (i.e., dispersion and dissolution).

2.3.7. Rehydration conditions

The selection of appropriate rehydration conditions (e.g., solvent temperature, total solids content, stirring rate, impeller design) can play an important role in optimising the dissolution of casein-dominant powders and thereby increase process efficiency for manufacturers. Jeantet et al. (2010) investigated the effect of

temperature (26-30 °C), total solids concentration (4.8-12%, w/w) and stirring rate (400-1000 rpm) on the rehydration characteristics of MCC powder. Temperature played a significant role in the process as it was shown that a 4 °C increase in temperature had the same effect on rehydration kinetics as doubling the stirring rate from 400 to 800 rpm. Increasing the concentration of solids significantly increased the stirring rate required but did not affect rehydration time to the same extent as temperature. Therefore, it was suggested that temperature is a crucial parameter to consider when rehydrating casein-dominant dairy powders.

Richard et al. (2013) monitored how temperature (25 and 30 °C), stirring speed (500-900 rpm) and agitator design (six-pitched-blade impeller or two impellers with right angled arrangement) influenced the rehydration behaviour of granulated and non-granulated NPC, WPI, NPC plus WPI and NPC plus lactose powders. Increasing stirring speed from 700 to 900 rpm reduced rehydration time by 25% on average; however, similar to previous work by Jeantet et al. (2010), rehydration was more sensitive to changes in temperature than stirring rate. Granulated powders required longer rehydration times, particularly for NPC powders, e.g., 380 min for granulated NPC compared to 220 min for non-granulated NPC at 900 rpm. The choice of impellar design impacted the rehydration of NPC powder in particular; the 6-pitched blade design resulted in greater particle breakdown due to greater energy dissipation, while the dual propeller design instead created more particle circulation. It is evident that higher temperatures and stirring rates are advantageous in accelerating the rehydration of micellar casein-dominant powders but would result in greater energy consumption.

2.4. Chemical modification and formulation strategies to enhance powder rehydration

2.4.1. Adjustment of pH before, during or after membrane filtration

Several studies have investigated the effect of reducing the pH of skim milk during membrane filtration and the subsequent solubility of the MPC powders produced. Liu et al. (2019) acidified skim milk (pH 6.7, 6.0, 5.7 and 5.4) using glucono-delta-lactone (GDL) before membrane filtration, followed by pH restoration of the retentate directly prior to spray drying. The amount of total calcium present in the reconstituted MPC powder was lowest for the sample pre-acidified to pH 5.4, which can be attributed to the passage of serum calcium through the membrane into the permeate following solubilisation of CCP. PSD analysis showed a decrease in particle size of MPC dispersions with decreasing pH from 6.7 to 5.4. Solubility values for the MPC dispersions increased with decreasing pH of pre-acidification and were slightly higher when the retentate pH was re-adjusted prior to spray drying compared to samples which were acidified only. The pH 6.7 sample had an initial solubility of 89% but this was just 19% after 84 d of storage at 40 °C; however, the pH 5.7 sample prepared from pH restored retentate had a solubility of 97 and 91% at these time points, respectively. Importantly, this demonstrates that storage-induced solubility loss can also be reduced when skim milk is acidified prior to membrane filtration and spray drying. Luo et al. (2016) acidified skim milk (pH 6.7, 6.3, 5.9 or 5.5) prior to UF and freeze drying. Lowering the pH of the skim milk feed from 6.7 to 5.5 before membrane filtration resulted in a significant decrease in solubility of the reconstituted MPC powders from 77 to 32%. However, upon restoration of the MPC dispersion to pH 6.7, this trend was reversed, e.g., ~90 and 73% solubility for pH 5.5 and 6.7 samples, respectively. In addition to the effects on powder solubility, lowering the pH of the feed to 5.5 significantly reduced membrane flux as pores became blocked, and the factors contributing to this included changes in casein micelle size, solubilisation

Table 2.2. Overview of literature regarding chemical modification and formulation strategies to enhance powder rehydration.

Strategy	Powder	Measurement techniques	Results	Reference
Adjustment of pH before, during or after membrane filtration				
Acidification (pH 6.7, 6.0, 5.7, 5.4)	MPC85	Dispersion: Particle size distribution (PSD) Solubility: Total solids (TS) before & after centrifugation (700*g* for 10 min)	↑ dispersion ↑ solubility	Liu et al. (2019)
Acidification (pH 6.7, 6.3, 5.9, 5.5)	MPC55	Solubility: Protein content before & after centrifugation (12000*g* for 25 min)	↑ solubility (pH restoration)	Luo et al. (2016)
Acidification (pH 6.6, 6)	MPC65 & 80	Dispersion: PSD Solubility: TS before & after centrifugation (700*g* for 10 min)	↔ dispersion ↑ solubility	Eshpari et al. (2014)
Alkalinisation (pH 6.9, 7.3, 7.6)	MCC75	Wettability: Contact angle Dispersion: PSD	↓ wettability ↑ dispersion	Panthi et al. (2021)
Ion exchange				
Before drying	MPI	Solubility: TS before & after centrifugation (700*g* for 10 min)	↑ solubility	Bhaskar et al. (2001)
Addition of calcium-binding agents				
Sodium phosphate (SP), trisodium citrate (TSC) or sodium pyrophosphate before membrane filtration	MPC80	Dispersion: PSD Solubility: TS before & after centrifugation (4400*g* for 10 min)	↑ dispersion ↑ solubility	Sun et al. (2017)
SP or TSC via co-drying, bi-drying & dry-mixing	NPC	Insolubility index Nuclear magnetic resonance (NMR)	↑ solubility ↓ rehydration time	Schuck et al. (2002)
Citrate before drying	MCC85	Dispersion: PSD	↑ solubility	Schokker et al. (2011)

		Solubility: TS before & after centrifugation (750*g* for 15 min)		
SP, TSC or sodium hexametaphosphate after drying	MPC80	Dispersion: PSD Solubility: TS before and after centrifugation (3000*g* for 10 min)	↑ dispersion ↑ solubility ↓ turbidity	McCarthy et al. (2017)
Addition of monovalent or divalent salts				
KCl or NaCl during diafiltration	MPC80	Solubility: Protein content before & after centrifugation (20000*g* for 30 min)	↑ solubility	Sikand et al. (2013)
NaCl during diafiltration	MPC80	Solubility: TS before & after centrifugation (700*g* for 10 min)	↑ solubility	Mao et al. (2012)
NaCl or $CaCl_2$ before drying	NPC		↑ solubility (NaCl)	Schuck et al. (2002)
NaCl or $CaCl_2$ before drying	MCC85		↑ solubility (NaCl)	Schokker et al. (2011)
NaCl before dying	NPC	Insolubility index Rehydration time: NMR	↑ solubility	Davenel et al. (2002)
NaCl or $CaCl_2$ after drying	NMC	Rehydration time: Turbidity sensor	↓ rehydration time	Hussain et al. (2011)
Enzymatic or chemical modifications of protein				
Crosslinking using transglutaminase before drying	MPC80	Wettability: Washburn method Diffusion: Confocal laser scanning microscopy	↑ wettability ↑ diffusion	Power et al. (2020)
Chymotrypsin, trypsin and papain after drying	MPC80	Solubility: Protein content before & after centrifugation (10000*g* for 10 min)	↑ solubility (pH 4.6-7)	Banach et al. (2013)
Flavourzyme™, Neutrase™ and Protamex™ after drying	MPI	Solubility: Protein content before & after centrifugation (3000*g* for 10 min)	↑ solubility (pH 6.5)	Ryan et al. (2018)

Succinylation after drying	MPC85	Dispersion: PSD Solubility: Protein content before & after centrifugation (1200*g* for 20 min)	↑ dispersion ↑ solubility	Shilpashree et al. (2015)
Addition of dairy proteins				
NaCas before diafiltration, before drying or dry blending with MC	MCC85		↑ solubility	Schokker et al. (2011)
NaCas via wet- or dry-blending	MPI	Dispersion: PSD	↑ dispersion	Bot et al. (2020)
Whey protein before drying	NPC	Rehydration time: NMR	↓ rehydration time	Davenel et al. (2002)
Whey protein-rich peptide hydrolysate before drying	MPC80	Protein solubility assay	↑ solubility	Torres Hernandez et al. (2018)
Addition of molecular spacers				
Addition of lecithin nanovesicles before drying using microfluidisation	MPC80	Solubility: TS before & after centrifugation (1000*g* for 10 min)	↑ solubility	Bansal et al. (2017)

Milk protein concentrate (MPC); milk protein isolate (MPI); native phosphocaseinate (NPC) native micellar casein (NMC); micellar casein concentrate (MCC); sodium caseinate (NaCas). The number following the powder abbreviation denotes the approximate protein content (%, w/w).

of salts from the micelle and increased viscosity. Eshpari et al. (2014) acidified skim milk to pH 6 using GDL prior to UF alone or UF combined with DF and reported that acidification caused a significant decrease in the calcium content of MPC from 1.84 to 1.59 g/100 g powder. Solubility was higher for the MPC which was acidified using GDL (~82%) before UF and DF compared to the control which received no GDL treatment (~72%). However, the PSD profiles following reconstitution of control and acidified MPC powders were similar, with monomodal peaks in the size range 10-300 µm. Thus, some disparities are apparent in the rehydration data available from experiments involving pH adjustment before membrane filtration and further work is required to determine the effects on both powder dispersibility and solubility. Alternatively, Panthi et al. (2021) increased the pH of MF retentates (pH 6.9 to pH 7.3 and 7.6) prior to freeze drying and reported that MCC powders had lower wettability but higher dispersibility with increasing retentate pH. The powder derived from the retentate that was re-adjusted from pH 7.6 to pH 6.9 had the highest dispersibility and this was attributed to changes in the ionic environment of the serum phase (e.g., higher calcium concentration resulting from partial solubilisation of CCP). This supports the positive effect of pH re-adjustment on powder rehydration performance that was reported in previous studies by Liu et al. (2019) and Luo et al. (2016).

The pH adjustment of dairy concentrate enables the mineral profile of the powder to be altered via a reduction in the CCP content, and this appears to enhance solubility of resultant powders, likely due to an increase in electrostatic repulsive forces between casein proteins. However, casein-dominant powders with reduced levels of micellar casein and calcium phosphate may not be suitable for applications such as cheese manufacture. Lucey and Fox (1993) discussed the significant role

played by calcium and phosphate in the production of several cheeses, including their impact on rennet coagulation and gel strength, while Lin et al. (2017) reported that an increased presence of non-micellar casein, generated by the addition of NaCas to skim milk, can adversely affect rennet gelation as it impairs the formation of a gel network. Another consequence of concentrate acidification to consider is that the permeate generated from such a process will contain higher levels of calcium and phosphorus, which may present challenges in down-stream processing (e.g., higher levels of demineralisation may be required).

2.4.2. Use of ion exchange and calcium-binding agents

Reducing the calcium content of micellar casein-dominant dairy concentrates before spray drying has proven to be an effective approach for increasing solubility of resultant powders. Bhaskar et al. (2001) described a process for producing a calcium-depleted MPI with improved solubility in water (20 °C); briefly, the retentate from UF of skim milk was acidified from pH 6.8 to 5.9 using citric acid and removal of calcium was performed using a strong cation exchange resin in the sodium form. After 1, 6, 15, 22 and 36 d of storage at 20 °C, the calcium-depleted powders (33, 50 and 83% calcium depletion) all showed 100% solubility. In comparison, control MPI powders had 70-80% solubility after storage for 1-6 d, and this was reduced to 50% after 15, 22 and 36 d.

In addition to ion exchange resins, calcium-binding agents have been used to reduce calcium contents and modify the functional properties of casein-dominant powders. Sun et al. (2017) added trisodium citrate (TSC), sodium pyrophosphate (SPP) and sodium phosphate (SP) to skim milk (0.3% of total solids) before membrane filtration. Calcium content was reduced significantly by the addition of each calcium-

binding agent. After stirring for 30 min, the median particle size was 40 µm for the control MPC, compared to 25, 20 and 25 µm for powders spray dried containing TSC, SP and SPP, respectively, while the solubility was 40, 67, 59 and 51% for control, TSC, SP and SPP powders, respectively. The sample with the highest solubility (83%) at that time point was one which contained a mixture of TSC and SPP (50:50). Schuck et al. (2002) produced NPC powders with added TSC or SP using three different manufacturing approaches: (i) co-drying (CD): calcium-binding agents added to NPC before spray drying, (ii) bi-drying (BD): mineral salt solution and NPC suspension spray dried together, and (iii) dry-mixing (DM): powders physically blended together after spray drying. NPC manufactured without additional calcium-binding agents had an ISI of 14.4 mL compared to <0.2 mL when SP (12 g/100 g solids) and TSC (30 g/100 g solids) were added before spray drying. Insolubility values were similar when SP and TSC were added using BD (1.8 and <0.2 mL, respectively) but higher when SP and TSC were added via DM (13.9 and 7.5 mL, respectively). This suggests that the addition of calcium-binding agents should be performed prior to spray drying. TSC was more effective than SP at increasing solubilisation, as measured using a nuclear magnetic resonance (NMR) relaxometry technique; however, it is important to note this powder had a lower protein content as greater amounts of this mineral salt were added. Similarly, Schokker et al. (2011) added citrate to the concentrate before drying and produced an MCC powder with solubility of 79.5%.

Calcium-binding agents have also been used to promote powder dissolution after spray drying. McCarthy et al. (2017) added sodium hexametaphosphate (SHMP), SP or TSC (0-150 mEq/L) to MPC solutions prepared from reconstituted powder. PSD analysis showed that TSC and SHMP significantly improved the dispersion of MPC powders, particularly with increasing concentration of SHMP, while SP did not have

a significant effect. Powder solubility was lower for the MPC control (89.7%) compared to 96.1 and 99.5% following the addition of 15 mEq/L of TSC and SHMP, respectively, with the changes in solubility attributed to the dissociation of casein micelles. Similarly, Nogueira et al. (2020) investigated the behaviour of demineralised and native casein micelle powders during rehydration, with calcium contents of 2.7 and 2.1 g/100 g powder for control and demineralised samples, respectively. Following stirring at 50 °C for 1 h, large particles (>10 μm) were present in both samples and further analysis using electrophoresis demonstrated that non-covalent interactions played an important role in the formation of these aggregates. However, it is not possible to fully elucidate the reason for this as the type of calcium-binding agent used to manufacture the demineralised powder was not given.

Despite the reports of ion exchange and calcium-binding agents generally improving powder rehydration, it would be important to consider the limitations of their use. With the removal of calcium using ion exchange, the composition, technological (e.g., gelation) and nutritional properties of the powder would be altered, and this should be carefully considered before their use in specific applications that require this micronutrient (e.g., clinical nutrition beverages). Moreover, the use of calcium-binding agents may alter ingredient listings, which may be undesirable in the food industry considering the increased consumer demand for more "clean label" products (Asioli et al., 2017).

2.4.3. Addition of monovalent or divalent salts

The incorporation of monovalent or divalent salts such as potassium chloride (KCl), sodium chloride (NaCl) and calcium chloride ($CaCl_2$) into dairy concentrates is a strategy that has been reported to modify powder dissolution. In a study by Sikand

et al. (2013), the addition of NaCl or KCl (150 mM) to UF retentate during DF improved the solubility of MPC powder, whereby NaCl and KCl treated MPC powders had 100% solubility compared to 53% when no salt was added. The higher solubility of these MPC powders was likely related to the significantly lower calcium content of the powders with salt added during DF, suggesting that some solubilisation of CCP may have occurred during membrane filtration due to a decrease in pH. Mao et al. (2012) added increasing concentrations of NaCl (0-150 mM) to the retentate at the DF step during the manufacture of MPC, with solubility increasing with increasing concentration of NaCl added, e.g., after reconstitution for 30 min, solubility was approximately 95% with the addition of 150 mM NaCl, compared with only 33% for 0 mM NaCl. The number of exposed hydrophobic regions on the MPC proteins increased significantly, while average particle size and disulphide bond formation decreased significantly, with the addition of 50, 100 and 150 mM NaCl. The change in surface hydrophobicity suggests that NaCl caused a change in protein structure, while the decrease in the number of disulphide bonds could possibly account for the measured improvements in powder rehydration. In the study by Schuck et al. (2002), NPC powders with added NaCl and $CaCl_2$ were also produced. The ISI was 0.9 mL when NaCl was added (12 g/100 g solids) by CD compared to 14.6 mL with $CaCl_2$ addition (11 g/100 g solids). The positive impact of NaCl addition on NPC rehydration was related to the hygroscopic strength of salt rather than its effect on casein micelle hydration and structure, likely because pH was re-adjusted following salt addition. Schokker et al. (2011) reported that an MCC powder which was manufactured by adding NaCl before DF had a solubility of 82.8%. Davenel et al. (2002) also produced NPC powders containing additional NaCl. The reconstitution time, measured using NMR, and ISI values were 22 min and 14.4 mL for the NPC control, compared to 9.5

min and 9 mL when NaCl was added (12 g/100 g solids) prior to spray drying, respectively. Carr et al. (2004) also reported a process whereby NaCl added to UF retentate prior to spray drying was shown to improve powder solubility.

Hussain et al. (2011) used NaCl and $CaCl_2$ solutions, ranging in concentration from 0-12% (w/v), to reconstitute native micellar casein (NMC) powder and turbidity measurements were used to provide rehydration times for each solution. NMC alone had a rehydration time of 467 min, as indicated by turbidity stabilisation, but this was reduced to 238 and 192 min when the concentration of NaCl and $CaCl_2$ was 6% (1034 mM), respectively. The shorter rehydration time for the sample containing $CaCl_2$ appears to contradict a previous report of this salt not enhancing solubility when added before spray drying (Schuck et al., 2002), possibly due to differences in the stage of addition, concentration and measurement techniques. When salt concentrations of 6% were used, no swelling stage was observed, possibly due to changes in micellar structure, and it has been reported by Famelart et al. (1999) that NaCl induced solubilisation of calcium and phosphorus when added to casein micelle suspensions but the addition of $CaCl_2$ did not cause any applicable modification. Similar to the removal of calcium as mentioned in Section 2.4.2., the addition of NaCl would negatively affect the nutritional content of the powder, particularly given its influence on cardiovascular health (Aaron and Sanders, 2013). However, KCl appears to be equally as effective for altering powder rehydration when added before spray drying and may represent a more consumer-friendly and health-conscious alternative for powder end-users.

2.4.4. Enzymatic or chemical modifications of protein

Enzymes are used to perform several functions in the dairy industry, most

notably the role of chymosin in cheese curd formation and proteinases to decrease allergenicity and improve the digestibility of infant formula (Nongonierma and Fitzgerald, 2011). Modifying dairy protein structure and functionality using enzymes has also been explored as a strategy to enhance the rehydration of high-protein powders. Power et al. (2020) produced MPC powders which were enzymatically crosslinked using transglutaminase (TGase) prior to spray drying to maintain micellar structure and control viscosity, as well as depleted in calcium using SHMP (0-25 mM) to improve rehydration performance of resultant powders. Capillary rise wetting and water sorption values were higher for TGase treated than control powders, which suggests this enzymatic treatment had a positive effect on water absorption. Diffusion was higher for TGase treated powders compared to control powders, which increased with increasing concentration of SHMP.

Alternatively, Banach et al. (2013) performed enzymatic hydrolysis of reconstituted MPC using three digestive enzymes (chymotrypsin, trypsin and papain) and one cysteine protease (papain). All enzyme treated samples displayed increased protein solubility in the pH range 4.6-7.0 compared to the control powder. Similarly, Ryan et al. (2018) investigated the influence of enzymatic modification on the functional properties of reconstituted MPI powders. The enzymes used were Flavourzyme™, Neutrase™ and Protamex™ and the solubility index of the MPI hydrolysates was measured over the pH range 2-8. At pH 6.5, the MPI control had ~35% solubility; however, after incubation for 180 min, the solubility was 90, 97 and 88% for the MPI samples enzymatically treated with Flavourzyme™, Neutrase™ and Protamex™, respectively. The authors attributed the increase in solubility to the formation of low molecular weight, hydrophilic peptides, while a limitation of protein hydrolysis in this case would be that it changes the product to an extent to which it

may no longer retain its original ingredient identification.

Aside from the use of enzymes to alter the chemistry of dairy proteins, Shilpashree et al. (2015) chemically modified the dairy proteins in MPC powder using succinylation, whereby succinyl groups were transferred to the ε-amino group of lysine residues, resulting in a change in amino acid charge from positive to negative. MPC proteins subjected to succinylation (90%) using succinic anhydride had a solubility of ~78% at pH 6 compared to 30% for the control. In addition, the average particle diameter was 200 and 720 nm for modified (i.e., 90% succinylation) and control MPC proteins, respectively. The improvements in solubility were attributed to changes in protein charge and a decrease in protein-protein interactions. Further research on the use of enzymatic or chemical modifications of dairy protein concentrates or powder dispersions, their feasibility and behaviour during pilot or industrial-scale processing (e.g., evaporation and spray drying) and their impact on other techno-functional and sensory properties of powders are required.

2.4.5. Addition of dairy proteins

The addition of whey or non-micellar casein proteins to high-protein, casein-dominant powders may appear counterintuitive but is based on the concept that lowering the concentration of micellar casein or partially dissociating casein micelles can promote solubilisation without reducing the total protein content of the powder. Schokker et al. (2011) added NaCas to the concentrate at different stages of the process and investigated the subsequent powder rehydration properties initially and after storage. The MCC powder produced when NaCas was added before DF (1.5%) had a solubility of 79.0% compared to 69.7% for the control. The solubility was higher when NaCas was added directly before drying compared to when NaCas was dry blended

with the spray-dried powder. The improvement in MCC reconstitution was attributed to increased levels of non-micellar casein and the two mechanisms proposed to explain this observation were: (i) non-micellar casein could preferentially adsorb at the air-water interface instead of casein micelles during spray drying which would prevent the formation of a network of casein micelles at the surface of the powder, and (ii) non-micellar casein may act as a physical spacer molecule and prevent the association of casein micelles with each other. Bot et al. (2020) compared the addition of NaCas to MPI powder, by wet- or dry-blending, on dispersion and solubility. The MPI control (i.e., no NaCas added) had a solubility of 89.6% but this was 92.3 and 97.5% when NaCas was added (15% of total protein) via the wet- and dry-blending approaches, respectively. The PSD profile for the MPI control and MPI plus NaCas wet-blended samples were similar, with both having a monomodal peak in the size range 6-100 µm. However, the MPI plus NaCas dry-blended powders all had bimodal distributions, with a peak <1 µm and a second peak between 6-100 µm. This suggests that dispersibility increased as the proportions of NaCas dry-blended with MPI powder increased.

Davenel et al. (2002) added whey proteins to NPC before freeze drying and measured its rehydration performance using NMR. Freeze dried NPC had a reconstitution time of 32 min but this was 13 min for the sample enriched with whey proteins (i.e., 12% of total solids). Torres Hernandez et al. (2018) reported that adding a whey proline-rich peptide hydrolysate (DISSEP), produced from enzymatic hydrolysis of WPI, to reconstituted MPC could improve protein solubility following storage at 4 °C. The addition of dairy proteins provides dairy manufacturers with a practical and convenient approach for improving powder rehydration and may add further value to the incorporated ingredients. Nevertheless, this approach alters the

original composition, physical state and often the protein profile of the powder (i.e., lower proportion of micellar casein) and does not resolve the fundamental issue of solubilising a micellar casein-dominant powder.

2.4.6. Addition of molecular spacers

The introduction of other food ingredients (e.g., soy lecithin) into high-protein concentrates to spatially separate micellar casein and reduce protein-protein interactions has recently been investigated by Bansal et al. (2017). Microfluidisation was applied to soy lecithin dispersions (5%, w/w) to create nanovesicles with an average hydrodynamic diameter of 82 nm. These dispersions were then added (1, 5 and 10% of milk solids, w/w) to the concentrate (11% total solids, w/w) prior to spray drying. The MPC powders containing 5% lecithin had significantly higher solubility at the beginning of the study and after 30 d of storage at 25 °C than the MPC powders containing 0 and 1% lecithin. Furthermore, after 90 d of storage at 25 °C, all powders containing lecithin nanovesicles had significantly higher solubility than the control MPC. However, after 180 d, no significant difference in solubility was observed between samples, while MPC powders containing 5 and 10% lecithin did not differ significantly during the study. Although this presents an interesting approach for modifying powder solubility, it alters the powders chemical composition which may limit its use in certain applications. The concept of adding molecular spacers or fillers such as Sephadex beads (Barden et al., 2015) or glass beads (Thionnet et al., 2017) to cheese has also been reported, whereby they were used to replace milk fat and investigate the subsequent rheological properties of low-fat cheese. Further research is required to evaluate if other molecular spacers, such as whey protein nanoparticles (Guralnick et al., 2021) could be used to design innovative dairy product structures

and enhance the rehydration properties of micellar casein-dominant powders.

2.5. Conclusion and perspectives for the future

Improving the rehydration performance of high-protein, micellar casein-dominant dairy powders remains a significant challenge and the selection of suitable processing strategies by manufacturers thereof is influenced by numerous, inter-related factors (e.g., capital and operating costs, bulk powder properties and end-user applications). Furthermore, any chemical or formulation changes made to the existing micellar casein-dominant powders available industrially need to be considered with respect to regulatory compliance and maintenance of established standards of identity, in addition to any potential changes to taste perception and consumer acceptance.

Although not the rate-limiting stage of rehydration, the wettability of these powders can be improved using food-grade surfactants (e.g., lecithin) or agglomeration. Altering dairy concentrate composition and physical state (e.g., dissociation of micellar casein and reduction of calcium content using ion exchange) or injecting gas directly prior to spray drying to influence powder particle structure, appear to be the most effective strategies at enhancing the dispersibility and solubility of micellar casein-dominant dairy powders. However, a strategy that successfully accelerates powder rehydration, without altering the chemical composition or physical properties of these types of powders, has not yet been developed. When the end-user needs to solubilise and rehydrate powders prior to their inclusion in food and beverage products, the use of high-shear or turbulence-inducing equipment (e.g., hydrodynamic cavitation) is essential. Further research is required to advance our knowledge of high-protein, micellar casein-dominant dairy powders, such as exploring additional or alternative drying technologies (e.g., electrostatic spray drying and spray freeze

drying), developing analytical techniques for characterising the stages of powder rehydration, creating an international system for categorising or grading powder dispersibility and solubility, and establishing a fundamental and comprehensive understanding of insolubility development during dehydration and storage (e.g., the mechanisms and nature of casein micelle interactions).

2.6. References

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Chapter 3

The influence of composition and manufacturing approach on the physical and rehydration properties of milk protein concentrate powders

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Abstract

This study investigated the physical and rehydration properties of milk protein concentrate (MPC) powders with five different protein contents (i.e., 38.9, 53.7, 63.6, 74.1, and 84.7%, w/w) prepared by recombining the ultrafiltration (UF) retentate and UF permeate of skim milk. Powder density and flowability increased, while the powder particle size decreased with decreasing powder protein content. The amount of non-wetting MPC powder decreased with decreasing protein content, demonstrating greater wettability for lower protein powders. At protein contents >65% (w/w), the dispersibility and solubility of the powders decreased significantly, likely due to the greater hydrophobic interactions between casein proteins and a lower concentration of lactose. Therefore, as the protein content of the MPC powders was decreased, their rehydration properties improved. The results obtained in this study provide novel insights into the relationship between the composition of recombined UF retentate and UF permeate streams on the subsequent powder particle size, density, and rehydration properties, and demonstrate that such powders possess similar properties to those prepared using conventional direct membrane filtration.

3.1. Introduction

The global demand for milk protein ingredients has increased greatly in recent years due to increased consumer awareness of the health benefits and importance of dietary protein as well as the economic development of countries in Europe and Asia (Henchion et al., 2017). Milk protein concentrate (MPC) ingredients are produced through the ultrafiltration (UF) of skim milk, followed by diafiltration to remove additional lactose and other low molecular weight material (i.e., to increase the protein content) before water removal through the use of evaporation and spray drying (Singh et al., 2007; Huffman and Harper, 1999; Mistry and Hassan, 1991). MPC generally contains 40–80% protein (Sikand et al., 2011) and possesses the same ratio of casein to whey as found in skim milk (i.e., ~80:20). The quantity of lactose, minerals, and water in the skim milk decreases as the protein content increases during membrane filtration (Sikand et al., 2012). The permeate stream generated from this process (i.e., the milk components that pass through the membrane) is collectively referred to as milk permeate.

The applications of MPC include infant milk formula, cheese, yogurt, and products designed for sports and medical nutrition; however, its uses are often limited by its inherent poor solubility (Mimouni et al., 2010; Crowley et al., 2015). This is associated with the presence of insoluble material formed by non-covalent (hydrophobic) protein–protein interactions that occur during the powder manufacturing process and subsequent storage. Therefore, hydration and dissolution of MPC powders is usually conducted in water at approximately 50 °C (Havea, 2006), whereby the increase in solvent temperature accelerates the release of material from the powder particles into the aqueous phase (Mimouni et al., 2010). In order to ensure complete protein hydration, solutions may need to be cooled to 4 °C in order to reduce

hydrophobic interactions between casein micelles and allow full hydration and swelling to occur. Furthermore, other high-protein dairy powders such as micellar casein concentrate, which is produced by the microfiltration of defatted milk and consists predominantly of casein proteins, also exhibits poor reconstitution properties (Schuck et al., 2007; Gaiani et al., 2007). Such rehydration challenges are compounded when powders are exposed to unfavourable environmental conditions such as high temperature and high relative humidity (Fyfe et al., 2011; Gazi and Huppertz., 2015; Thomas et al., 2004; Le et al., 2011). The deterioration in solubility over time has been attributed to the presence of cross-linked casein micelles at the surface of the powder particles, which can reduce the transfer of water and thus inhibit dissolution (Havea, 2006; Anema et al., 2006). Rehydration of casein-dominant powder is characterised by five stages: (a) wetting, (b) swelling, (c) sinking, (d) dispersion, and (e) dissolution (Crowley et al., 2016). These steps can be influenced by several factors: (i) pre-treatment of the concentrate (e.g., using high-shear; Augustin et al., 2012), (ii) processing conditions such as spray drying temperatures (Fang et al., 2012), and (iii) the relative humidity and temperature at which the powder is stored (Haque et al., 2010). Furthermore, the powder surface composition (e.g., presence of fat), particle structure (e.g., porosity), and rehydration conditions (e.g., stirring rate and solvent temperature) also play important roles in powder dissolution (Gaiani et al., 2006; Schuck et al., 2013).

The standardisation of high-protein dairy concentrates through the addition of milk permeate to UF retentate could allow for an approach to manufacture targeted MPC ingredients with a wide range of compositions, particularly for academic researchers with limited access to membrane filtration technology, while the influence of this manufacturing process on powder functionality has not been reported.

Therefore, the aim of this study was to first determine the influence of the protein content of MPC powders, prepared from blends of UF retentate and UF permeate, on the powder density, air content, particle size, flowability, microstructural properties, and subsequent powder rehydration. Second, these results were compared to previous studies from the literature that assessed high-protein dairy (mainly MPC) powders produced via conventional direct UF, without the addition of milk permeate, to determine whether or not this novel manufacturing approach would produce powders with the same properties.

3.2. Materials and methods

3.2.1. Manufacture of milk protein concentrate powders

Milk protein concentrate (MPC) powders were produced in the Bio-functional Food Engineering Facility at Teagasc Food Research Centre (Moorepark, Fermoy, Co. Cork, Ireland) using a similar method as that described by Maidannyk et al. (2020). Liquid MPC (19.5 and 16.6% w/w, total solids and protein, respectively, i.e., MPC85) and concentrated milk permeate (24% w/w, total solids) were obtained from a local dairy supplier directly after ultrafiltration (UF) and reverse osmosis, respectively. Milk permeate was then combined with the UF retentate to dilute the protein content to ~75, 65, 55, and 40% protein (w/w). The subsequent five (i.e., MPC85, 75, 65, 55, and 40) MPC batches were stored overnight at 4 °C under gentle agitation. MPC batches were then pre-heated to 45 °C and spray dried using a single-stage spray dryer (Anhydro F1 Lab Dryer; Copenhagen, Denmark) equipped with a two-fluid nozzle atomisation system (Type 1/8 JAC 316ss) under counter-flow drying conditions. The atomisation pressure was set at ~2–3 bar. Air inlet and outlet temperatures were maintained at 185 and 85 °C, respectively. After spray drying, powders were stored in polyethylene

plastic bags at 4 °C for the duration of the study.

3.2.2. Compositional analysis of milk protein concentrate powders

The free moisture and ash content of the MPC powders was determined using a TGA701 thermogravimetric analyser (LECO Corporation, St Joseph, MI, USA). The protein nitrogen values of the MPC powders were obtained by the Dumas method using a LECO FP628 nitrogen analyser (LECO Corporation, St Joseph, MI, USA); the protein content was determined by multiplying the nitrogen concentration by a nitrogen-to-milk protein conversion factor of 6.38. The fat content of the MPC powders was analysed using the Rose Gottlieb method (ISO/IDF, 2008). The lactose contents were calculated by difference. All analysis was carried out in triplicate, except for fat determination, which was performed in duplicate.

3.2.3. Bulk density, particle density, occluded and interstitial air

The loose and tapped (100 taps) bulk density of the MPC powders were measured as per GEA Niro (2006a) using a jolting volumeter STAV II (Funke Gerber, Berlin, Germany). Particle density of MPC powders was measured using an AccuPyc II 1340 gas pycnometer (Micromeritics Instrument Corporation, Norcross, GA, USA), according to the air pycnometer method of GEA Niro (2006b). The volume of interstitial air and occluded air was calculated as outlined in the GEA Niro method (2006b).

3.2.4. Powder particle size distribution

The particle size of the MPC powders was determined using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd, Malvern, Worcestershire,

UK) equipped with an Aero S dry dispersion unit. The refractive index was set at 1.45 (Murphy et al., 2013). The air pressure was set at 2 bar for all samples, and the feed rate was adjusted (from 25-100%), depending on the cohesiveness of the sample. Size measurements were recorded as the median diameter (D_{50}) and cumulative diameters (D_{90} and D_{10}) whereby 10, 50 and 90% of the powder volume is represented by powder particles smaller than the size indicated. The volume weighted mean particle diameter ($D_{[4,3]}$) was also calculated.

3.2.5. Powder flowability and compressibility

A Powder Flow Tester (PFT; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to measure the flowability, bulk density, and compressibility of the MPC powders. Samples were prepared for analysis by filling each into an aluminium trough (volume of 230 cm^3 and 15.2 cm internal diameter). A curved blade was then used to bring the powder into the required conformation for flow function testing and a vane lid was attached to the compression plate before testing. Samples were analysed in triplicate.

A flow function (FF) test was carried out to determine the flowability of the MPC powders. This involved applying five normal stresses (1.0, 1.9, 2.9, 3.9, and 4.8 kPa) and three over-consolidation stresses at each normal stress. A FF graph was obtained by plotting major principal consolidating stress (MPCS) as a function of unconfined failure strength (UFS). This corresponds to the strength that develops within a powder when consolidated, which must be overcome to enable powder flow (Fitzpatrick et al., 2005). Flow index (i) values were calculated from the inverse of the slope of the FF curve. Loose bulk density (p_b) and tapped bulk density (p_t) were recorded at minimum and maximum MPCS, respectively. The Hausner ratio was

calculated by dividing the tapped or compressed bulk density by the loose bulk density. The compressibility index (Eq. 1) was calculated as the percentage increased from the loose bulk density to tapped bulk density (Schuck et al., 2012):

$$C = \frac{p_t - p_b}{p_t} \text{ X } 100 \quad (1)$$

3.2.6. Scanning electron microscopy

Samples of each MPC powder were attached to double-sided adhesive carbon tabs mounted on scanning electron microscope stubs, and then coated with chromium (K550X, Emitech, Ashford, UK). Scanning electron microscopy images were collected using a Zeiss Supra 40P field emission SEM (Carl Zeiss SMT Ltd., Cambridge, UK) at 2.00 kV. Representative micrographs were taken at 5000× magnification.

3.2.7. Wettability of milk protein concentrate powders

Wettability was first measured using the method of GEA Niro (2009) with a slight modification; 4 g of each sample was added to a beaker of water (25 °C) instead of 10 g. Wettability was also assessed using the method of Fitzpatrick et al. (2016) with some modifications; 250 mL of water (25 °C) was used and the test duration was 20 min. Briefly, 10 g of powder was placed onto the surface of 250 mL of water (25 °C) in a 600 mL volume glass beaker. After 20 min, the remaining surface powder was carefully removed using a spatula. This powder was dried in an oven (102 °C) and its original water content was determined. Wettability (%; Eq. 2) was defined as:

$$100 \text{ x } \frac{\text{mass of powder disappeared}}{\text{mass of initial powder}} \quad (2)$$

3.2.8. Particle size distribution of milk protein concentrate dispersions

The particle size distribution of the MPC dispersions were measured using static light scattering with a laser-light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens. Particle and dispersant (i.e., water) refractive indices were set at 1.45 and 1.33, respectively. MPC powders were rehydrated (4% total solids, w/w) in ultrapure water under two different conditions: (a) high-speed mixing for 30 s at 23 °C and (b) high-speed mixing for 30 s at 50 °C. High-speed mixing (3600 ± 100 rpm) was carried out using a solubility index meter (Labinco-BV, Breda, the Netherlands). Each sample was introduced into ultrapure water re-circulating at 20 °C in the dispersion unit (Hydro MV) at 1750 rpm. Size measurements were recorded as the D_{10}, D_{50} and D_{90}. Size distributions were obtained using polydisperse analysis. Measurements were recorded at a laser obscuration of 3–4% and all particle size measurements were performed in triplicate.

3.2.9. Powder solubility

MPC powders were dispersed in ultrapure water (23 °C; 4%, w/w, total solids) for 30 s using a solubility index meter (Labinco BV, Breda, the Netherlands). Aliquots (30 mL) of these solutions were then centrifuged at 3000 × g for 10 min (23 °C) and the total solids content of the supernatant was then determined using a moisture analyser (CEM Smart System5™, 3100 Smith Farm Road, Matthews, NC, USA). The solubility of the powders was given by the total solids content of the supernatant expressed as a percentage of the total solids content of the initial dispersion.

3.2.10. Statistical analysis

Measurements of the powder physical and rehydration characteristics were performed in triplicate. Analysis of variance (ANOVA; Tukey's HSD) was carried out using the IBM SPSS (Version 24, Armonk, New York, USA) statistical analysis package. The level of significance was determined at $P < 0.05$.

3.3. Results and discussion

3.3.1. Composition of milk protein concentrate powders

A process flow diagram comparing conventional milk protein concentrate (MPC) production with the novel approach used in this study is displayed in Fig. 3.1, with the composition of the resultant MPC powders shown in Table 3.1.

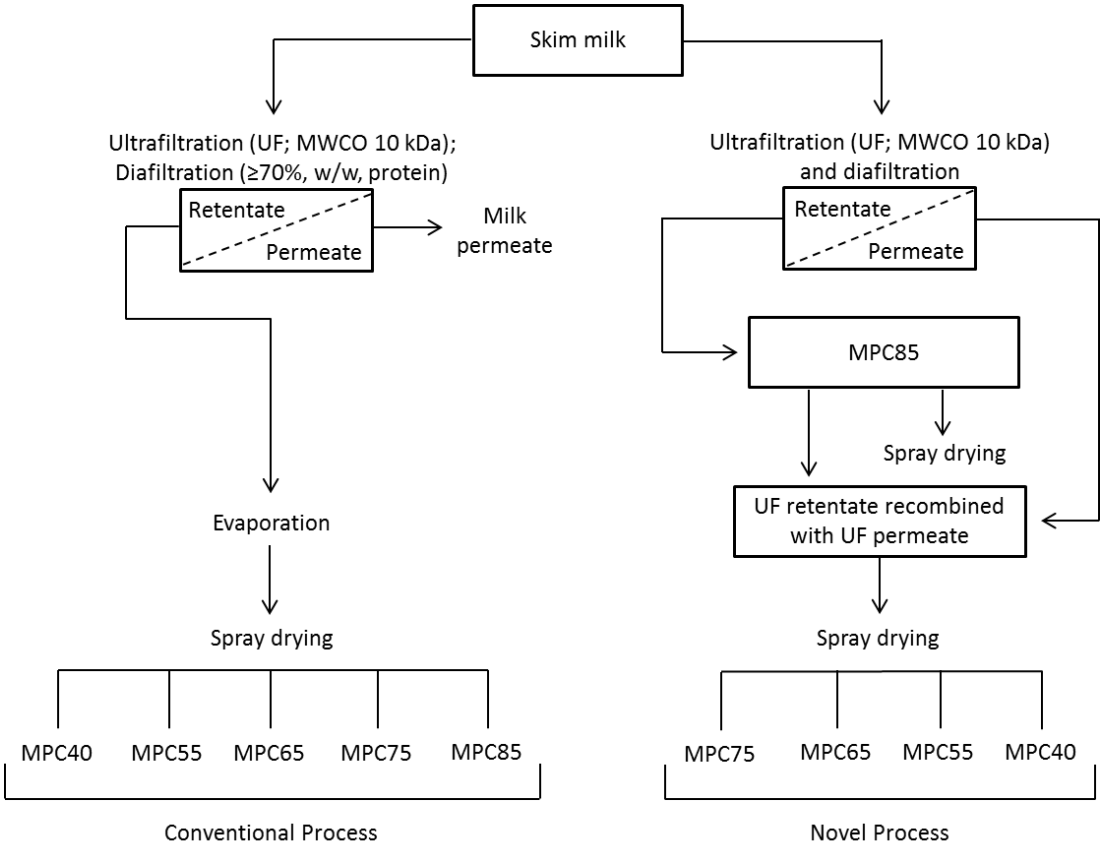

Fig. 3.1. Process flow diagram of conventional and novel approaches for the production of milk protein concentrate (MPC) powders.

Table 3.1. Composition of milk protein concentrate (MPC) powders.

MPC	Protein	Lactose	Fat	Ash	Moisture	Ash:Protein
			%			
MPC85	84.7 ± 0.9	1.37	2.07	6.88[a] ± 0.1	6.68[a] ± 0.3	0.08
MPC75	74.1 ± 0.8	12.6	1.59	6.99[b] ± 0.0	5.19[b] ± 0.1	0.09
MPC65	63.6 ± 0.7	22.8	1.34	7.17[c] ± 0.0	5.49[b] ± 0.1	0.11
MPC55	53.7 ± 1.3	33.4	1.17	7.43[d] ± 0.0	5.09[b] ± 0.0	0.14
MPC40	38.9 ± 0.6	48.2	0.87	7.82[e] ± 0.0	4.59[c] ± 0.0	0.20

[a–e] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

The recombination of the milk permeate with UF retentate resulted in a progressive decrease in the protein concentration of the MPC powders, with the powder moisture content tending to decrease with decreasing protein content. This was due to the higher viscosity of the feed prior to drying because of the higher protein content (Karlsson et al. 2005; Sauer et al. 2012). A high viscosity feed can result in larger spray droplets being produced during atomisation with reduced surface area available for the removal of moisture. Crowley et al. (2014) reported a moisture content of 4.6% (w/w) for MPC80 powder, compared to 3.4% (w/w) for MPC35. In the present study, significant ($P < 0.05$) differences in ash content were measured for the MPC powders, with the values ranging from 6.88% for MPC85 to 7.82% for MPC40 (Table 3.1). Deeth and Hartanto (2009) reported similar ash results of 7.5 and 7.1% (w/w) for MPC42 and MPC85, respectively. In the present study, there was an increase in ash:protein with decreasing protein content, whereby the ash:protein ratio increased from 0.08 for MPC85 to 0.20 for MPC40 (Table 3.1). In a similar manner, Crowley et al. (2015) reported an ash:protein ratio of 0.23 for MPC35 compared to 0.10 for MPC85.

3.3.2. Physical properties of milk protein concentrate powders

3.3.2.1. Powder particle size

Powder particle size distribution analysis displayed a significant decrease in particle size with decreasing protein content (Fig. 3.2); MPC85 had a $D_{[4,3]}$ of 57.3 μm compared to 18.9 μm for MPC40 (Table 3.2). This is most likely caused by differences in the protein content of the concentrates prior to spray drying; as mentioned in Section 3.3.1, with high-protein concentrates possessing a higher viscosity, thereby generating larger droplets during the atomisation step of spray drying (Walstra et al. 2006). Rupp et al. (2018) reported that the $D_{[4,3]}$ of the MPC powder increased significantly from

31 to 50 μm with an increase in the protein content of the concentrate from 19 to 23% (w/w). Crowley et al. (2014) reported D_{90} values of 64.6 μm for MPC35 and 51.9 μm for MPC80 spray dried under similar conditions to the present study; however, this difference may be explained by the large differences in the concentrate total solids before spray drying, i.e., 35.5% (w/w) for MPC35 and 14.7% (w/w) for MPC85.

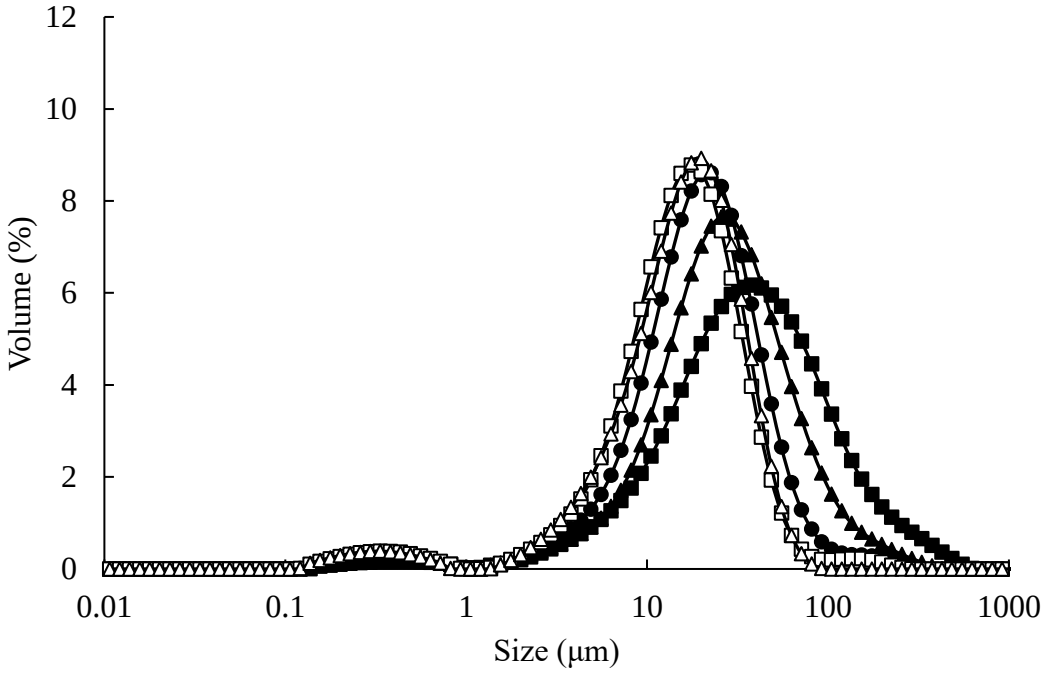

Fig. 3.2. Particle size distribution of milk protein concentrate (MPC) 85 (■), MPC75 (▲), MPC65 (●), MPC55 (□), and MPC40 (Δ) powders.

3.3.2.2. Density

Particle, loose and tapped bulk density values for the MPC powders increased with decreasing protein content (Table 3.2). For instance, the particle density increased from 1.00 g/cm^3 for MPC85 to 1.18 g/cm^3 for MPC55, while tapped bulk density increased from 0.35 to 0.44 g/cm^3, respectively. This finding is supported by the results of Crowley et al. (2014), who reported that particle density increased from 0.84 g/cm^3 for MPC85 to 1.25 g/cm^3 for MPC50, while tapped bulk density increased from

Table 3.2. Particle density (p_p), loose bulk density (p_b), tapped bulk density (p_t), volume of interstitial air (V_{ia}), volume of occluded air (V_{oa}), particle size below which 90% of material volume exists (D_{90}), and the volume weighted mean particle diameter ($D_{[4,3]}$) values for milk protein concentrate (MPC) powders.

MPC	p_p	p_b	p_t	V_{ia}	V_{oa}	D_{90}	$D_{[4,3]}$
	(g/cm^3)			mL/100 g		μm	
MPC85	$1.00^{a} \pm 0.0$	$0.29^{a} \pm 0.0$	$0.35^{a} \pm 0.0$	$190^{a} \pm 7.8$	$32.2^{a} \pm 0.1$	$127^{a} \pm 4.5$	$57.3^{a} \pm 2.9$
MPC75	$1.08^{b} \pm 0.0$	$0.32^{b} \pm 0.0$	$0.38^{b} \pm 0.0$	$173^{a} \pm 5.6$	$25.5^{b} \pm 0.4$	$76.1^{b} \pm 1.4$	$37.5^{b} \pm 0.7$
MPC65	$1.14^{c} \pm 0.0$	$0.34^{c} \pm 0.0$	$0.41^{c} \pm 0.0$	$155^{b} \pm 3.1$	$20.5^{c} \pm 0.8$	$47.4^{c} \pm 1.0$	$25.5^{c} \pm 0.4$
MPC55	$1.18^{d} \pm 0.0$	$0.39^{d} \pm 0.0$	$0.44^{d} \pm 0.0$	$141^{b} \pm 10$	$17.5^{d} \pm 1.1$	$36.3^{d} \pm 0.8$	$19.9^{d} \pm 0.6$
MPC40	$1.14^{c} \pm 0.0$	$0.40^{d} \pm 0.0$	$0.43^{cd} \pm 0.0$	$143^{b} \pm 0.8$	$21.1^{c} \pm 0.7$	$35.9^{d} \pm 0.3$	$18.8^{d} \pm 0.2$

[a–d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

0.29 g/cm^3 for MPC85 to 0.59 g/cm^3 for MPC50. Eshpari et al. (2014) reported similar results to the present study with a particle density value of 1.07 g/cm^3 for the MPC80 powder. There was a corresponding increase in both the interstitial and occluded air content of the powders as the density decreased. MPC85 powder had the lowest density (i.e., particle, loose, and tapped) and the highest interstitial (190 mL/100 g) and occluded (32.2 mL/100 g) air content, which may be accounted for by the greater powder particle size of this sample (Skanderby et al. 2009). The increase in particle density with a decrease in the protein content could be accounted for by the concomitant increase in lactose in the powders. Furthermore, the MPC40 in the current study had a loose bulk density value of 0.40 g/cm^3, which is lower than the value of 0.65 g/cm^3 recorded by Fitzpatrick et al. (2005) for a commercial skim milk powder. This difference in bulk density may be due to the difference in the total solids content of the concentrate between the MPC40 sample (21.7%) and a typical commercial skim milk concentrate (e.g., 50%).

3.3.2.3. Flowability

The flow index values obtained were similar for all powders (Table 3.3). For example, the flow index value for MPC65-85 was approximately 2.1. MPC40 had the highest flow index value of 2.6. However, as these values were all less than 4, the powders were categorised as cohesive according to the Jenike classification system for powder flowability. The poor flowability of the low-protein MPC sample (i.e., MPC40) is possibly related to the use of a two-fluid nozzle during spray drying, or the drying of this concentrate at a relatively lower total solids content than would be used for a typical commercial product with a similar protein content (e.g., skim milk). Crowley et al. (2014) reported that flow index was higher for MPC35 (13.4) than for

Table 3.3. Flow and rehydration (wettability and solubility) properties of milk protein concentrate (MPC) powders.

MPC	i	JC	CI (%)	HR	Wettability (%)	Solubility (%)
MPC85	2.1 ± 0.1	Cohesive	41.2^{a} ± 1.5	1.71	14.7^{a} ± 1.8	83.0^{a} ± 2.2
MPC75	2.1 ± 0.0	Cohesive	42.1^{a} ± 0.7	1.73	17.5^{a} ± 2.0	92.9^{b} ± 1.6
MPC65	2.0 ± 0.3	Cohesive	41.9^{a} ± 2.6	1.73	49.3^{b} ± 1.1	98.0^{c} ± 1.3
MPC55	2.2 ± 0.2	Cohesive	35.0^{b} ± 1.3	1.55	48.3^{b} ± 1.1	98.5^{c} ± 1.1
MPC40	2.6 ± 0.2	Cohesive	32.4^{b} ± 1.8	1.50	48.3^{b} ± 0.9	98.1^{c} ± 0.8

$^{a–c}$ Values within a column not sharing common superscripts differ significantly ($P < 0.05$). i = flow index, JC = Jenike classification, CI = compressibility index, HR = Hausner ratio.

MPC85 (3.5), while Fitzpatrick et al. (2005) reported a flow index value of 6.1 for a commercial skim milk powder. The Hausner ratio (HR) values correlated with the flowability results, which demonstrated that high-protein powders had poorer flowability than low-protein powders. According to Turchiuli et al. (2005), a HR greater than 1.4 corresponds to a non-free flowing powder. Furthermore, the compressibility of MPC65-85 was significantly greater than that for both the MPC40 and MPC55 powders. This is most likely caused by the greater interstitial air content of the higher protein powders as these voids between powder particles would have been reduced considerably during compaction, resulting in a greater change in density.

3.3.2.4. Microstructure

Scanning electron microscopy images of each MPC powder are shown in Fig. 3.3. Low-protein powders (e.g., MPC40) had a collapsed structure with wrinkled, concaved surfaces. However, for MPC75 and MPC85, the surface morphology changed significantly, with the surfaces of these powder particles appearing smoother and more dimpled. These results are supported by the findings of Kelly et al. (2015), who observed similar differences between the microstructures of spray-dried MPC powders (MPC35–90). The distinct differences in the microstructure of low- and high-protein MPC powders may be caused by several factors. Crowley et al. (2014) stated that lower protein MPC powders (i.e., MPC40) contained a lower volume of occluded air in comparison to higher protein MPC (i.e., MPC85), similar to the results of the current study, and possibly accounts for the collapsed appearance of the particles. The smooth surface of high-protein powders possibly arises from the compaction of casein micelles during the spray drying process (Tamime et al. 2007). Moreover, Sadek et al. (2014) and Tan et al. (2019a) showed that protein type also plays an important role in

powder particle morphology, with casein-dominant powder particles appearing more wrinkled compared to whey protein powders that possessed a spherical shape. Furthermore, spray drying temperatures can also affect particle morphology, with Tan et al. (2019b) showing that an increase in drying inlet temperature could produce particles with wrinkled surfaces, while lower drying temperatures produced more spherical particles.

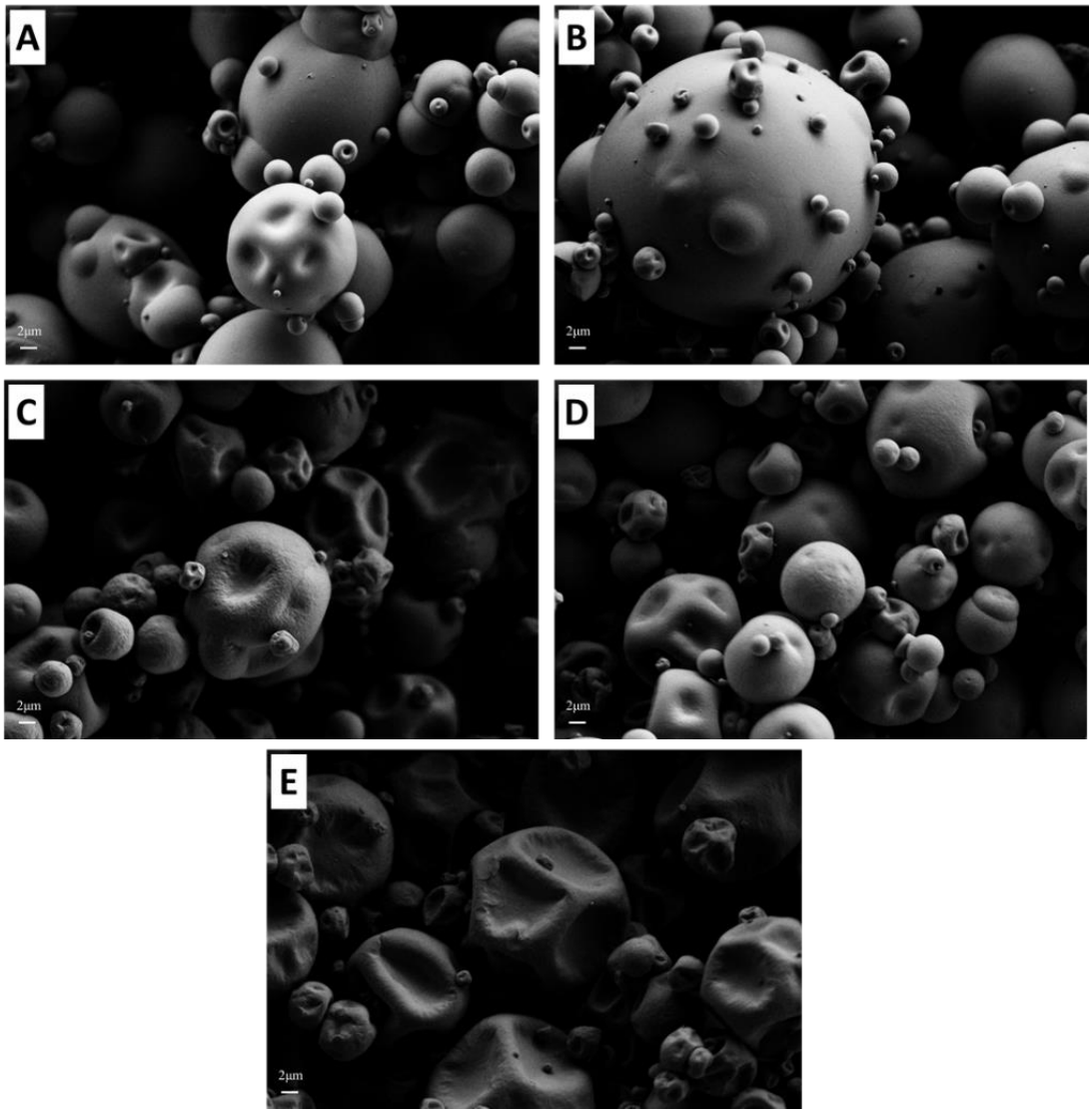

Fig. 3.3. Scanning electron microscopy images of milk protein concentrate (MPC) 85 (A), MPC75 (B), MPC65 (C), MPC55 (D), and MPC40 (E) powders at 5000× magnification.

3.3.3. Wettability of milk protein concentrate powders

Wettability analysis showed that MPC85 and MPC75 had the lowest wettability at 14.7% and 17.5% after 20 min, respectively, compared to approximately 47% for MPC40–65 (Table 3.3). Poor wetting behaviour of the MPC powders has previously been attributed to the hydrophobic, protein-rich surface of these ingredient powders (Crowley et al., 2015; Fyfe et al., 2011). Despite possessing similar protein content to skim milk powder, the MPC40 in the current study displayed poor wetting behaviour. Fitzpatrick et al. (2016) found that a skim milk powder completely wetted after 55 s at 20°C, likely due to its large D_{50} value (132 μm) and a tapped bulk density of 0.55 g/cm^3. MPC powders did not completely wet and sink within the time period measured; however, a visual difference was observed between samples (results not shown) with a smaller quantity of the low-protein powders (i.e., MPC40 and MPC55) remaining on the surface of the water, with the water becoming more turbid, compared to the high-protein powders (i.e., MPC75 and MPC85) that remained on the surface of the water and formed a surface film layer. This may also be accounted for by the differences in carbohydrate content between powders, with powders containing ≥22.8% lactose (w/w) likely being more hydrophilic, resulting in greater water transfer into and between proteins.

3.3.4. Dissolution and solubility of milk protein concentrate

The particle size distribution data indicated the presence of large, poorly dispersible particles in high-protein MPC powders (Fig. 3.4). This was most apparent for MPC85 and MPC75 when dispersed in water at 23 °C as they exhibited monomodal size distribution in the range 5–100 μm (Fig. 3.4A). Dispersion of powder particles is considered the rate limiting stage in the rehydration of MPC (Mimouni et

al., 2010), and this is most likely caused by protein-protein (e.g., hydrophobic) interactions between casein micelles in close proximity and the low concentration of lactose facilitating close packing (Anema et al., 2006; Horne, 1998).

Table 3.4. Mean particle size of milk protein concentrate (MPC) dispersions after high-speed mixing at 23 °C and 50 °C.

MPC	D_{90} (μm)		$D_{[4,3]}$ (μm)	
	23 °C	50 °C	23 °C	50 °C
MPC85	$68.9^{a} \pm 5.4$	$156^{a} \pm 11$	$40.7^{a} \pm 2.9$	$76.4^{a} \pm 4.3$
MPC75	$92.6^{b} \pm 4.2$	$98.2^{b} \pm 2.2$	$51.7^{b} \pm 1.9$	$36.7^{a} \pm 3.5$
MPC65	$59.7^{c} \pm 2.1$	$25.6^{c} \pm 11$	$18.3^{c} \pm 1.6$	$6.68^{a} \pm 1.9$
MPC55	$13.1^{d} \pm 4.6$	$0.39^{d} \pm 0.0$	$4.57^{d} \pm 0.3$	$1.98^{b} \pm 0.2$
MPC40	$6.30^{e} \pm 5.8$	$0.41^{d} \pm 0.1$	$4.25^{d} \pm 0.3$	$2.06^{b} \pm 0.4$

[a–d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). D_{90} = the size of particles below which 90% of the sample lies. $D_{[4,3]}$ = volume weighted mean diameter.

On the other hand, bimodal distributions were observed for MPC40-65, which suggests the presence of both casein micelles (<1 μm) and primary powder particles (>1 μm).

The volume of primary particles generally decreased with the reducing protein content of the powders. MPC55 and MPC40 displayed the highest dispersibility, which corresponded to a small volume of large particles in the range of 5–100 μm, and a larger volume of sub-micron (<1 μm) particles. Additionally, the $D_{[4,3]}$ value generally decreased as the protein content of the powders was reduced, (e.g., 51.7 μm for MPC75 compared with 4.25 μm for MPC40 when the samples were reconstituted at 23 °C (Table 3.4). The target particle size profile for a rehydrated MPC would be a

monomodal distribution in the size range of casein micelles, (i.e., <1 μm). It has been reported that a mean particle size of 0.08–0.2 μm represents the presence of casein micelles, providing evidence that the hydration of powder particles has taken place (Mimouni et al., 2009; Bouvier et al., 2013).

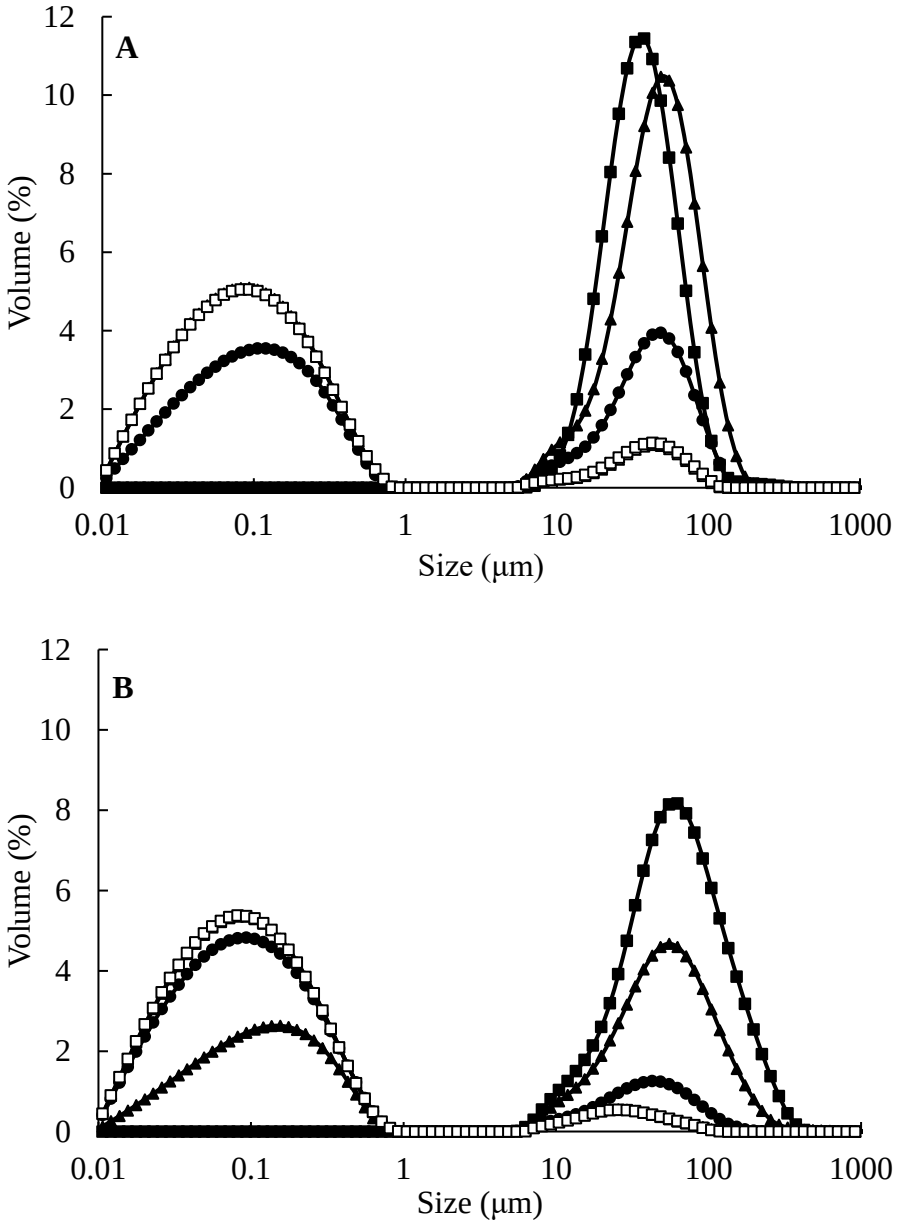

Fig. 3.4. Particle size distribution of milk protein concentrate (MPC) 85 (■), MPC75 (▲), MPC65 (●), MPC55 (□), and MPC40 (Δ) powders after reconstitution in ultrapure water at (A) 23 °C and (B) 50 °C.

Reconstitution of MPC85 and MPC75 powder in water at 50 °C reduced the volume of primary powder particles but resulted in the occurrence of some particles with a size >100 μm (Fig. 3.4B). This may be accounted for by powder particle swelling caused by greater water uptake and hydration at 50 °C than at 23 °C; however, even though hydration occurred, it is suggested that complete particle dissociation did not occur as a large volume of particles remained in the 10–500 μm size range. The swelling stage of powder rehydration had previously been observed by Gaiani et al. (2007) during the rehydration of micellar casein powder, whereby swelling was recorded as a peak in particle size following powder wetting. The short period of reconstitution (30 s) in 50 °C water appears to have been sufficient to allow wetting of high-protein powders to occur, but insufficient to enable complete dispersion of powder particles. It is possible that particle swelling would not be detected when the reconstitution time is longer. Conversely, MPC40-65 powders had lower $D_{[4,3]}$ values when dispersed at 50 °C, compared to at 23 °C, indicating that after water sorption, the powder particles began to dissociate. The solubility was greater for the low-protein powders, (i.e., MPC40 and MPC55) in comparison to the higher protein powder (i.e., MPC85; Table 3.3). The MPC40–65 powders all displayed solubility of approximately 98%, compared with just 83% for MPC85. These results support those recorded during the particle size distribution analysis; high-protein MPC powders (75–85%, w/w) displayed poor dispersion and solubility properties in water. (Note: Lactose crystallisation, which is an important factor to consider in relation to the solubility of the MPC powders, did not occur in the current study (results not shown). Maidannyk et al. (2020) reported that MPC powders, ranging in protein content from 40-80% (w/w), did not show lactose crystallisation in their amorphous state following spray drying, but this process did occur for MPC40, 50, and 60 powders stored at high

relative humidity).

3.4. Conclusion

This study provided new information on the physical properties of milk protein concentrate powders prepared through the novel combination of milk permeate and high-protein UF retentate to create MPC powders at different protein contents, but with comparable physical and rehydration characteristics to those produced by conventional direct UF concentration and drying. Powder particle size decreased with a decrease in the protein content of the concentrate, most likely due to differences in concentrate viscosity. Decreasing the protein content also brought about an increase in bulk, tapped and particle density of the MPC powders. The wetting and dispersion of the powders were improved by decreasing the protein and increasing the lactose content of the blends. The rehydration and physical properties of the MPC powders were significantly altered by changes in concentrate composition but did not appear to be affected by the method of manufacture (i.e., concentrate standardisation with milk permeate compared with direct membrane concentration).

3.5. References

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Chapter 4

Heat treatment of high-protein, liquid ultrafiltration concentrate influences the physical and functional properties of milk protein concentrate powders

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Abstract

Liquid milk protein concentrate (MPC; 18.3 and 16.5%, w/w, total solids and protein, respectively), derived from industrial ultrafiltration (UF) of pasteurised skim milk, was heat treated at 80 °C (low-heat), 100 °C (medium-heat) and 120 °C (high-heat) for 30 s, or did not undergo heat treatment (control), prior to spray drying at pilot-scale. With increasing temperature of heat treatment, the viscosity of liquid MPC increased, while pH and particle size did not change significantly. The physical properties of the MPC powders were influenced by heat treatment, particularly increased size of powder particles with increasing temperature up to 100 °C. Loose bulk density was higher and interstitial air was lower for heat-treated powders, likely due to higher concentrate viscosity. Protein profiles obtained by electrophoresis showed a reduction in κ-casein band intensity for the high-heat (HH) treated MPC, while the band intensity for β-lactoglobulin reduced upon medium-heat treatment, followed by complete loss of band intensity upon HH treatment. Heat treatment of the UF concentrate influenced the heat stability of MPC powders, with HH-MPC having higher heat stability at pH 6.9 and 7.0 (140 °C). However, particle size distribution profiles demonstrated a decrease in powder dispersion with the increase in heat treatment temperature, with large particles remaining undissolved despite overnight stirring. Centrifugation of MPC dispersions showed that less powder constituents were present in the supernatant when heat treatment temperature was increased, likely due to the sedimentation of protein aggregates. This study demonstrates the effect of heat treatment of liquid UF concentrate on the physical and functional properties of MPC powders and suggests that heat treatment at temperatures ≥100 °C should be avoided to optimise powder rehydration performance.

4.1. Introduction

Milk protein concentrate (MPC) powder is used in nutritional food and beverage formulations as it contains a significant amount of high-quality protein. However, it is recognised as having sub-optimal dispersibility and solubility in water, generally attributed to non-covalent interactions between micellar caseins that are facilitated by the low concentration of lactose (Anema et al., 2006; Havea, 2006), and this phenomenon is accelerated by elevated storage temperatures (>37 °C; Mimouni et al., 2010). Ultrafiltration (UF) and diafiltration (DF) are used to create protein-enriched concentrates from skim milk, which are evaporated and spray dried to create MPC powder. Considering that liquid concentrate derived from membrane filtration is quite soluble (e.g., particle size distribution in the casein micelle range), but the product obtained following spray drying has significantly impaired rehydration performance, it seems important to explore the intermediate processing steps and what effect they could have on the physicochemical properties of such powder. One of these unit operations commonly performed is heat treatment, whereby the concentrate derived from membrane filtration is heated at a pre-determined temperature, for a defined time, prior to evaporation, likely to alter functional properties of the powder and therefore the ability to meet customer requirements. For example, in the case of skim milk powder, whey protein denaturation may be favourable at this stage to improve gelation or heat stability in the final product (Kelly and Fox, 2016).

Interestingly, despite heat treatment having a considerable role in dairy processing, the number of scientific studies reporting its effects on the functional properties of liquid and spray-dried MPC is quite limited. Ho et al. (2018) investigated the influence of pH (6.2-7.2) and heat treatment (45, 55, 65 and 75 °C for 20 min) on the viscosity and heat stability of liquid MPC (19.8%, w/w, total solids). Heat stability

at 130 °C was highest at pH 6.7 (32.3 min), but significantly decreased to 12.5 min at pH 7 and 11.5 min at pH 7.2, while viscosity and particle size increased significantly after heat treatment at 75 °C (pH 6.7). Another study by Ho et al. (2019) investigated the effect of heat treatment (85, 100 and 120 °C for 15-200 s) on the viscosity of liquid MPC derived from UF. Viscosity increased significantly with increasing temperature and holding time, which would have implications for atomisation of such concentrates during industrial spray drying. Tari et al. (2021) heat treated (85 °C for 5 min or 125 °C for 15 s) liquid MPC (18.5%, w/w, total solids; 13%, w/w, protein) at different pH values (6.5, 6.7 and 6.9) and reported that viscosity was significantly higher after heat treatment, while β-lactoglobulin denaturation was greater than that of α-lactalbumin. Crowley et al. (2014) measured the heat coagulation time of reconstituted (3.5%, w/w, protein) MPC powders ranging in protein content from 35-90% (w/w) and reported that calcium ion activity played a significant role in reducing heat stability. A further study by Crowley et al. (2015) evaluated the behaviour of MPC powders, reconstituted to 8.5% protein (w/w), during heating at 120 °C to simulate in-container sterilisation, and reported that MPC containing approximately 85% protein (w/w) was extremely unstable to heating between pH 6.3-7.1.

Lin et al. (2018) performed low-heat (LH; 72 °C for 15 s) and medium-heat (MH; 85 °C for 30 s) treatment of skim milk prior to membrane filtration and spray drying and reported that MPC powder solubility was marginally higher for MH-MPC (96.8%) than LH-MPC (95.4%). Gazi and Huppertz (2015) produced MPC powder from LH (72 °C for 15 s) and MH (95 °C for 45 s) treated skim milk and reported no difference in initial solubility, despite higher whey protein denaturation (25 and 65% for α-lactalbumin and β-lactoglobulin, respectively) in the MH-MPC compared to LH-MPC. However, the solubility of the MH-MPC powder decreased more rapidly when

stored at elevated temperatures (>37 °C), compared to LH-MPC. Khalesi and FitzGerald (2021b) recently compared the physicochemical properties of commercial MPC powders, differing in the level of denatured whey proteins generated by heat treatment, and reported higher solubility and lower particle size values for the powder with higher whey protein denaturation when dispersed in water at 50 °C. Most studies investigating the relationship between thermal processing and solubility of subsequent MPC powders have only applied heat treatment to the skim milk before UF, despite heat treatment commonly being applied to the liquid UF concentrate. Carr (1999) applied a range of heat treatments (72-130 °C for 30-45 s) to skim milk prior to UF and investigated the solubility of MPC powders produced, with heat treatment generally leading to a decrease in powder solubility.

It is evident that the influence of thermal processing prior to spray drying, particularly high-heat (HH) treatment, on the functional properties of MPC powders, has not been fully elucidated and further research is necessary. While the denaturation of native whey proteins is likely one factor that may reduce powder rehydration performance, the extent to which rehydration deteriorates (or whether it does significantly) as the intensity of heat treatment applied to liquid UF concentrate increases (i.e., LH versus HH treatment) is not well defined (Gazi and Huppertz, 2015; Lin et al., 2018; Khalesi and FitzGerald, 2021b). Therefore, the objective of this research study was to investigate the influence of heat treatment after membrane filtration on the physical and functional properties of MPC powders

4.2. Materials and methods

4.2.1. Heat treatment of liquid milk protein concentrate and powder manufacture

Milk protein concentrate (MPC; 18.3 and 16.5%, w/w, total solids and protein,

respectively) was obtained from a dairy ingredient manufacturer following UF and DF of pasteurised skim milk. Three heat treatments were applied to the liquid MPC (18 L) using a pilot-scale Microthemics UHT/HTST system (MicroThermics, Raleigh, NC, USA) in the Bio-functional Food Engineering facility in Teagasc Food Research Centre (Moorepark, Co. Cork, Ireland): (i) low-heat (LH) treatment of 80 °C for 30 s, (ii) medium-heat (MH) treatment of 100 °C for 30 s, and (iii) high-heat (HH) treatment of 120 °C for 30 s, while the starting material was considered the control as it did not undergo additional heat treatment (i.e., it was only pasteurised). Powders were produced using an Anhydro single-stage spray dryer (SPX Flow Technology, Denmark), equipped with a two-fluid nozzle atomization system and configured in a counter-current flow mode, while the air inlet and outlet temperatures were set at 180 and 85 °C, respectively. The free moisture and ash contents of the MPC powders were determined using a TGA701 thermogravimetric analyser (LECO Corporation, St Joseph, Michigan, USA) at 102 and 550 °C, respectively. The protein nitrogen values of the liquid MPC and powders were obtained using a LECO FP628 nitrogen analyser (LECO Corporation, St Joseph, MI, USA) and the protein content was determined by multiplying the nitrogen concentration by a nitrogen-to-protein conversion factor of 6.38.

4.2.2. Colour and pH of liquid milk protein concentrates

The pH of liquid MPC samples was measured at 25 °C using a SevenCompact pH meter S210 (Mettler Toledo, Greifensee, Switzerland). The colour of each MPC sample was measured using a Chroma Meter CR-400 (Konica Minolta Sensing Europe B.V., Nieuwegein, the Netherlands). The colour measurement was determined according to the three colour coordinates: L*, a*, and b*. The value L* represents the

sample luminosity or brightness, varying from black (0) to white (100); a* represents the colour varying from green (-) to red (+); b* represents the colour varying from blue (-) to yellow (+). The total colour difference (ΔE) was calculated using the formula reported by Kelleher et al. (2020).

4.2.3. Viscosity of liquid milk protein concentrates

Viscosity of the control and heat-treated liquid MPC was measured under cold (5 °C) and warm (40 °C) conditions using an AR-G2 controlled-stress rheometer (TA Instruments, Crawley, UK), equipped with a parallel plate geometry. Investigating the rheological behaviour of MPC at 5 °C could be relevant for transport of liquid MPC, while analysis at 40 °C provides insight into viscosity prior to evaporation. Samples were pre-sheared at a shear rate of 200 s^{-1} for 30 s, followed by a shear rate ramp from 0.1 to 300 s^{-1} over 5 min, with the temperature (5 or 40 °C) controlled using a Peltier system (± 0.1 °C).

4.2.4. Calcium ion concentration

The concentration of ionic calcium in liquid MPC samples was determined using a Sension+ MM340 benchtop meter equipped with a Sension+ 9660C calcium ion selective electrode (Hach Co., CO, USA). The ion selective calcium probe was calibrated with standard calcium solutions of 0.5, 1.0, 2.5 and 5.0 mM at 25 °C (Lin et al., 2016). A standard curve was obtained using the linear relationship between electrical output (mV) and the logarithm of ionic calcium concentration. Analysis was performed in duplicate.

4.2.5. Particle size of liquid milk protein concentrates

The particle size distribution of control and heat-treated liquid MPC was determined by dynamic light scattering using a Zetasizer nano (Malvern Instruments, Worcestershire, UK). MPC samples were diluted (1:20) in ultrapure water (25 °C) and placed in disposable cuvettes for analysis. The dispersant refractive index used was 1.33, the viscosity parameter was 0.89 cP, and the sample refractive and absorption indices were set at 1.45 and 0.001, respectively.

4.2.6. Particle density, bulk density, interstitial and occluded air

Loose bulk density, tapped density (100 taps), particle density, interstitial air and occluded air were determined, as described by McSweeney et al. (2020). All measurements were recorded in duplicate.

4.2.7. Powder particle size distribution analysis

The particle size distributions of the MPC powders were determined using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with an Aero S dry powder dispersion unit. The refractive index and absorption index were set at 1.45 and 0.1, respectively. The air pressure was set at 2 bar and the obscuration range was 0.1-6%. Measurements were recorded as the median particle diameter (D_{50}) and cumulative diameters (D_{10}) and (D_{90}), whereby 10, 50 and 90% of the sample volume is represented by particles smaller than the size indicated. The volume-weighted mean particle diameter ($D_{[4,3]}$) was also calculated.

4.2.8. Particle size and solubility of milk protein concentrate dispersions

The particle size distribution of MPC dispersions was measured using a laser-

light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens, as described by McSweeney et al. (2020). Particle size measurements were recorded when the laser obscuration reached 3-4%. The solubility of MPC powders was measured at 23 and 50 °C using a traditional solubility method, as described by McSweeney et al. (2020); powder solubility was given by the total solids content of the supernatant (obtained following centrifugation at 3000*g* for 10 min), expressed as a percentage of the total solids content of the initial dispersion. In addition, to investigate the rehydration behaviour of these powders under more industrially relevant conditions, powders were reconstituted for 1 h in ultrapure water at 50 °C using a 4-blade overhead stirrer operating at 500 rpm and then stirred magnetically (250 rpm) at 4 °C for 21 h. This rehydration procedure was also used to prepare separate MPC dispersions for electrophoresis, chromatography, and heat stability experiments. Finally, to investigate the relationship between heat treatment and storage, powders were also placed in sealed plastic containers and stored at 37 °C for 14 d, and the solubility after mixing (50 °C water for 30 s) was measured.

4.2.9. Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

MPC powders were reconstituted (1 h in 50 °C ultrapure water followed by magnetic stirring for 21 h at 4 °C) to 3.5% protein (w/w) and diluted (1:10) to give a concentration of 3.5 µg protein/µL. Samples for electrophoresis were prepared by combining the MPC solution with lithium dodecyl sulphate buffer and ultrapure water in eppendorf tubes. For reduced samples, reducing agent (dithiothreitol) was added and samples heated at 80 °C for 10 min at 200 rpm. A precast 12% Bis-Tris Nu-PAGE gel was placed in an XCell Surelock Mini-Cell (Invitrogen, ThermoFischer Scientific,

Dublin, Ireland) containing running buffer and antioxidant, and 10 µL of each sample was added to the wells. Analysis was performed at a constant voltage of 200 V for 50 min (Buggy et al., 2017). Gels were then stained overnight using SimplyBlue Safe Stain (Thermo Fisher Scientific, Ireland) and de-stained using ultrapure water.

4.2.10. Quantification of native whey proteins

MPC powder dispersions (3.5%, w/w, protein) for reverse-phase high performance liquid chromatography (RP-HPLC) analysis were prepared as described in Section 4.2.8. Sodium acetate buffer (0.1 M; pH 4.6) was added to MPC dispersions to give a final protein concentration of 0.25% (w/w) and these were centrifuged at 20000g (4 °C) for 20 min to precipitate casein and non-native whey proteins. Prior to injection of the samples, the supernatants were filtered through Captiva 0.2 µm filters (PES 25 mm; Agilent Technologies, Ireland). β-Lactoglobulin, α-lactalbumin and BSA standards (Sigma Aldrich, Ireland) were used for column calibration. RP-HPLC (1200 series; Agilent Technologies) was used to quantify native whey proteins, in unheated and heat-treated MPC samples, using a Waters 2487 dual wavelength absorbance detector at 214 nm. A silica-based C-18 RP-HPLC column (ZorBax 300SB-C18 5 µm, 4.6 x 150 mm; Agilent Technologies) was used for separation of native whey proteins using a gradient solvent program of 82% solvent A (99.9 % MilliQ water + 0.1% trifluoroacetic acid) and 18% solvent B (99.9% of acetonitrile + 0.1% trifluoroacetic acid). The column temperature was 40 °C and the eluent flow rate was 1 mL min^{-1} for 45 min. Data was processed using Waters Empower® software.

4.2.11. Heat coagulation time

The heat coagulation time of MPC dispersions (3.5%, w/w, protein) was

determined over the pH range 6.7-7.2 at 140 °C, with the pH adjusted twice prior to analysis using 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. Glass tubes containing 3 mL of sample were immersed in a silicone oil bath and the time elapsed between placing samples in the oil bath and visible coagulation was recorded. All measurements were performed in duplicate.

4.2.12. Statistical data analysis

Measurements were performed in triplicate unless otherwise stated, with results presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was carried out using IBM SPSS (Version 28; Armonk, New York, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

4.3. Results and discussion

4.3.1. Physicochemical properties of liquid milk protein concentrates

The physicochemical properties of the liquid milk protein concentrate (MPC) samples are shown in Table 4.1. The pH did not change with heat treatment, with all MPC samples having a pH value of approximately 6.7. The z-average diameter increased slightly, but not significantly with heating. Lin et al. (2018) reported z-average diameters of 190 and 209 nm for low-heat (72 °C for 15 s) and medium-heat (85 °C for 30 s) MPC powder dispersions that were pH adjusted to 6.65, while Tari et al. (2021) reported that MPC heat treated at pH 6.7 for (i) 85 °C for 5 min or (ii) 125 °C for 15 s did not significantly alter the particle size (volume-weighted mean diameters of 0.15 and 0.16 μm, respectively). Calcium ion concentration increased significantly following LH and MH treatments, e.g., it increased from 3.23 for the control (C) to 3.79 and 4.08 mmol/L after LH and MH treatment, respectively, while

Table 4.1. Physicochemical properties of liquid control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate samples prior to spray drying.

MPC	pH	z-average diameter (nm)	Calcium ion concentration (mmol/L)	L*	a*	b*	ΔE	Apparent viscosity (mPa·s)
C	6.68	183 ± 8.8^{a}	3.23 ± 0.06^{a}	75.7 ± 0.14^{a}	-3.33 ± 0.02^{a}	-0.77 ± 0.05^{a}	-	6.68 ± 0.04^{a}
LH	6.71	181 ± 5.8^{a}	3.79 ± 0.05^{b}	77.8 ± 0.13^{b}	-3.61 ± 0.01^{b}	-0.03 ± 0.05^{b}	2.23 ± 0.02^{a}	19.1 ± 0.64^{b}
MH	6.71	189 ± 7.1^{a}	4.08 ± 0.08^{c}	79.2 ± 0.08^{c}	-4.03 ± 0.02^{c}	-0.28 ± 0.02^{c}	3.61 ± 0.06^{b}	23.3 ± 1.94^{c}
HH	6.72	195 ± 4.6^{a}	4.11 ± 0.02^{c}	80.3 ± 0.02^{d}	-4.11 ± 0.01^{d}	-0.54 ± 0.01^{d}	4.65 ± 0.12^{c}	25.1 ± 0.79^{c}

$^{a-d}$ Values within columns not sharing common superscript letters differ significantly ($P < 0.05$). All measurements were recorded at 25 °C, with the exception of apparent viscosity, which was measured at 40 °C (shear rate of 300 s^{-1}).

there was no difference between MH and high-heat (HH) samples. This may have been caused by differences in viscosity, combined with the relatively high total solids content of the samples, which affected the measurements obtained by the probe. While Ho et al. (2018) reported that calcium ion activity of MPC increased following heat treatment at 75 °C for 5 min, it is widely reported that heat treatment decreases the concentration of ionic calcium in the serum phase of milk (Lewis, 2011). Future work involving mineral analysis of the colloidal and serum phases after ultracentrifugation would provide more clarity on this result. Changes in colour measurements were also observed post heat treatment. Total colour difference, which takes each of the colour values into account, demonstrated that the colour increased as heat treatment temperature increased, e.g., 2.23 for LH-MPC and 4.65 for HH-MPC. Kelleher et al. (2020) reported a colour difference of 2.02 for a milk protein beverage containing a casein:whey ratio of 80.20 which had received a final heat treatment of 120 °C for 30 s, compared to an unheated control.

Analysis of viscosity demonstrated the significant effect of temperature on the rheological properties of liquid MPC (Fig. 4.1). When measurements were performed at 5 °C, C-MPC had the lowest viscosity of all samples, with an apparent viscosity of 126 mPa·s, and this increased with heat treatment temperature (e.g., 317, 379 and 426 mPa·s for LH-, MH- and HH-MPC, respectively). This may be relevant in relation to the transport of liquid protein concentrates for use in food and beverage formulations (Dunn et al., 2021). Viscosity was considerably lower when measured at 40 °C (Table 4.1), but the same trend persisted (i.e., concentrate viscosity increased with the temperature of heat treatment): apparent viscosity at 40 °C was 6.68 for C-MPC, and this increased to 19.1, 23.3 and 25.1 mPa·s for LH-, MH- and HH-MPC. Higher viscosity after heat treatment would likely limit the total solids content

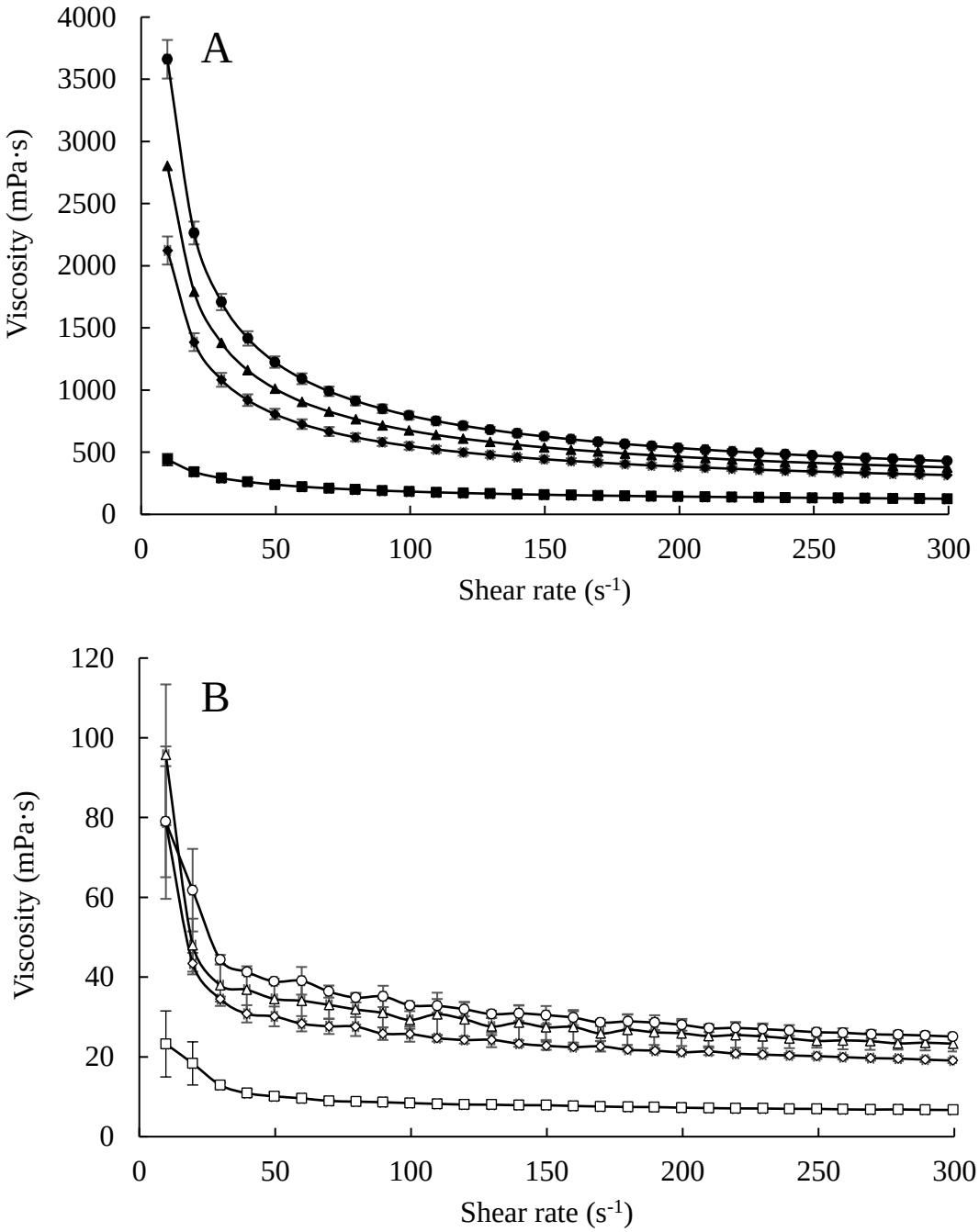

Fig. 4.1. Viscosity as a function of shear rate at (A) 5 °C for control (■), low-heat (♦), medium-heat (▲) and high-heat (●) milk protein concentrates and at (B) 40 °C for control (□), low-heat (◊), medium-heat (Δ) and high-heat (○) milk protein concentrates (18.3%, w/w, total solids).

attainable during subsequent evaporation, thereby increasing energy costs during spray drying. Ho et al. (2019) reported that viscosity of liquid MPC (19.8%, w/w, total solids) increased with increasing temperature of heat treatment (85, 100 and 120

°C), and this was likely caused by higher levels of whey protein denaturation and aggregation. For example, when measured at 45 °C, the control MPC had a viscosity of ~8 mPa·s at a shear rate of 300 s^{-1} and this increased to ~21 mPa·s after heat treatment at 120 °C for 30 s. Warncke et al. (2022) reported higher apparent viscosity for MPC following heat treatment for 30 min at 80 °C. Anema et al. (2014) suggested that viscosity of skim milk concentrate increased after heat treatment, particularly at pH 6.5 and 6.7, due to the association of denatured whey proteins with the casein micelles, increasing their voluminosity. It is important to mention that viscosity analysis was performed in the current study after storing MPC samples at 4 °C overnight and not immediately after heat treatment as it was reported by Tari et al. (2021) that viscosity of liquid MPC increased during storage.

4.3.2. Composition and physical properties of milk protein concentrate powders

The moisture contents of the MPC powders were 5.0, 5.6, 5.1 and 6.1% (w/w) for C-, LH-, MH- and HH-MPC, respectively, while the ash content was 7.8% for all four powders. Furthermore, the protein content was 87.1, 86.1, 86.9 and 85.9% (w/w) for C-, LH-, MH- and HH-MPC, respectively. The physical properties of the MPC powders are shown in Table 4.2. Particle density was highest for C-MPC (1.17 g/cm^3), followed by LH- (1.09 g/cm^3), HH- (1.03 g/cm^3) and MH-MPC (1.02 g/cm^3). However, loose bulk density was lower for C-MPC (0.22 g/cm^3) than for heat-treated MPC powders (0.25-0.26 g/cm^3), while following 100 taps, bulk density increased by approximately 0.06 g/cm^3 for all powders. Regarding the air content of the powders, C-MPC had the highest interstitial air (272 mL/100 g) but lowest occluded air (16.9 mL/100 g) values, while there were little differences between powders produced from heat-treated concentrate, e.g., 221 mL/100 g for LH-MPC compared

to 216 mL/100 g for HH-MPC. The slightly lower interstitial air content and higher bulk density of heat-treated MPC powders compared to C-MPC may have been caused by the higher viscosity of these concentrates.

Powder particle size increased with increasing temperature of heat treatment (Table 4.2). The volume-weighted mean particle diameter ($D_{[4,3]}$) increased from 27.4 µm for C-MPC to 43.9 and 71.4 µm for LH- and MH-MPC powders, respectively, while there was no difference in the $D_{[4,3]}$ between MH- and HH-MPC powders. The increased size of powder particles generated is most likely accounted

Table 4.2. Physical properties of control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate powders.

MPC	p_p	p_b	p_t	V_{ia}	V_{oa}	$D_{[4,3]}$
	g/cm^3			mL/100 g		µm
C	1.17 ± 0.00	0.22 ± 0.01	0.28 ± 0.00	272 ± 0.3	16.9 ± 0.1	27.4 ± 0.6^a
LH	1.09 ± 0.00	0.26 ± 0.00	0.32 ± 0.00	221 ± 0.0	22.9 ± 0.9	43.9 ± 3.8^b
MH	1.02 ± 0.00	0.26 ± 0.00	0.33 ± 0.00	205 ± 1.0	29.1 ± 1.1	71.4 ± 2.6^c
HH	1.03 ± 0.00	0.25 ± 0.00	0.32 ± 0.00	216 ± 0.7	28.4 ± 0.3	73.4 ± 3.5^c

$^{a-c}$ Values within columns not sharing common superscript letters differ significantly ($P < 0.05$). Particle density (p_p), loose bulk density (p_b), tapped bulk density (p_t), volume of interstitial air (V_{ia}), volume of occluded air (V_{oa}), particle size below which 90% of material volume exists (D_{90}), and the volume-weighted mean particle diameter ($D_{[4,3]}$).

for by the higher concentrate viscosity after heat treatment (Fig. 4.1) as it would be more difficult for the nozzle to form small uniform droplets. Rupp et al. (2018) reported that when the protein content of liquid MPC was increased from 19 to 21 and 23% (w/w) using evaporation, there was a corresponding increase in the viscosity of the concentrate and the $D_{[4,3]}$ values of the spray-dried powders (31, 37 and 50 µm, respectively). Similarly, Park et al. (2016) reported higher $D_{[4,3]}$ for MPC powders

produced from concentrate at 22% total solids (46.8 µm) compared to concentrate at 12% total solids (34.2 µm).

4.3.3. Protein profile by electrophoresis

The protein profile of MPC under reducing and non-reducing conditions is displayed in Fig. 4.2. For the non-reduced samples in lanes 1-4, protein aggregates were visible at the top of the gel in the loading wells. These were likely disulphide-linked whey proteins as they were not visible in lanes 5-8 due to the addition of reducing agent (dithiothreitol) which breaks disulphide bonds between cysteine residues. Heat treatment did not appear to have a substantial effect on the α-caseins as the band intensity was similar for all samples. However, the intensity of the κ-casein band was lower for HH-MPC (lane 4), which suggests it dissociated from the casein micelle during heating and formed aggregates with whey proteins in the serum phase. Dissociation of κ-casein from the micelle has been reported by Sauer and Moraru (2012) during heating of micellar casein concentrate at 110-150 °C. Anema and Li (2000) reported that dissociation of κ-casein in reconstituted skim milk generally increased with increasing temperature from 60-120 °C. SDS-PAGE protein profiles reported by Tari et al. (2021) and Ho et al. (2018) did not show any differences in the intensity of the κ-casein bands between the control and MPC heated at 125 °C for 15 s and 75 °C for 20 min, respectively. Crowley et al. (2014) reported higher levels of non-sedimentable κ-casein for an MPC85 powder reconstituted to 3.5% protein at pH 6.5 and 6.8 when no heating was applied compared to 90 °C for 30 min, while this trend was reversed at pH 7.1. Under non-reducing conditions in the current study, the intensity of the β-lactoglobulin (β-lg) band decreased significantly with heat treatment, particularly from LH to MH-MPC, with no band

present for HH-MPC, while for α-lactalbumin (α-la), faint bands were visible for all samples except HH-MPC. Ho et al. (2018) reported lower band intensities for β-lg and α-la following heat treatment at 75 °C for 20 min. Similarly, the SDS-PAGE gels

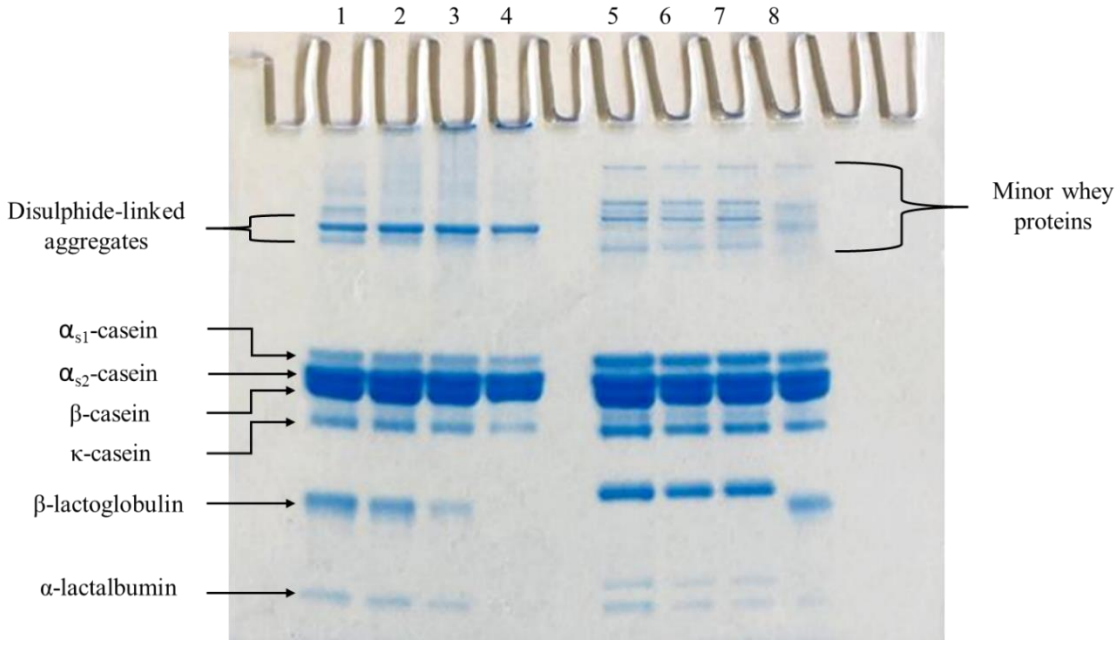

Fig. 4.2. Sodium dodecylsulphate-polyacrylamide gel electrophoresis profiles of milk protein concentrate under non-reducing (1-4) and reducing (5-8) conditions: control (lanes 1 and 5), low-heat (lanes 2 and 6), medium-heat (lanes 3 and 7) and high-heat (lanes 4 and 8).

produced by Tari et al. (2021) showed faint bands for these two whey proteins when MPC was heat-treated at 125 °C for 15 s (pH 6.7 and 6.9) compared to the control. For the reduced samples in lanes 5-8, the κ-casein and β-lg bands were restored following the reduction of disulphide bonds, while the intensity of the bands corresponding to α-la only changed slightly.

4.3.4. Quantification of native whey proteins

The quantity of native whey proteins in MPC and the extent of whey protein denaturation induced by each heat treatment is presented in Table 4.3. Denaturation

of both α-la and β-lg increased with increasing heat treatment temperature in the range 80-120 °C, and to a greater extent for β-lg. Vasbinder and de Kruif (2003) previously reported that β-lg denatured more readily than α-la (70 and 40%, respectively) when skim milk was heated at 80 °C for 10 min at pH 6.7. In the current study, the quantity of α-la present was low overall and decreased from 0.045 mg/100 mL for C-MPC to 0.017 mg/100 mL for HH-MPC, corresponding to a 62% decrease in the concentration of native α-la (Table 4.3). This is supported by the SDS-PAGE results in Fig. 4.2, whereby α-la was not visible for HH-MPC under non-reducing conditions (lane 4). Similarly, extensive β-lg denaturation occurred following HH treatment, with the concentration decreasing from 0.313 to 0.029 mg/100 mL, and also correlates with the absence of a β-lg band on the gel in Fig. 4.2. Similarly, using RP-HPLC, Gazi and Huppertz (2015) reported denaturation values of 25 and 65% for α-la and β-lg following heat treatment of skim milk at 95 °C for 45 s prior to MPC

Table 4.3. Concentration (mg/100 mL) and denaturation (%) of whey proteins in control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate (MPC) samples following heat treatment.

MPC	α-Lactalbumin	β-Lactoglobulin
C	0.045 ± 0.001	0.313 ± 0.002
	-	-
LH	0.039 ± 0.002	0.222 ± 0.004
Denaturation	14.4 ± 1.7	29.0 ± 1.4
MH	0.030 ± 0.004	0.098 ± 0.014
Denaturation	33.0 ± 9.5	68.6 ± 4.4
HH	0.017 ± 0.000	0.029 ± 0.001
Denaturation	62.2 ± 0.7	90.7 ± 0.4

Denaturation is expressed as the percentage decrease in concentration of native whey proteins relative to the control sample.

manufacture. However, Tari et al. (2021) reported considerably lower denaturation values of 3.02 and 33.4% for α-la and β-lg following heat treatment (125 °C for 15 s) of MPC (70%, w/w, protein) at pH 6.7, when measured using ion exchange chromatography.

Heat treatment is often used to alter the quantity of native whey proteins in a resultant powder as this can influence its industrial applications. Carr (1999) reported that increasing the extent of whey protein denaturation by heat-treating skim milk prior to MPC manufacture led to an increase in rennet coagulation time. However, denatured whey proteins play an important role in the rheological properties of yogurt. For example, Lucey et al. (1997) reported that gelation time of reconstituted skim milk powders decreased as whey protein denaturation increased (up to 95 and 81% for α-la and β-lg, respectively) with the intensity of heat treatment applied before spray drying. The association of denatured whey proteins with casein micelles via disulphide bonding was identified as an important factor for increasing the stiffness of acid milk gels (Lucey et al., 1998). Further research investigating the influence of native whey protein content in MPC powder on its functionality in different food systems (e.g., ability to form rennet and acid gels) would provide useful data for ingredient producers and end-users.

4.3.5. Heat stability

The stability of MPC dispersions to heating over the pH range 6.7-7.2 at 140 °C is shown in Fig 4.3, with heat stability generally increasing with increasing pH. This resembles the pH-heat coagulation time (HCT) profiles of type B milk and serum-protein free casein micelle dispersions described by Singh (2004). All MPC samples were unstable to heating at pH 6.7, with coagulation occurring after

approximately 1 min. At pH 6.8, heat stability remained low (<2 min) for LH-, MH- and HH-MPC, but was slightly higher for C-MPC. Heat stability increased considerably to ~9 min at pH 6.9 for C-, LH- and MH-MPC, while HH-MPC did not

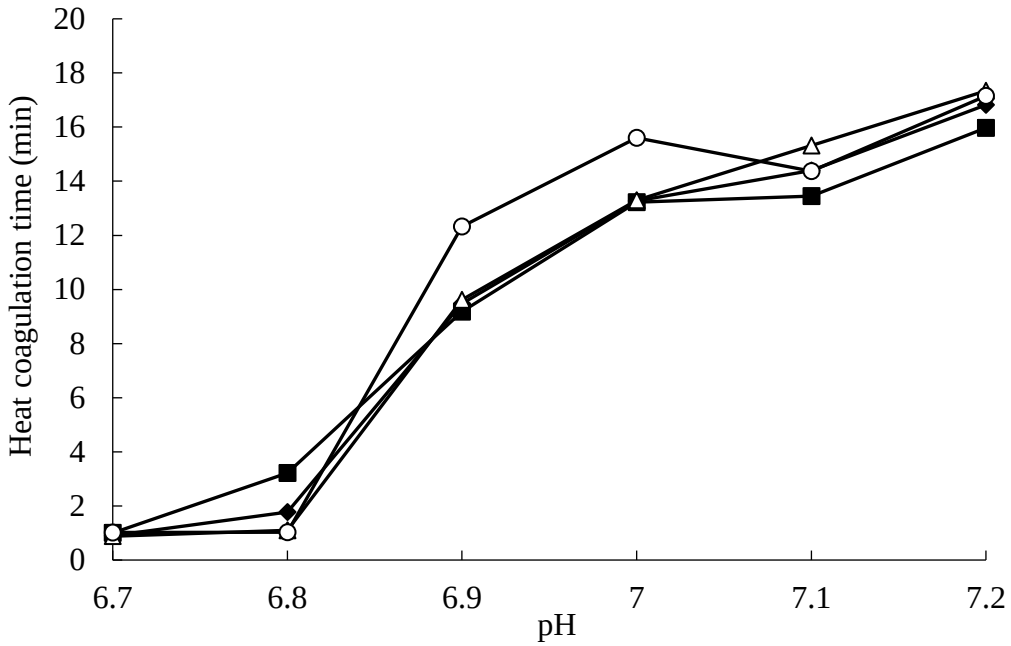

Fig. 4.3. pH-heat coagulation time profiles at 140 °C for control (■), low-heat (♦), medium-heat (Δ) and high-heat (○) milk protein concentrate powders reconstituted to 3.5% protein (w/w).

coagulate until 3 min later. This trend was also observed at pH 7, whereby HH-MPC remained stable for 2 min more than the other samples, however; a slight reduction in HCT was observed for HH-MPC at pH 7.1, but this increased again at pH 7.2. The calcium ion concentration in MPC solutions at pH 7.2 was 3.04, 2.95, 2.92 and 3.10 mmol/L for C-, LH-, MH- and HH-MPC, respectively, which suggests calcium ion concentration did not play a large role in the heat stability observed. The results suggest that denatured whey proteins in MPC can provide some improvements in heat stability at certain pH values (i.e., 6.9 and 7). Crowley et al. (2014) reported that MPC powder (84%, w/w, protein) reconstituted to 3.5% protein (w/w) had extremely

poor heat stability at or below pH 7 (HCT <1 min), while it increased to ~10 and 13 min at pH 7.1 and 7.2, respectively, likely due to decreased calcium ion activity. Crowley et al. (2015) reported that MPC powder produced from skim milk heated at 95 °C for 45 s showed higher heat stability at 120 °C in the pH range 6.8-7.1 than MPC produced from pasteurised skim milk, but it decreased at pH 7.2 and 7.3. Carr (1999) did not report a significant change in the heat stability (120 °C) of MPC powders produced from heat-treated skim milk up to whey protein denaturation levels of 86%, but that heat stability decreased significantly once denaturation reached 90%. Sunkesula et al. (2021) measured heat stability of reconstituted MPC (10% protein) at 140 °C and reported HCT of 13.02, 20.29 and 8.37 min at pH 6.7, 6.9 and 7.1, respectively. Khalesi and FitzGerald (2021a) reported HCT values of 2.2 and 2.7 min at 140 °C for MPC containing 16.6 and 6.0 g/100 g of native whey proteins, respectively, with heat stability remaining higher for the powder containing less native whey at 110, 120 and 130 °C also. It is important to consider that heat stability results are difficult to compare across studies given the subjective nature of the test, particularly at specific pH values, but can provide useful information regarding trends as pH changes.

4.3.6. Particle size distribution and solubility of milk protein concentrate dispersions

The particle size distribution profiles of MPC powders following reconstitution are shown in Fig. 4.4. When mixed in 23 °C ultrapure water for 30 s, each sample had a monomodal distribution in the size range 10-100 μm (Fig. 4.4A) and there was no significant difference between samples in relation to the D_{90} and $D_{[4,3]}$ values (Table 4.4). However, when the temperature of the water was increased

to 50 °C, the volume of large particles (10-100 μm) was reduced for all powders, but only C- and LH-MPC had a new second peak in the size range 0.01-1 μm (Fig. 4.4B), suggesting that some of these powder particles dispersed. The overall size distribution was expanded, possibly due to powder particle swelling (Table 4.4), and there was a significant difference between C-MPC and samples that were heat treated. For example, when the water temperature was 23 °C, the $D_{[4,3]}$ values were 35.3, 39.9 and 43 μm for C-, LH-, MH-MPC, but when the temperature was increased to 50 °C, the $D_{[4,3]}$ values were 24.8, 65.6 and 74 μm, respectively. To further elucidate the effect of heat treatment on MPC powder rehydration, overhead stirring was performed instead and for a longer duration, with the particle size distribution profiles for all samples shown in Fig. 4.4C. Under these conditions, all samples displayed a bimodal distribution. There was little difference in the dispersion of C- and LH-MPC, with the $D_{[4,3]}$ value being 11.2 and 8.95 μm, respectively. However, MH- and HH-MPC had significantly higher $D_{[4,3]}$ values of 51.5 and 55.5 μm, respectively. The dispersions prepared for 1 h were subsequently stirred magnetically in the fridge overnight to investigate if the powders would eventually disperse and solubilise to the same extent, and the distribution is shown in Fig. 4.4D. The trend observed previously for C- and LH-MPC remained the same, with both distributions overlapping, but a greater difference was recorded between the MH- and HH-MPC, with $D_{[4,3]}$ values of 22.9 and 40.3 μm, respectively (Table 4.4).

The solubility values obtained following centrifugation of MPC dispersions, prepared in 23 and 50 °C water, generally followed the same trends as those observed in the particle size distribution data, as shown in Fig. 4.5. When measured at 23 °C, solubility was highest for C-MPC (87.8%) but decreased to 64.7, 45.9 and 48.8% for LH-, MH- and HH-MPC, respectively. When solubility was evaluated after

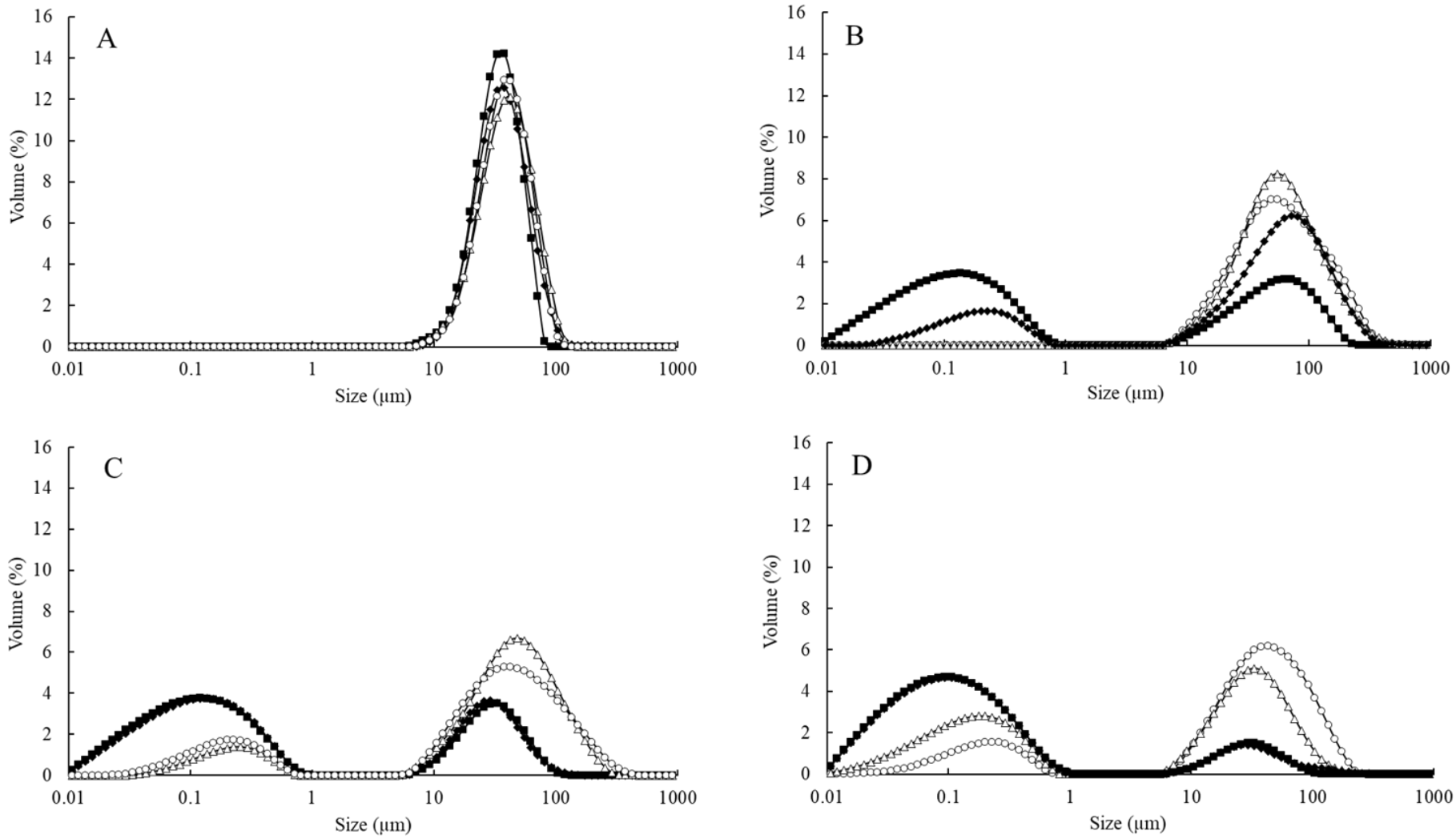

Fig. 4.4. Particle size distribution profiles of control (■), LH (♦), MH (Δ) and HH (○) milk protein concentrate powders measured after reconstitution in ultrapure water for (A) 30 s at 23 °C, (B) 30 s at 50 °C (C) 1 h at 50 °C and (D) overnight stirring

reconstitution in 50 °C water, C-MPC remained the most soluble (95.6%), followed by LH- (92%), MH- (75.5%) and HH- (74.2%) MPC, demonstrating that heat treatment impaired the rehydration performance of MPC. Furthermore, solubility measurements of MPC powders stored for 2 weeks at 37 °C demonstrated that heat treatment accelerated the deterioration in solubility. The solubility values were 93.6, 89.1, 61.1 and 33.2% for C-, LH-, MH- and HH-MPC, respectively. The greatest decrease in solubility was recorded for HH-MPC, as it was 74.2% initially, but only 33.2% after storage. These result agree with the study by Gazi and Huppertz (2015) which reported higher storage-induced solubility loss for MPC produced from skim milk heated at 95 °C for 45 s.

Carr (1999) applied several heat treatments to skim milk prior to membrane filtration and reported that MPC powder (83-86%, w/w, protein) solubility decreased with increasing temperature of heat treatment. Solubility, evaluated after stirring powders for 1 h in 50 °C water, was 95.5% for MPC produced from pasteurised skim milk, 81.2% when the heat treatment was 80 °C for 30 s, 81.1% for the skim milk processed at 100 °C for 30 s and only 22.5% for the sample heat treated at 120 °C for 45 s. This supports the results presented in the current study whereby powder solubility decreases with the increased intensity of heat treatment applied before spray drying. Furthermore, Carr (1999) reported that a higher homogenisation pressure (i.e., 200 bar instead of 150 bar) was generally required to promote powder solubilisation as the heat treatment temperature increased. Gazi and Huppertz (2015) did not report a difference in solubility between MPC produced from pasteurised skim milk compared to an MPC powder derived from skim milk that was heat-treated at 95 °C for 45 s. Similarly, Lin et al. (2018) did not find a significant difference between MPC manufactured from pasteurised and medium-heat (85 °C for 30 s) treated skim milk;

Table 4.4. Particle size values for control (C), low heat (LH), medium heat (MH) and high heat (HH) milk protein concentrate (MPC) dispersions after high-shear mixing at 23 °C and 50 °C for 30 s using a solubility index meter, overhead stirring (500 rpm) in 50 °C ultrapure water for 1 h and magnetic stirring (250 rpm) for 21 h (4 °C).

Rehydration procedure	MPC	D_{90}	$D_{[4,3]}$
		μm	
23 °C for 30 s	C	55.0 ± 1.2[a]	35.3 ± 0.6[a]
	LH	65.3 ± 2.7[b]	39.9 ± 1.7[b]
	MH	71.4 ± 3.4[c]	43.0 ± 1.7[c]
	HH	66.6 ± 2.8[bd]	41.2 ± 2.0[bcd]
50 °C for 30 s	C	85.2 ± 3.6[a]	24.8 ± 2.2[a]
	LH	147 ± 8.2[b]	65.6 ± 4.1[b]
	MH	151 ± 15[bc]	74.0 ± 6.4[c]
	HH	171 ± 14[d]	78.6 ± 4.4[cd]
50 °C for 1 h	C	39.6 ± 4.5[a]	11.2 ± 2.2[a]
	LH	32.7 ± 5.3[a]	8.95 ± 2.9[a]
	MH	115 ± 12[b]	51.1 ± 4.3[b]
	HH	128 ± 18[b]	51.5 ± 8.1[b]
4 °C for 21 h	C	21.4 ± 3.5[a]	4.90 ± 0.68[a]
	LH	23.1 ± 5.2[a]	6.61 ± 2.03[a]
	MH	60.9 ± 7.9[b]	22.9 ± 3.0[b]
	HH	95.0 ± 9.3[c]	40.3 ± 6.8[c]

[a-d] Values within columns, for each rehydration procedure, not sharing common superscript letters differ significantly ($P < 0.05$). The dispersions used for magnetic stirring were those prepared by overhead stirring powders in 50 °C ultrapure water for 1 h.

however, solubility was evaluated after stirring in 50 °C water for 2 h followed by overnight stirring, a point at which differences would be unlikely, as suggested by the particle size distribution data from overnight stirring for C- and LH-MPC in the current study (Table 4.4). Khalesi and FitzGerald (2021b) investigated the rehydration performance of MPC powders (~85%, w/w, protein) containing different quantities of native whey proteins (16.6 and 6.0 g/100 g) due to different heat treatments (conditions

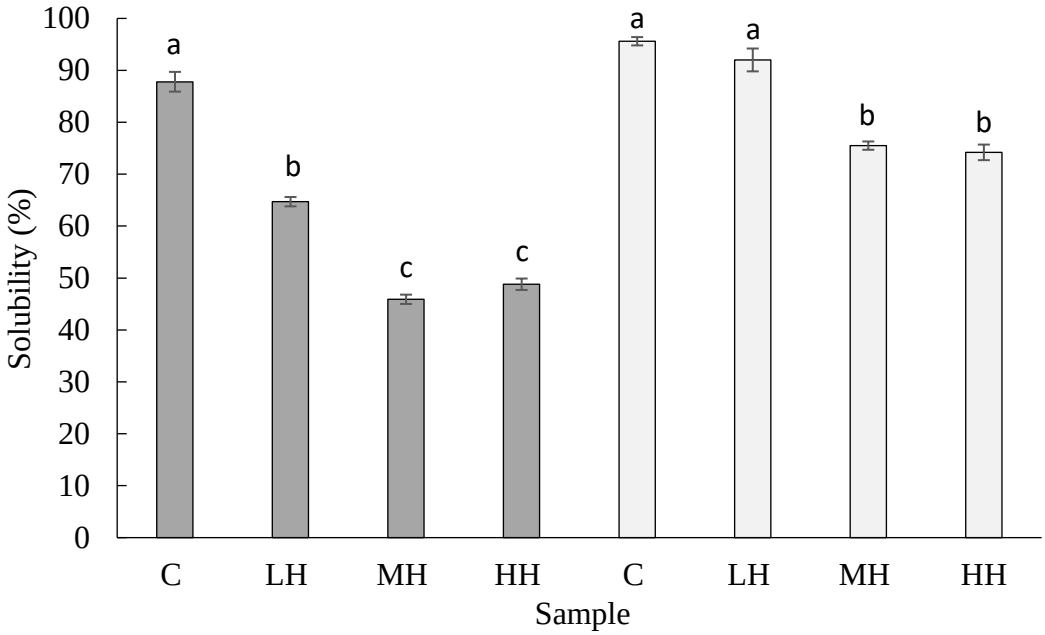

Fig. 4.5. Solubility values for control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate dispersions after mixing for 30 s in 23 °C (dark grey bars) and 50 °C (light grey bars) ultrapure water, followed by centrifugation at 3000*g* for 10 min. Values within each temperature category not sharing a common superscript differ significantly ($P < 0.05$).

not disclosed). Particle size distribution was determined by stirring powders in 50 °C water for 1 h (5%, w/v, protein) and the D_{90} value reported for the MPC with more native whey protein was 3.13 µm, compared to 0.25 µm for the other powder. These values are considerably lower than those reported in the current study, which ranged from 32.7 to 128 µm depending on the heat treatment applied (Table 4.4). Particle size

values within that range (i.e., 0.25-3.13 µm) are usually only attainable following intense high-shear treatment, such as the value (0.4 µm) reported by Pathania et al. (2018) when MPC was reconstituted using hydrodynamic cavitation. The D_{90} value of 39.6 µm for C-MPC is similar to the result of 59.4 µm reported by McCarthy et al. (2014) for an MPC (81.4%, w/w, protein) prepared by overhead stirring in 50 °C water for 1 h. Khalesi and FitzGerald (2021b) also measured solubility of MPC powders after dispersing them for 30 min in water. Following a 4 h holding time, the average solubility was ~73 and 77% for the sample with more native whey protein at 25 and 50 °C, and ~66 and 88% for the powder with less native whey protein at these temperatures, respectively. This seems contrary to the results presented in the current study which demonstrate that rehydration performance of MPC is impaired by the application of an intensive thermal process to the liquid concentrate directly after membrane filtration and prior to spray drying, particularly upon reaching heat treatment parameters of 100 ℃ for 30 s. This is likely a result of the formation of whey protein and whey-casein aggregates which do not dissolve readily and remain even after overnight stirring, thereby further impairing MPC powder dissolution. Overall, the results suggest that heat treatment of ultrafiltration concentrate at ≥100 °C for 30 s can present processing challenges for users of MPC powder (i.e., will be more difficult to solubilise the powder). One approach for addressing this challenge may be the use of high-shear technologies to accelerate powder dissolution. It is important to consider this as other powder applications may require high levels of denatured whey proteins, such as yogurt manufacture.

4.4. Conclusion

Heat treatment of liquid milk protein concentrate (MPC) after ultrafiltration

can play an important role in modifying its functional properties and those of the resulting powders. Heating MPC at 100-120 °C for 30 s significantly increased viscosity. Physical properties of MPC powders were altered by heat treating the concentrate, most notably an increase in bulk density and powder particle size. Protein profile analysis demonstrated a significant decrease in the proportion of native whey proteins as the temperature of heat treatment increased, which appeared to confer higher heat stability to MPC at pH 6.9 and 7. However, the creation of protein aggregates impaired the dispersion and solubilisation of MPC powders, with large particles remaining after extensive mixing. The results presented in this study will inform dairy ingredient researchers and manufacturers of the benefits and disadvantages of applying heat treatments to liquid milk protein concentrates prior to spray drying. Further studies involving the effect of both heat treatment and evaporation of MPC prior to spray drying on powder rehydration performance are required, and how this influences end-product functionality.

4.5. Acknowledgements

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Chapter 5

Influence of nitrogen gas injection and agglomeration during spray drying on the physical and bulk handling properties of milk protein concentrate powders

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Abstract

This study investigated the influence of injecting nitrogen gas (N_2) under high pressure into milk protein concentrate (80%, w/w, protein; MPC) prior to spray drying and examining the physical and bulk handling properties of regular (non-agglomerated) and agglomerated powders. MPC powders produced using the N_2 injection (NI) process had significantly lower bulk density and flowability, higher wall friction angles and increased levels of interstitial and occluded air. Agglomerated MPC powders had higher flow index values, lower wall friction angles, but were more friable, compared to regular powders. Surface composition analysis of MPC powders showed that NI caused fat to preferentially migrate to the surface in comparison to powders spray dried without NI. The results obtained in this study demonstrate that the injection of N_2 into liquid MPC directly prior to spray drying, as well as agglomeration by fines return, can produce ingredients with unique particle and bulk powder properties.

5.1. Introduction

The adoption of membrane filtration technology has enabled the dairy industry to produce high-protein, casein-based dairy powders such as milk protein concentrate (MPC). In the preparation of MPC using ultrafiltration and diafiltration of skim milk, caseins and whey proteins are retained, while lactose and minerals pass through the membrane as permeate. This high-protein retentate is then evaporated and spray dried to form a powder (Mistry and Hassan, 1991). MPC ingredients are incorporated into a range of products due to their functional, sensory and nutritional properties, e.g., yogurt, cheese, low lactose beverages and medical nutrition products (Agarwal et al., 2015). However, commercially available high-protein, casein-dominant powders (e.g., MPC and micellar casein concentrate) generally have poor powder rehydration properties. This has mainly been attributed to hydrophobic interactions occurring between micellar casein proteins in close proximity and the low concentration of lactose facilitating close packing (Havea, 2006; Anema et al., 2006). Several approaches have previously been developed to improve the rehydration properties of MPC powders. These include chemical modifications such as the use of calcium chelating agents (McCarthy et al., 2017) and ion-exchange (Bhaskar et al., 2001), and physical high shear treatments such as microfluidisation or homogenisation of concentrates before spray drying (Augustin et al., 2012). However, limited research has been performed regarding the use of gas injection to alter powder particle structure and improve the subsequent rehydration of high-protein dairy ingredients.

Gas injection has been utilised in dairy products to modify the functionality of milk powders, butter and cheese (Bisperink et al., 2004; Adhikari et al., 2018). Hanrahan et al. (1962) investigated the influence of nitrogen gas (N_2) injection into whole milk concentrate before atomisation on the characteristics of the spray-dried

powder and reported an improvement in dispersibility and an increase in powder particle size. Similarly, Bell et al. (1963) produced a skim milk powder with higher dispersibility via the injection of compressed air into the concentrate between the high-pressure pump and nozzle. More recently, Bouvier et al. (2013) used a novel technology known as extrusion-porosification to create MPC powders with improved dispersibility due to increased particle porosity. This involved mixing carbon dioxide gas with a high solids concentrate using a twin-screw extrusion-aeration system. The influence of carbonation on the physical and functional properties of whole milk powder has been reported by Kosasih et al. (2016), whereby the addition of CO_2 prior to spray drying increased powder porosity, occluded air content and dispersibility. Aside from dairy, confectionary products (e.g., marshmallows, nougat and taffy) are often aerated during manufacture to modify relative density, texture and appearance (Hartel et al., 2018).

Modifying powder particle structure may influence the physical attributes of the bulk powder (e.g., density, porosity, friability, particle size and morphology), and these factors can play an important role in bulk handling of powders industrially. After spray drying, powder is usually transferred to storage containers (e.g., bins, silos) and can undergo numerous handling (e.g., pneumatic conveying) and processing (e.g., packaging) steps (Ilari, 2002). To alter the physical and bulk handling properties of powders, the process of agglomeration may be used whereby small powder particles, collected from the cyclone (i.e., fines), can be pneumatically conveyed and returned to the top of the spray dryer main chamber and introduced near the nozzles to combine with atomised milk droplets (Gianfrancesco et al., 2008; Murrieta-Pazos et al., 2012). Agglomeration is used extensively in the dairy industry for whole milk, fat-filled and infant formula powders in which the physical (e.g., flow behaviour, density and

porosity) and rehydration (e.g., wetting) characteristics are significantly improved (Palzer, 2007). However, agglomeration is seldom used in high-protein dairy ingredients as it generally increases rehydration times (Crowley et al., 2016; Gaiani et al., 2007). The combined effect of gas injection and agglomeration on the physical properties of high-protein powders has not been previously investigated but may facilitate the manufacture of MPC powders with improved flow behaviour and rehydration performance. Therefore, the objective of this paper was to characterise the physical and bulk handling properties of MPC powders produced using N_2 injection prior to spray drying and agglomeration.

5.2. Materials and methods

5.2.1. Rehydration of milk protein concentrate powder

Milk protein concentrate (MPC) powder (80%, w/w, protein) was supplied by a local dairy ingredient manufacturer. All subsequent processing was carried out using the pilot-plant facilities at Moorepark Technology Limited (Teagasc, Moorepark, Fermoy, Co. Cork, Ireland). To obtain a rehydrated MPC dispersion, reverse osmosis water (1800 kg) was weighed into a 5000 L capacity, jacketed, stainless steel tank, attached to a continuous in-line Crepaco high shear mixer (APV Pulvermixer, SPX Flow Technology, Pasteursvej, Silkeborg, Denmark), configured in a "squirrel cage" design. MPC powder (~500 kg) was inducted directly into the recirculating water stream (50 °C) as it passed through a high shear mixing head. Once dispersed, it was recirculated for 30 min and stored at 5 °C overnight under gentle agitation.

The MPC dispersion (21.2%, w/w, total solids) was passed once through an SPX hydrodynamic cavitator (Model P286184-12 R4; SPX Flow Technology, Pasteursvej, Silkeborg, Denmark) equipped with a proprietary dispersion head (300

mm diameter), consisting of 160 discrete fluid channels, at a rotational speed of 2914 rpm to ensure complete rehydration of the powder (Pathania et al., 2018). The rotor speed, which determines the extent of cavitation, was driven by a 30 kW motor at a frequency of ~40 Hz. The MPC dispersions were transferred through the cavitator using a centrifugal pump at a feed flow rate of 1287 L/h. The flow rate, and thereby residence time in the cavitation zone, was controlled by a manual back-pressure valve on the system outlet (1.18 bar) with a product change in temperature of 15 °C.

5.2.2. Nitrogen gas injection, spray drying and agglomeration

Immediately after hydrodynamic cavitation, the MPC dispersion was heated from 18 to 70 °C using a scraped surface heat exchanger and passed through two filters (pore size of 800 μm), before being pumped to the atomisation nozzles using a high-pressure pump (HPP). Nitrogen gas (N_2) was injected (3.5 kg/h) at a pressure of ~190 bar into the feed line, after the HPP and prior to atomisation, using a pressurised injection unit (Carlisle Process Systems, Farum, Denmark). Concentrates were dried using a NIRO Tall Form spray dryer (TFD-0025-N, Soeborg, Denmark), with air inlet and outlet temperatures set at 185 and 75 °C, respectively, for manufacture of regular and agglomerated powders. Air inlet and outlet temperatures for concentrates with N_2 injection were set at 180 and 75 °C, respectively, for both regular and agglomerated variants. First- and second-external fluid bed temperatures were set at 50 and 25 °C, respectively. Agglomeration was performed by returning all fines collected in the cyclone to the top of the spray dryer. For regular powders, all fines were returned to the second external fluid bed. A process flow diagram for the production of powders is provided in Fig. 5.1. Four MPC powders were produced in total: regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN).

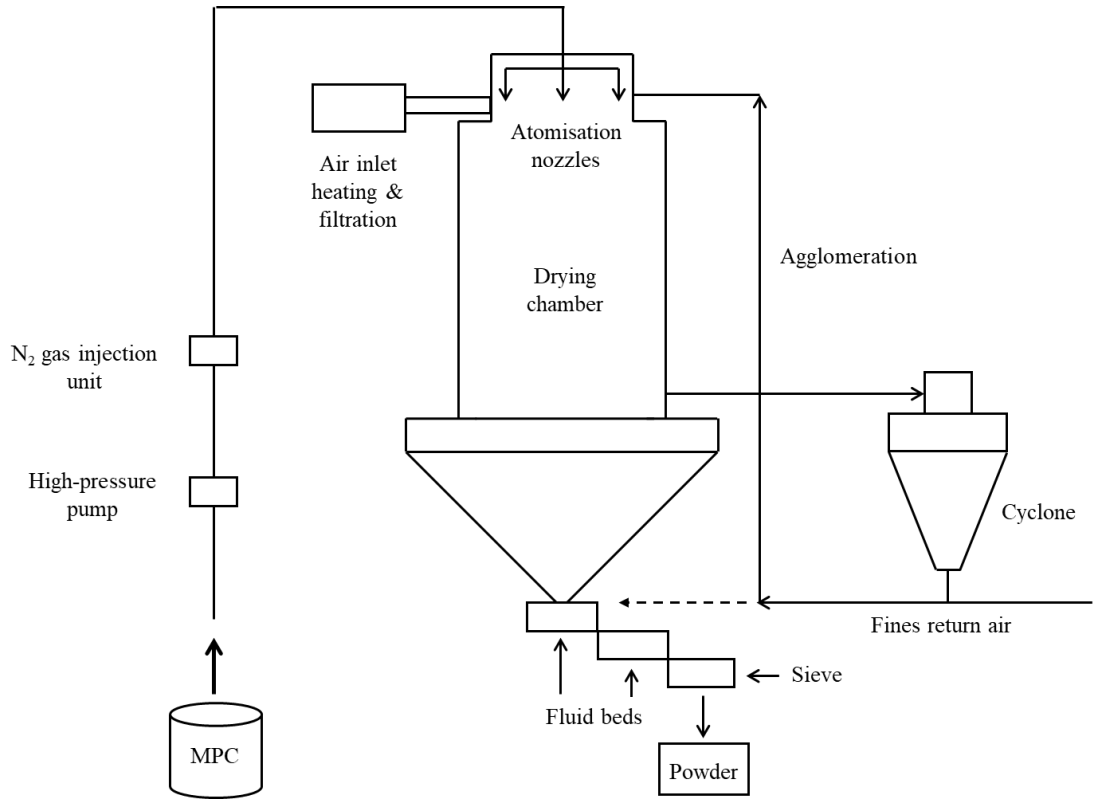

Fig. 5.1. Process flow diagram for the production of milk protein concentrate (MPC) powders.

5.2.3. Compositional analysis

The free moisture and ash content of the MPC powders were determined using a TGA701 thermogravimetric analyser (LECO Corporation, St Joseph, Michigan, USA) at 102 and 550 °C, respectively. The protein nitrogen values were obtained using a LECO FP628 nitrogen analyser (LECO Corporation, St Joseph, Michigan, USA); the protein content was determined using a nitrogen-to-protein conversion factor of 6.38. The fat content was determined using the Rose Gottlieb method (ISO, 2008). The lactose content was calculated by difference. All analyses were carried out in triplicate, except for fat, which was conducted in duplicate. The mean protein, lactose, fat, and ash content of the MPC powder was 80.5, 5.10, 1.54, and 7.49% (w/w), respectively.

5.2.4. Scanning electron microscopy

Samples of each MPC powder were attached to double-sided adhesive carbon tabs mounted on scanning electron microscope (SEM) stubs, and then coated with chromium (K550X, Emitech, Ashford, UK). Images were collected using a Zeiss Supra 40P field emission SEM (Carl Zeiss SMT Ltd., Cambridge, UK) at 2.00 kV. Representative micrographs were taken at 1000× magnification.

5.2.5. Surface composition

Surface composition analysis of the powders was determined using a Kratos Axis Ultra X-ray photoelectron spectrophotometer (XPS; Kratos Analytical, Manchester, UK), equipped with a monochromatic Al Ka X-ray source (1486.58 eV) at 150 W (15 kV, 10 mA). Using elemental composition, i.e., carbon (C), oxygen (O) and nitrogen (N), data derived from experimental analysis of milk protein isolate (C = 68.4, O = 17.6 and N = 12.85%), lactose (C = 55.75, O = 44.25 and N = 0%), and anhydrous milk fat (C = 90.3, O = 9.7 and N = 0%) reference samples, a matrix formula was used to determine relative amounts of protein, lactose and fat on the MPC powder particle surface, as described by Faldt et al. (1993).

5.2.6. Colour

The colour of each MPC powder was measured using a Chroma Meter CR-400 (Konica Minolta Sensing Europe B.V., Nieuwegein, the Netherlands). The colour measurement was determined according to the three colour coordinates: L*, a*, and b*. The value L* represents the sample luminosity or brightness, varying from black (0) to white (100); a* represents the colour varying from green (-) to red (+); b* represents the colour varying from blue (-) to yellow (+). Each reported colour value

was the mean of three different measurements. Total colour difference (ΔE) was calculated for RN, A and AN-MPC powders using equation (1), as reported by Kelleher et al. (2020):

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (1)$$

5.2.7. Particle density, bulk density, porosity, occluded and interstitial air

Particle density of the MPC powders was measured using an AccuPyc II 1340 gas pycnometer (Micromeritics Instrument Corporation, Norcross, Georgia, USA) according to the air pycnometry method of GEA Niro (2006a). The volume of interstitial and occluded air was calculated as described in the GEA Niro method (2006a). The loose and tapped (100 taps) bulk density of the MPC powders was measured as per the GEA Niro method (2006b), using a jolting volumeter STAV II (Funke Gerber, Berlin, Germany). The porosity (ε) of each MPC was calculated using equation (2), as described by Li et al. (2016):

$$\varepsilon = 1 - (\text{tapped density}/\text{particle density}) \quad (2)$$

5.2.8. Powder particle size and friability

The particle size and friability of the MPC powders were determined using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with an Aero S dry powder dispersion unit. The refractive index and absorption index were set at 1.45 and 0.1, respectively. The air pressure used was 2 bar and the feed rate was adjusted (from 25-100%) to compensate for innate differences in flowability of the powder samples. Size measurements were

recorded as the median particle diameter (D_{50}) and cumulative diameters (D_{10}) and (D_{90}), whereby 10, 50 and 90% of the sample volume is represented by particles smaller than the size indicated. The volume-weighted mean particle diameter ($D_{[4,3]}$) was also calculated.

Friability, the ability of powder particles to fragment during processing, was measured according to the method of Schuck et al. (2012b), using a Malvern Mastersizer equipped with an Aero S dry powder dispersion unit. The compressed air pressure was set at either 0.5 or 4 bar, the feed rate was adjusted (from 20-100%) to compensate for innate differences in flowability of the powder samples, and the D_{50} was subsequently recorded. Each powder was analysed in triplicate and friability was calculated using equation (3) as follows:

$$F = \left(\frac{[d(0.5@50\ kPa)-d(0.5@400\ kPa)]}{d(0.5@50\ kPa)}\right) \times 100 \qquad (3)$$

5.2.9. Specific surface area

Specific surface area (SSA) values for MPC powders were determined using a Gemini VI Surface Area Analyzer (Micromeritics, Norcross, GA, USA). Powder particles (0.1-0.5 g) were first loaded into a glass tube and degassed at 25 °C, overnight, before analysis using a FlowPrep™ 060 degassing unit (Micromeritics, Norcross, GA, USA); nitrogen was used as the adsorbate and the operating pressure set at 1 bar. The SSA was calculated from a nine-point sorption isotherm (liquid nitrogen at -196 ºC was used to maintain isothermal conditions) using the Brunauer-Emmett-Teller equation (Brunauer et al., 1938). The technique determined SSA of powder particles by correlating it to the flow of nitrogen through the column of packed particles (Buma, 1971a). All measurements were carried out in triplicate. SSA can be

inferred from particle size distribution data but are representative only of the SSA of equivalent spheres, while analysis of SSA by nitrogen adsorption does not include an assumption of sphericity (Crowley et al., 2014).

5.2.10. Bulk powder properties

The powder bulk handling and flowability properties (i.e., flow index, the effective angle of internal friction, bulk density and compressibility) were measured using a Brookfield Powder Flow Tester (PFT; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). Powder samples were prepared for analysis as described by Crowley et al. (2014). Briefly, a standard flow function (FF) test was carried out to determine the flowability of MPC powders by applying five normal stresses (1.0-4.8 kPa) and three over-consolidation stresses at each normal stress. Values for the effective angle of internal friction were obtained from FF analysis, and the value at 4.8 kPa was reported. Loose bulk density (p_b) and tapped bulk density (p_t) were recorded at the minimum and maximum major principal consolidating stress, respectively. A standard wall friction test was performed whereby ten normal stresses (0.5-4.8 kPa) were applied to determine wall friction angle ($ø_w$) values. The $ø_w$ was reported at a normal stress of 4.8 kPa. Compressibility index (CI) was calculated using equation (4) as described by Schuck et al. (2012a):

$$CI = \frac{p_t - p_b}{p_t} X\ 100 \quad (4)$$

Powder flowability was also measured by determining the time required for a defined volume of powder to leave a rotating drum (GEA Niro, 2019). The flowability of powder was expressed using equation (5) as follows:

$$F_d = (g_{p1} - g_{p2})/\text{time} \quad (5)$$

where F_d is the drum flowability (g/s) and g_{p1} and g_{p2} correspond to the amount (g) of powder in the container at the beginning and end of the test, respectively (Murphy et al., 2020).

5.2.11. Statistical analysis

Measurements of powder characteristics were performed in triplicate, with results presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was carried out using IBM SPSS (version 24; Armonk, New York, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

5.3. Results and discussion

5.3.1. Microstructure

Scanning electron microscopy images showed significant differences between the morphology of regular and agglomerated milk protein concentrate (MPC) powders (Fig. 5.2). Regular (R) MPC powder displayed shrivelled or collapsed particles (Fig. 5.2A), which resembled the wrinkled native phosphocaseinate (NPC) particles reported by Sadek et al. (2014), while agglomeration resulted in the formation of clearly defined powder clusters composed of several closely linked particles (Fig. 5.2C). The injection of nitrogen gas (N_2) resulted in significantly different morphology for regular and agglomerated powders, as evidenced by the spherical, fractured and porous appearance of the particles (Fig. 5.2B and D). The higher porosity was likely a result of the foam structure formed in the liquid MPC following the injection of N_2, and the subsequent rapid removal of N_2 from the atomised droplets in the drying

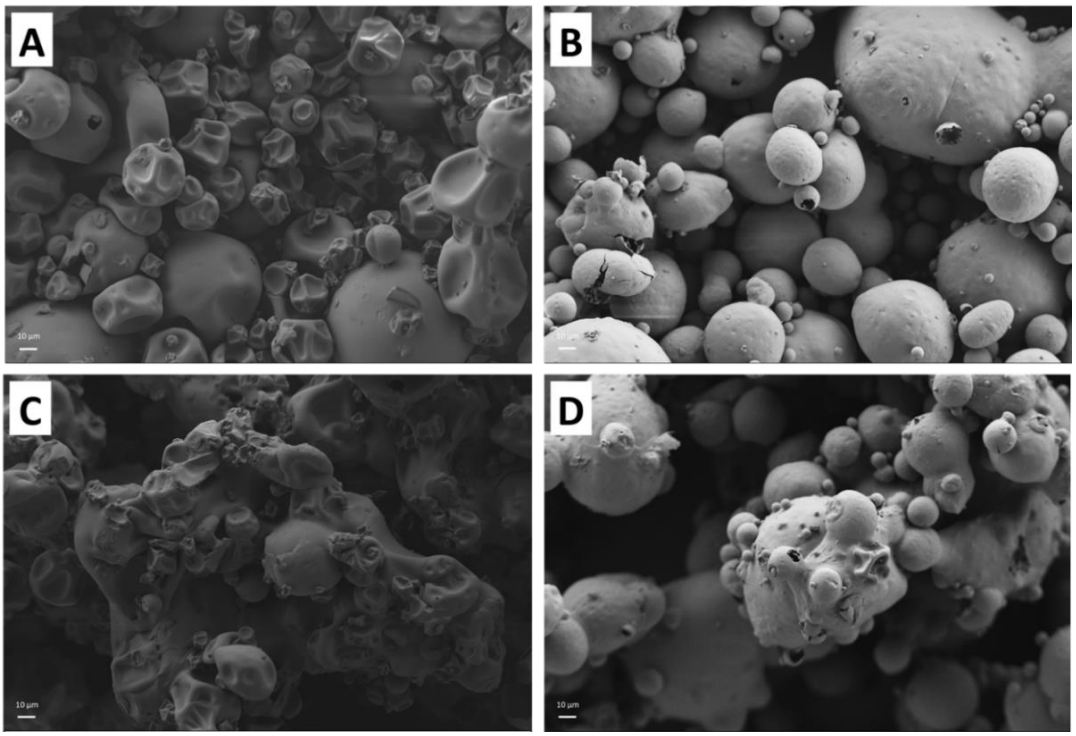

Fig. 5.2. Scanning electron micrographs showing the microstructure of (A) regular (R), (B) regular with N_2 injection (RN), (C) agglomerated (A) and (D) agglomerated with N_2 injection (AN) milk protein concentrates powders at a magnification of 1000x. Scale bar represents 10 µm.

chamber, as suggested by Bouvier et al. (2013) for an MPC into which carbon dioxide gas was incorporated. Furthermore, the change in particle shape following N_2 injection (NI), whereby the particles were puffed and inflated, could be explained by increases in occluded air content. Bouvier et al. (2013) showed that extrusion-porosification produced particles with a more spherical appearance compared to a conventionally spray-dried MPC powder. Spray drying temperature can also play a role in powder morphology as Fang et al. (2012) reported that smooth MPC powder particles were produced at low drying temperature (i.e., 77 °C) whereas higher drying temperatures (i.e., 178 °C) generated wrinkled powder particles, likely due to differences in the rate of water removal and particle shrinkage.

5.3.2. Surface composition

In this study, x-ray photoelectron spectroscopy (XPS) was used to investigate whether NI altered powder particle surface composition as it has been reported that higher levels of surface fat result in greater inter-particle cohesiveness and impaired powder flowability (Fitzpatrick et al., 2007; Kim et al., 2005; Silva and O'Mahony, 2017). Surface composition analysis showed that all MPC powders had a greater coverage of fat at the particle surface compared to the bulk fat content of the powders (Table 5.1). It is worth noting that as the melting point of milk fat is ~36 °C (O'Callaghan et al., 2016), significantly lower than the temperature of the concentrate to the dryer (i.e., 70 °C), it was in liquid form throughout the spray drying process (Liu et al., 2020). R-MPC powder particles had the highest proportion of surface protein (96.9%) and the lowest amount of surface fat (2.0%). However, for regular with N_2 injection (RN) MPC, surface protein and fat coverage were 87.9% and 11.1%, respectively. Therefore, the NI process had a significant impact on the migration of fat to the surface of the powder particles. Lactose was present in significantly higher proportions on the surface of agglomerated MPC powders, compared to regular powders; 0.9% for RN-MPC and 4.7% for agglomerated with N_2 injection (AN) MPC. Previous studies have shown that for dairy powders such as MPC, the level of fat at the surface of particles is over-represented when compared to the level of fat in the bulk powders. For example, Kelly et al. (2015) reported that fat was present in a higher quantity at the surface of an MPC powder (8.2%) compared to the bulk (1.2%), while for a NPC powder, Gaiani et al. (2006) reported surface and bulk fat values of 5.3 and 0.4%, respectively. Kim et al. (2009) demonstrated that spray drying temperature plays an important role in surface fat content of skim milk powder, with a decrease in the air inlet temperature causing an increase in the migration of fat to the surface of the powder particle, possibly due to slower particle skin formation. Furthermore,

Table 5.1. Surface composition (%) of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders, as determined by X-ray photoelectron spectroscopy (XPS) analysis.

MPC	Protein	Lactose	Fat
R	$96.9^{a} \pm 0.55$	$0.91^{a} \pm 0.70$	$2.02^{a} \pm 0.02$
RN	$87.9^{bc} \pm 1.10$	$0.90^{a} \pm 0.32$	$11.1^{b} \pm 1.27$
A	$89.5^{b} \pm 2.20$	$2.61^{b} \pm 0.29$	$7.68^{b} \pm 1.96$
AN	$83.3^{c} \pm 1.10$	$4.72^{c} \pm 0.03$	$11.4^{b} \pm 1.13$

[a-c] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

Gaiani et al. (2009) attributed the migration of lipids to the surface of NPC powder during storage, measured using XPS, to the development of pores throughout the powder matrix. In the current study, the increased porosity of the powder particles that were produced using the NI process may have facilitated the movement of fat to the powder surface. Another contributing factor may be that as N_2 bubbles within the liquid droplets escaped, creating pores and voids in the powder particles, they promoted the transfer of hydrophobic lipids towards the surface. Although surface free fat differs from surface fat measurements by the use of organic solvents to extract fat from the powder, it should be mentioned that Buma (1971b) reported a strong correlation between surface free fat and porosity of whole milk powder. Additionally, Hansen (1980) suggested that fat-filled milk powders had higher surface free fat contents when the powders were more aerated, with numerous capillaries and vacuoles, as the fat was less protected.

5.3.3. Moisture, colour, density, porosity, occluded and interstitial air

The mean moisture contents of MPC powders were 5.37, 5.59, 4.96 and 5.16%,

for R-, RN-, A- and AN-MPC, respectively. The significantly lower moisture content of A-MPC compared to R-MPC powder, could be due to the recirculation of fines through the dryer main chamber, resulting in the removal of additional moisture. Similarly, Gaiani et al. (2007) reported moisture contents of 5.4 and 4.5% for non-agglomerated NPC and whey protein isolate (WPI) compared to 4.8 and 3.9% for agglomerated NPC and WPI, respectively. The injection of N_2 did not significantly increase the moisture content of MPC powders.

Significant ($P < 0.05$) differences were observed in the colour of the MPC powders (Table 5.2). The L* values were higher for RN- and AN-MPC (95.5 and 93.6, respectively) compared to R- and A-MPC powders (93.3 and 91.5, respectively), indicating higher overall whiteness of the NI powders. The b* values were higher for both R- and A-MPC powders (i.e., 11.0 and 12.0, respectively) compared to RN- and AN-MPC (5.48 and 6.47, respectively; Table 5.2), indicating a significant reduction in the yellowness of NI powders. Overall, the ΔE was highest for RN-MPC (6.08), followed by AN- and A-MPC (4.64 and 2.11, respectively), suggesting that N_2 injection influenced powder colour more than agglomeration. The differences in colour may be explained by the density data presented in Table 5.3. The process of agglomeration produced powders with lower loose bulk density values, e.g., 0.29 and

Table 5.2. Colour space values (L*, a*, b*) and total colour difference (ΔE) for regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

MPC	L*	a*	b*	ΔE
R	$93.3^{a} \pm 0.52$	$-2.28^{a} \pm 0.01$	$11.0^{a} \pm 0.02$	-
RN	$95.5^{b} \pm 0.06$	$-1.07^{b} \pm 0.01$	$5.48^{b} \pm 0.02$	6.08 ± 0.19
A	$91.5^{c} \pm 0.01$	$-2.68^{c} \pm 0.02$	$12.0^{c} \pm 0.02$	2.11 ± 0.46
AN	$93.6^{a} \pm 0.01$	$-1.42^{d} \pm 0.02$	$6.47^{d} \pm 0.01$	4.64 ± 0.04

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

0.18 g/cm^3 for R- and A-MPC powders, respectively (Table 5.3). Similarly, Chever et al. (2017) reported lower loose (0.37 g/cm^3) and tapped (0.50 g/cm^3) bulk density values for agglomerated whole milk powders compared to non-agglomerated powders (0.41 and 0.72 g/cm^3, respectively). The loose bulk density was significantly lower for RN-MPC (0.09 g/cm^3) than for R-MPC (0.29 g/cm^3) due to the injection of N_2 into the concentrate. The tapped bulk density was significantly lower for RN- and AN-MPC (0.11 and 0.08 g/cm^3, respectively) compared to the R- and A-MPC powders (0.34 and 0.21 g/cm^3, respectively). The particle density of MPC powders produced using NI was also significantly lower. For example, the particle density was 1.09 g/cm^3 for R-MPC but 0.96 g/cm^3 for RN-MPC, while it was 0.99 and 0.87 g/cm^3 for A- and AN-MPC, respectively. With the decrease in powder density, there was a corresponding increase in the interstitial (between particles) and occluded (within particles) air content values (Table 5.3). The interstitial air was higher for RN-MPC (771 mL/100 g) and AN-MPC (1078 mL/100 g) compared to 202 and 372 mL/100 g for R- and A-MPC, respectively.

Finally, the porosity was higher for RN- and AN-MPC (0.88 and 0.90, respectively) compared to R- and A-MPC powders (0.68 and 0.79, respectively). The higher porosity occurred as a result of the lower tapped and particle densities for NI powders. Previously, Bouvier et al. (2013) showed that non-agglomerated MPC powders produced using extrusion-porosification had loose bulk density, tapped bulk density and occluded air values of 0.22 g/cm^3, 0.43 g/cm^3 and 146 mL/100g, respectively, compared to 0.36 g/cm^3, 0.52 g/cm^3, and 107 mL/100g, respectively, for a conventionally spray-dried MPC powder. A challenge of producing dairy powders with a low loose bulk or particle density is their suitability for export due to volume constraints during handling and packaging (Barbosa-Canovas et al., 2005).

Table 5.3. Physical properties of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

MPC	p_p	p_b	p_t	V_{ia}	V_{oa}	ε
	g/cm^3			mL/100 g		
R	$1.09^{a} \pm 0.02$	$0.29^{a} \pm 0.01$	$0.34^{a} \pm 0.01$	$202^{a} \pm 8.00$	$24.2^{a} \pm 1.27$	$0.69^{a} \pm 0.01$
RN	$0.96^{b} \pm 0.00$	$0.09^{b} \pm 0.00$	$0.11^{b} \pm 0.00$	$771^{b} \pm 16.3$	$36.3^{b} \pm 0.33$	$0.88^{b} \pm 0.00$
A	$0.99^{b} \pm 0.01$	$0.18^{c} \pm 0.00$	$0.21^{c} \pm 0.00$	$372^{c} \pm 9.38$	$33.7^{b} \pm 1.47$	$0.79^{c} \pm 0.00$
AN	$0.87^{c} \pm 0.01$	$0.07^{d} \pm 0.00$	$0.08^{d} \pm 0.01$	$1078^{d} \pm 70.8$	$47.5^{c} \pm 1.91$	$0.90^{d} \pm 0.01$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). p_p = particle density; p_b = loose bulk density; p_t = tapped bulk density; V_{ia} = volume of interstitial air; V_{oa} = volume of occluded air; ε = porosity.

5.3.4. Powder particle size and friability

The effect of NI and agglomeration on powder particle size is displayed in Fig. 5.3, whereby the D_{90} values were 134 and 148 μm for R- and RN-MPC compared to

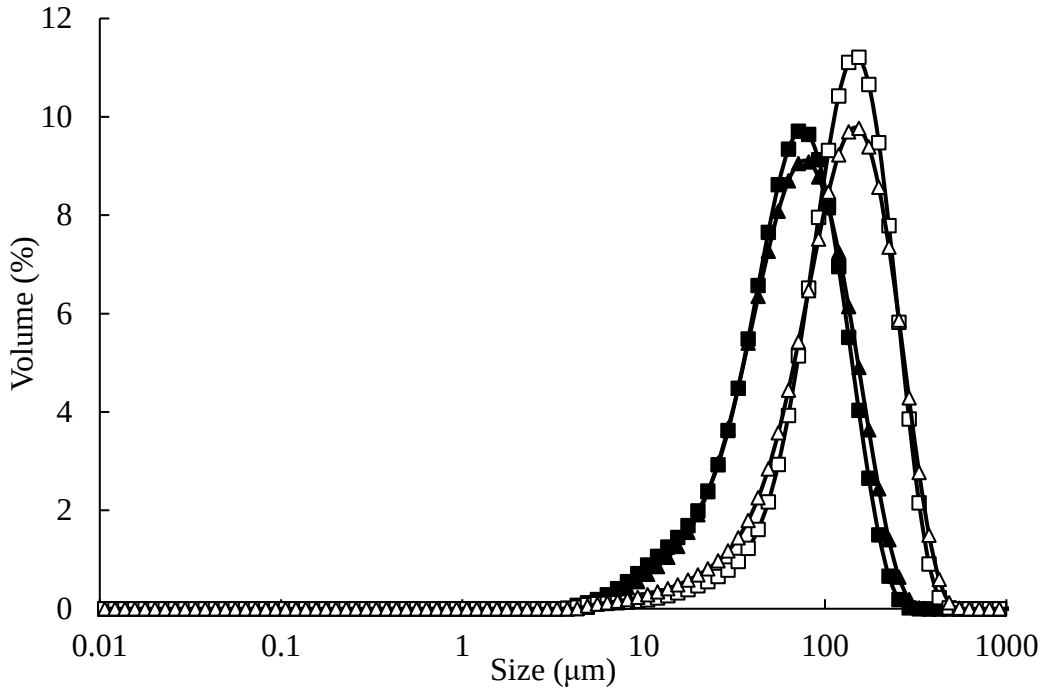

Fig. 5.3. Particle size distribution of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders.

244 and 256 μm for A- and AN-MPC powders, respectively (Table 5.4). The incorporation of N_2 into the concentrate significantly ($P < 0.05$) increased the size of the regular MPC powder particles across all size measurements (Table 5.4). This slight increase in particle size for NI powders may be due to the expansion of gas bubbles within the liquid droplets directly after exiting the spray nozzles. As expected, agglomerated samples had a significantly higher particle size than regular MPC powders due to the return of fines through the drying chamber. The R-MPC powder had a higher friability value (20.6%) compared to RN-MPC (19.1%; Table 5.4).

However, the injection of N_2 did not influence the breakdown of the agglomerated samples as they both had a friability of approximately 33% (Table 5.4). Therefore, the friability results suggest that regular MPC powders would retain their shape and structure to a greater extent and be less likely to break during handling and processing, as they possessed the higher particle strength, in comparison to the agglomerated powders. Attrition of agglomerated products can negatively impact powder functionality, with Hazlett et al. (2020) reporting that pneumatic conveying of an agglomerated whey protein concentrate powder (80% protein, w/w) caused agglomerate breakdown, resulting in lower powder bulk density, flowability, wettability and dispersibility. The significant difference in friability between regular MPC powders is most likely due to the greater cohesiveness or poorer flow of the RN-MPC powder, thus making it less friable than R-MPC. In addition, the significantly lower particle density and higher occluded air content of RN-MPC may also play a role in its lower friability.

5.3.5. Flowability, specific surface area, wall friction angle and compressibility

The injection of N_2, as well as agglomeration by fines return, altered the flow properties of the MPC powders (Fig. 5.4). The flow index values were 5.14, 2.71, 7.73 and 3.68 for R-, RN-, A- and AN-MPC, respectively (Table 5.5). According to the Jenike classification, powders with a flow index value between 4 and 10 are easy flowing, while cohesive powders present flow index values of less than 4 (Jenike, 1964). Therefore, both powders which underwent NI were categorised as cohesive over the range of consolidating stresses applied and as mentioned in Section 5.3.2., surface fat can play a detrimental role in powder flowability. Conversely, the regular and agglomerated MPC powders were easy flowing. Assessment of powder

Table 5.4. Particle size distribution parameters, friability (F) and specific surface area (SSA) of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

MPC	D_{10}	D_{50}	D_{90}	$D_{[4,3]}$	F	SSA
	μm				%	m^2/g
R	$22.8^{d} \pm 0.0$	$65.3^{d} \pm 0.1$	$134^{a} \pm 0.0$	$73.0^{a} \pm 0.2$	$20.6^{a} \pm 0.1$	$0.65^{b} \pm 0.03$
RN	$24.8^{c} \pm 0.2$	$68.5^{c} \pm 0.3$	$148^{b} \pm 1.5$	$78.9^{b} \pm 0.6$	$19.1^{b} \pm 0.3$	$2.82^{a} \pm 0.05$
A	$55.5^{a} \pm 0.2$	$132^{a} \pm 0.0$	$244^{c} \pm 1.5$	$142^{c} \pm 1.0$	$32.6^{c} \pm 0.3$	$0.50^{b} \pm 0.02$
AN	$45.5^{b} \pm 0.1$	$126^{b} \pm 0.0$	$256^{d} \pm 3.0$	$140^{c} \pm 1.5$	$33.1^{c} \pm 0.4$	$2.62^{a} \pm 0.16$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). D_{10} = particle size below which 10% of sample volume exists; D_{50} = particle size below which 50% of sample volume exists; D_{90} = particle size below which 90% of sample volume exists; $D_{[4,3]}$ = volume-weighted mean particle diameter.

flowability by the drum method supported the results obtained by the Brookfield powder flow tester; agglomeration improved flowability while the injection of N_2 into the concentrate produced a powder with poorer flowability, e.g., 2.24 g/min for R-MPC compared to 0.31 g/min for RN-MPC (Table 5.5). The size of powder particles

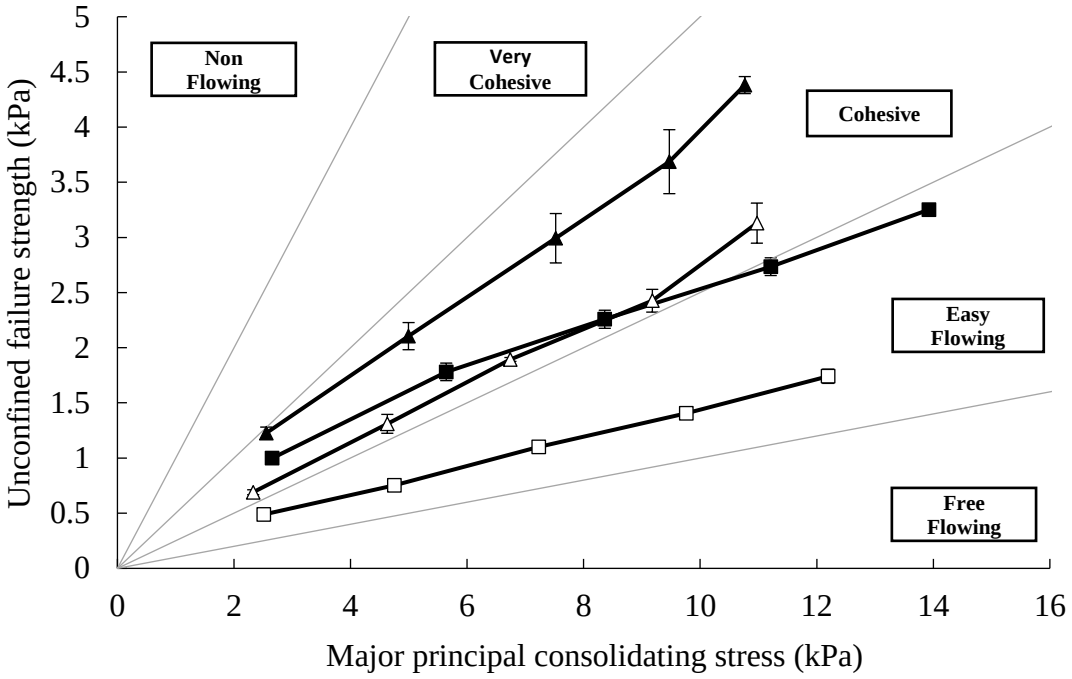

Fig. 5.4. Flow function profiles showing unconfined failure strength as a function of major principal consolidating stress (kPa) for regular (■), regular with N_2 injection (▲) agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders.

affects the bulk properties (e.g., flowability) and it has been suggested by Rennie et al. (1999) that powder cohesiveness decreases as particle size increases. However, AN-MPC was classified as cohesive, despite having a D_{90} of 256 µm compared to A-MPC which was easy-flowing with a D_{90} of 244 µm. This suggests that the NI process counteracts the improved flowability that agglomeration typically provides. The differences in flowability between NI and non-NI powders could be due to a difference in powder particle shape, as shown in Fig. 5.2, but was most likely caused by the

significant difference in specific surface area (SSA). The SSA was almost four times higher for the NI samples in comparison to non-NI MPC powders (Table 5.4), which may be due to the higher porosity of the NI MPC powders. A-MPC had a lower SSA than R-MPC, likely due to its larger powder particle size. The greater SSA of RN- and AN-MPC would facilitate a greater number of attractive surface interactions between powder particles and restrict movement. It was previously found by Fu et al. (2012) that lactose powder with the highest sphericity had better flowability. However, in this study, RN-MPC had the most spherical powder particles (Fig. 5.2B) but had the lowest flow index value (Table 5.5). These differences in particle structure and shape appear to have a large impact on its physical characteristics and will likely influence the rehydration properties also.

The NI process did not alter the effective angle of internal friction, however; it was significantly different for the agglomerated powders (~42°) in comparison to the regular MPC powders (~46°; Table 5.5). This suggests that less resistance to flow occurs between the agglomerated powder particles in comparison to regular MPC powders. In this study, the wall friction angle was increased by the NI process as it was highest for the RN-MPC and lowest for the A-MPC powder (Table 5.5 and Fig. 5.5). An increase in wall friction can cause greater stress on the perimeter or wall of silos and significantly hinder the removal and emptying of powders (Fitzpatrick et al., 2004). The wall friction angle obtained for A-MPC (13.6°) is similar to that reported by Teunou et al. (1999) for an agglomerated skim milk powder (13.0°). Furthermore, Crowley et al. (2014) reported a wall friction angle of 21.7° for a regular MPC80 powder, which differs from the value of 16.2° for R-MPC in the current study, possibly due to differences in powder particle size; D_{90} of 134 μm in the current study (Table 5.4) compared to 58 μm reported by Crowley et al. (2014). Of all powders analysed,

Table 5.5. Bulk-handling and flowability properties of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with nitrogen injection (AN) milk protein concentrate (MPC) powders.

MPC	i	JC	F_d (g/min)	δ_e (°)	$ø_w$ (°)	CI (%)
R	$5.14^{a} \pm 0.16$	Easy flowing	$2.24^{a} \pm 0.14$	$46.4^{a} \pm 0.6$	$16.2^{a} \pm 0.6$	$41.5^{a} \pm 2.4$
RN	$2.71^{b} \pm 0.18$	Cohesive	$0.31^{b} \pm 0.02$	$46.8^{a} \pm 2.4$	$18.3^{b} \pm 0.6$	$50.4^{b} \pm 3.2$
A	$7.73^{c} \pm 0.36$	Easy flowing	$8.30^{c} \pm 0.11$	$42.0^{b} \pm 0.1$	$13.6^{c} \pm 1.3$	$24.9^{c} \pm 0.8$
AN	$3.68^{d} \pm 0.38$	Cohesive	$1.29^{d} \pm 0.04$	$42.7^{b} \pm 0.2$	$14.1^{c} \pm 0.4$	$48.2^{ab} \pm 3.4$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). i = flow index; JC = Jenike classification; F_d = drum flowability; δ_e = effective angle of internal friction; $ø_w$ = wall friction angle; CI = compressibility index.

RN-MPC had the highest wall friction angle and the highest effective angle of internal friction. The strong attractive forces between both the powder and a surface, and between the powder particles themselves, were probably attributed to the larger SSA of RN-MPC. Furthermore, NI-MPC powders were found to be more compressible; RN-MPC had a compressibility index of 50.4% compared to 41.5% for R-MPC (Table 5.5). This can be explained by the large volume of interstitial air present between these

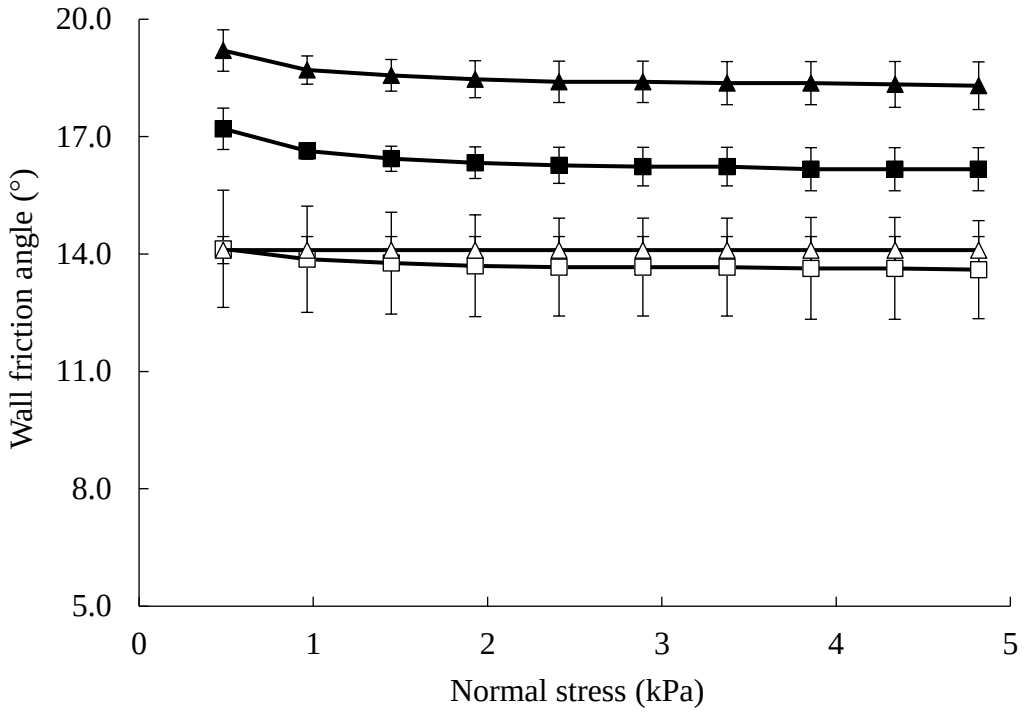

Fig. 5.5. Wall friction angle as a function of normal stress (kPa) for regular (■), regular with N_2 injection (▲) agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders.

powder particles. The voids would be largely removed during compaction, with a corresponding decrease in the distance between powder particles. It was evident that the more compressible a powder was, the lower its flow index. A relationship between poor flowability and high compressibility was also previously reported by Crowley et al. (2014) for high-protein MPC powders. Compression of these MPC powders during

handling and storage is known to have important implications for the functionality of such powders for end-users, e.g., changes in powder density and loss of dispersibility due to removal of air voids.

5.4. Conclusion

The injection of nitrogen gas (N_2) into the concentrate prior to spray drying can significantly alter the physical and bulk handling characteristics of milk protein concentrate (MPC) powders. In particular, the bulk density and flowability of MPC powders were significantly changed by this process. Flow index values were lower and wall friction angles were higher with the use of N_2 injection (NI). This was attributed to the alterations in surface composition and powder particle structure, as well as the higher specific surface area and porosity. The NI process significantly increased the compressibility of MPC powders which may cause changes in powder properties during handling and storage and subsequently alter their reconstitution properties. Agglomeration by fines return during spray drying generated powders with improved flowability but increased friability, which suggests their functional properties could be impaired during bulk powder handling and conveying. This study has provided essential information regarding the influence of NI and agglomeration on powder functionality and will support both the processing operations (i.e., storage, handling) and technological development of milk protein ingredients.

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Chapter 6

Rehydration properties of regular and agglomerated milk protein concentrate powders produced using nitrogen gas injection prior to spray drying

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Abstract

This study evaluated the effect of high-pressure nitrogen gas (N_2) injection prior to spray drying on the subsequent rehydration properties of regular and agglomerated milk protein concentrate (MPC) powders. Conductivity measurements demonstrated a slower release of ions for powders produced using N_2 injection (NI) as they took longer to wet and sink due to their lower density. However, analysis of particle size distribution on reconstitution at both 23 and 50 °C showed an improvement in powder dispersion with NI. Powder solubility, when measured at 23 °C, was higher for the NI powders, while agglomeration negatively impacted solubility. Confocal laser scanning microscopy analysis showed a faster diffusion of dye into MPC powder particles produced using NI. The improvement in powder dissolution with NI was attributed to higher porosity and the presence of air voids which facilitated increased water transfer and accelerated the breakdown of primary powder particles.

6.1. Introduction

The rehydration of high-protein, casein-dominant dairy powders, including milk protein concentrate (MPC), is currently a significant challenge encountered by the food and beverage industry. These value-added ingredients provide unique nutritional (e.g., high protein and calcium, low lactose content) and functional (e.g., heat stability, gelation) properties. To exploit the functionality of dry dairy ingredients, rapid and complete powder rehydration is generally required. However, this is impaired by reduced water transfer due to non-covalent protein-protein interactions (Havea, 2006) and high micellar casein content (Schuck et al., 1998, 2002), with dispersion of primary powder particles regarded as the rate-limiting stage of rehydration due to the presence of a network of casein micelles at the powder particle surface (Mimouni et al., 2009).

A water temperature of approximately 50 °C, in combination with high-shear treatment and extended mixing times, are normally required to accelerate the rehydration of casein-dominant powders (Gaiani et al., 2006b; McCarthy et al., 2014), but this is not desirable for ingredient manufacturers and end-users. Ideally, rehydration should take place within a short time period at ambient temperature (~20 °C) and low shear to minimise manufacturing time and production costs (Saggin and Coupland, 2002). Previous research has proposed several processing and formulation strategies to promote the rehydration of casein-dominant powders, including cold microfiltration during micellar casein concentrate manufacture (Crowley et al., 2018), high-pressure treatment (Udabage et al., 2012), acidification of skim milk before membrane filtration (Liu et al., 2019), and the incorporation of monovalent salts (e.g., sodium chloride) into the concentrate before spray drying (Schuck et al., 2002; Sikand et al., 2013).

Gases have been used in dairy processing to alter the functional properties of a range of products. For example, carbon dioxide (CO_2) has been used to improve the shelf life and quality of milk, cheese and fermented beverages (Hotchkiss et al., 2006). However, only a limited number of studies have reported their impact on the rehydration properties of dairy powders. Marella et al. (2015) investigated the effect of CO_2 injection into skim milk before and during membrane filtration and reported the subsequent characteristics of the MPC powder. An improvement in cold water (10 °C) solubility was observed, which was attributed to the solubilisation of colloidal calcium phosphate during membrane fractionation due to the decrease in pH and a reduction in micellar casein interactions. However, the incorporation of CO_2 into dairy streams during processing may change product composition, presenting challenges for some existing applications. Bouvier et al. (2013) used CO_2 during extrusion-porosification to manufacture MPC powders with enhanced dispersibility compared to MPC produced using conventional spray drying. The achievement of a sub-micron particle size distribution after only 2 h of rehydration was attributed to the partial dissociation of casein micelles as well as increased porosity of powder particles. Kosasih et al. (2016a, 2016b) investigated the addition of dry ice (i.e., solid CO_2) to whole milk concentrate prior to spray drying and showed an improvement in the dispersibility of powder particles. Nitrogen gas (N_2) has also been used in dairy processing to modify ingredient functionality (Adhikari et al., 2018). One apparent benefit of using N_2 is that, unlike CO_2 and compressed air, it is inert, so is unlikely to alter the pH of the dairy concentrate or promote oxidation in the final product. Hanrahan et al. (1962) reported an improvement in whole milk powder dispersion when N_2 was incorporated into the concentrate before spray drying. Similarly, Bell et al. (1963) enhanced the dispersibility of skim milk powder by injecting compressed

air into the concentrate between the high-pressure pump and atomisation nozzle.

Aside from the incorporation of gas into dairy streams, powder particle structure and physical properties can be modified via a process known as agglomeration. It can be performed by returning fine powder particles from the cyclone to the top of the drying chamber during droplet dehydration or by combining the spray-dried powder with water or a binder in the fluidised bed (Gianfrancesco et al., 2008). The process of intentionally mixing the atomised spray with small, dry powder particles is known as forced secondary agglomeration (Pisecky, 2012). The effects of fluid bed agglomeration on the physicochemical properties of milk protein isolate powders have been reported by Ji et al. (2015, 2016, 2017), whereby improvements in powder wettability were achieved, with no improvement in solubility. Gaiani et al. (2007) reported that agglomeration using fines return was effective in accelerating rehydration of whey protein powder, while it resulted in impaired rehydration performance for casein-dominant powder. Furthermore, the rehydration characteristics of MPC powders produced using both agglomeration and N_2 injection have not been established. A previous study (McSweeney et al., 2021) by the current authors investigated the influence of N_2 injection directly prior to spray drying, agglomeration by fines return, and a combination of these approaches, on the physical and bulk handling properties of MPC powders. The MPC powder produced using N_2 injection had lower density and flow index values, with higher air content, specific surface area, porosity and surface fat, compared to the powders produced without N_2 injection, while agglomeration also decreased powder density but improved flowability. Given the significant changes to the structure of the powder particles, the current study was designed to investigate the rehydration properties of these MPC ingredients. Several techniques were employed to elucidate the impact of

these processing modifications on the performance of the powders throughout the main stages of rehydration (i.e., wetting, dispersion and dissolution).

6.2. Materials and methods

6.2.1. Powder manufacture

The manufacture, composition and basic physical properties (e.g., density, morphology, porosity, powder particle size) of the regular (R), regular with nitrogen gas injection (RN), agglomerated (A) and agglomerated with nitrogen gas injection (AN) milk protein concentrate (MPC) powders used in this study were described by McSweeney et al. (2021). Briefly, liquid concentrate (21.2% total solids, w/w) was first prepared from MPC powder using high-shear treatment and hydrodynamic cavitation. Prior to spray drying, the concentrate was pre-heated to 70 °C and pumped to the atomisation nozzle using a high-pressure pump (HPP). Regular (R) MPC powder was produced using a conventional spray drying process. Agglomerated (A) powders were manufactured by returning all fines collected in the cyclone to the atomisation zone of the spray dryer main chamber. For MPC powders produced with nitrogen (N_2) gas injection (i.e., RN- and AN-MPC), N_2 was injected (3.5 kg/h) at a pressure of ~190 bar into the feed line, after the HPP and prior to atomisation, using a pressurised injection unit (Carlisle Process Systems, Farum, Denmark).

6.2.2. Immersional and capillary rise wetting behaviour

Immersional wetting was measured using the GEA Niro method (GEA Niro, 2009) with one modification; 4 g of each powder sample was added to the beaker of water (250 mL; 25 °C). Capillary rise wetting was measured using a modified Washburn method with 2 g of each powder sample added to a cylindrical stainless-

steel tube (diameter = 2.4 cm) with an open base covered by filter paper and parafilm (Ji et al., 2015). The analysis was first carried out with no powder to determine the quantity of water absorbed by the filter paper and parafilm (i.e., control), and subsequently this value was subtracted from the test values. The weight of the tube was recorded before and after the addition of powder. The top of the tube was submerged in 25 °C ultrapure water and the wettability was quantified by measuring the additional mass of the wetted powder after 20 min, with results presented as the mean of three independent measurements.

6.2.3. Confocal laser scanning microscopy and liquid phase water diffusion

A Leica TCS SP5 confocal laser scanning microscope (CLSM; Leica Microsystems CMS GmbH, Wetzlar, Germany) was used for the real-time visualisation of dye penetration into powder particles, as described by Power et al. (2020). Liquid phase water diffusion in MPC powders was measured using the novel method presented by Maidannyk et al. (2019). Rhodamine B was added to anhydrous powders which allowed diffusion of the dye molecules into the particles without changing particle morphology and preventing solubilisation, thereby providing an indicator of powder hydration. The CLSM images were obtained at fixed time intervals and represent real-time water diffusion. Diameters of particles were detected using Leica TCS SP5 software in the size range 6-142 μm. The areas of individual powder particles were measured using spherical approximation and this information, combined with the time of dye penetration, enabled the local effective diffusivity of the liquid phase in individual powder particles to be calculated. Initially, powder particles appear as dark particles with a dark green background. However, during the water diffusion process, the fluorescent dye penetrates the particles and changes their

colour to bright green.

6.2.4. Water sorption isotherms

Water sorption analysis was carried out as described by Maidannyk et al. (2020), with one modification: powders were weighed at intervals of 0, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 h. The water content in each system was plotted as a function of time, and the Guggenheim-Anderson-de Boer (GAB) relationship was fitted to data to relate water activity and water content of anhydrous powders, as shown in equation (1):

$$\frac{m}{m_0} = \frac{Cka_w}{(1-ka_w)(1-ka_w+Cka_w)} \quad (1)$$

Where m is the water content (g of water/100 g of dry solids), m_0 – the monolayer value of water content, C, k – constants, which can be calculated from m_0.

6.2.5. Measurement of mineral release using conductivity

Conductivity of MPC dispersions (1.5% protein, w/w; 300 mL of ultrapure water in a 400 mL beaker) was measured using a Titrando autotitrator equipped with a five-ring conductivity measuring cell and accompanying Tiamo v2.3 software (Metrohm Ireland Ltd, Athy Road, Co. Carlow, Ireland). The probe was calibrated at 25 °C with a KCl solution of known conductivity (12.9 mS/cm) and a temperature coefficient of 2.07 was used (Crowley et al. 2015). Before the addition of powder to the beaker of water, 1 min was allowed to elapse to establish a baseline reading of conductivity and powder was then added over a period of 1 min. It is expected that cations and anions found in the serum phase (e.g., H^+ and Cl^-) would contribute most

to conductivity measurements rather than minerals found in the colloidal phase, e.g., calcium (Zhuang et al., 1997; Schuck et al., 2007).

6.2.6. Particle size distribution of milk protein concentrate dispersions

The particle size distribution of MPC dispersions was measured using a laser-light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens, as described by McSweeney et al. (2020). Additionally, powders were reconstituted using low-speed mixing for 1 h at 23 °C. Size measurements were recorded as the volume-weighted mean particle diameter ($D_{[4,3]}$), median diameter (D_{50}) and cumulative diameters (D_{90} and D_{10}), whereby 10, 50 and 90% of the sample volume is represented by particles smaller than the size indicated. Particle size measurements were recorded when the laser obscuration reached 3-4%.

6.2.7. Powder solubility

The solubility of MPC powders was measured as described by McSweeney et al. (2020). Powder solubility was given by the total solids content of the supernatant (obtained following centrifugation at 3000*g* for 10 min) expressed as a percentage of the total solids content of the initial dispersion.

6.2.8. Viscosity of dispersions

MPC powders were reconstituted (8%, w/w, total solids) using magnetic stirring (550 rpm) for (i) 1 h at 23 °C only or (ii) 45 min at 45 °C followed by overnight stirring (250 rpm) at 4 °C to facilitate powder solubilisation. The viscosity of MPC dispersions were measured using an AR-G2 controlled-stress rheometer (TA

Instruments, Crawley, UK), equipped with a parallel plate geometry. Samples were pre-sheared at a shear rate of 100 s^{-1} for 30 s and a shear rate ramp of 0.1 to 300 s^{-1} for 5 min was performed, with the temperature (23 °C) controlled using a Peltier system (± 0.1 °C).

6.2.9. Foaming capacity

The capacity of MPC powders to foam upon reconstitution was measured by dispersing the samples in ultrapure water (23 and 50 °C) using a solubility index meter (Labinco BV, Breda, the Netherlands) operating at approximately 3600 ± 100 rpm for 1 min. The dispersion (8%, w/w, total solids; 100 mL) was poured into a 500 mL graduated cylinder and the height of the foam formed was measured. The foam expansion index was calculated as described by Voronin et al. (2021).

6.2.10. Statistical analysis

Measurements of powder rehydration, viscosity and foaming were performed in triplicate and results presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was performed using the IBM SPSS (Version 24; Armonk, NY, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

6.3. Results and discussion

6.3.1. Wetting behaviour of milk protein concentrate powders

Wettability analysis by the GEA method showed that all milk protein concentrate (MPC) powders did not completely wet or submerge below the surface of the water within 10 min. However, the water became increasingly turbid for the regular

(R) and agglomerated (A) nitrogen (N_2) injection powders (i.e., RN- and AN-MPC) compared to R- and A-MPC, in which the water remained relatively clear (data not shown). Bouvier et al. (2013) reported that extrusion-porosification, which created MPC powder particles with high porosity, did not improve wetting time as it had the same wettability index (>120 s) as a conventionally spray-dried MPC powder. The capillary rise wetting behaviour, observed using the modified Washburn method, is shown in Fig. 6.1. A-MPC absorbed the most water (1.0 g) and R-MPC absorbed the

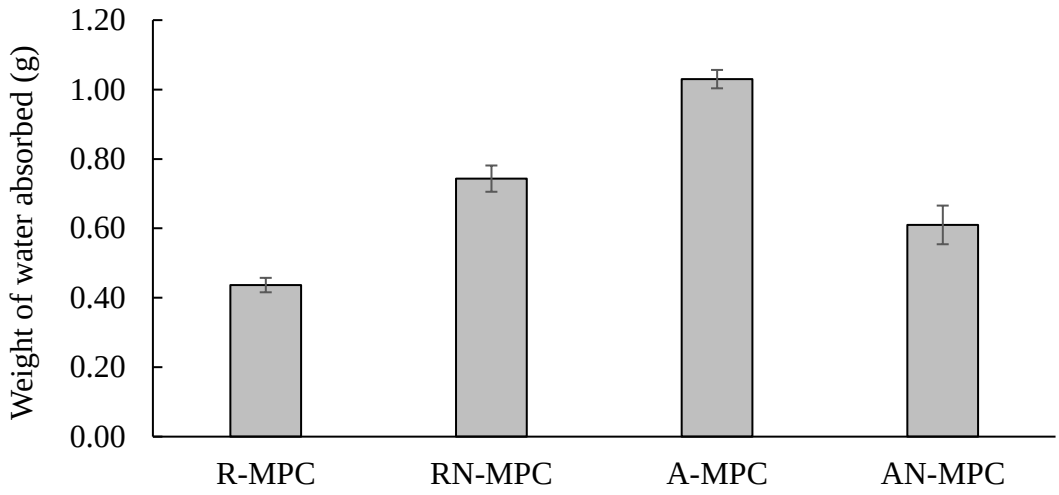

Fig. 6.1. Mean weight of water absorbed for regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders after submerging in ultrapure water (25 °C) for 20 min using a modified Washburn method.

least (0.43 g). The N_2 injection (NI) process appeared to improve capillary rise wetting for the regular powders as RN-MPC absorbed 0.74 g of water. However, for the agglomerated powders, AN-MPC absorbed a lower quantity of water (0.61 g) than A-MPC, despite having a higher porosity. The difference in capillary rise wetting between R- and A-MPC may be explained by the differences in powder particle size; A-MPC had a $D_{[4,3]}$ of 142 μm while R-MPC had a $D_{[4,3]}$ of 79 μm. Similarly, Ji et al.

(2016) reported water absorption levels of 0.24 and 1.0 g for non-agglomerated and agglomerated milk protein isolate (MPI) powders, respectively. It has been previously reported that agglomeration improved the wetting behaviour of a native phosphocaseinate (NPC) powder due to the large powder particle size (mean = 285 μm) and high porosity (Gaiani et al., 2005, 2007). One of the main factors influencing powder wettability is the surface composition (Gaiani et al., 2006a), and the presence of fat specifically on the surface of spray-dried powders would be expected to influence the wetting behaviour by increasing surface hydrophobicity. The surface composition of the powders in the current study were established previously by McSweeney et al. (2021), where it was reported that the NI powders had significantly higher amounts of surface fat (e.g., 2.02% for R-MPC and 11.1% for RN-MPC), while these samples still performed relatively well in powder wetting experiments. Kim et al. (2002) reported that surface fat had a strong, negative impact on the wettability of several dairy powders (e.g., cream, skim and whole milk powder), while Gaiani et al. (2006a) did not find a clear relationship between the surface fat of NPC powders and wetting times.

6.3.2. Visualisation of liquid phase water diffusion and effective diffusivity

Confocal laser scanning microscopy images showing the movement of the rhodamine B dye into R- and RN-MPC powder particles are displayed in Fig. 6.2. Complete diffusion of rhodamine dye into R-MPC powder particles took 1563 s (Fig. 6.2A) compared to 196 s for RN-MPC (Fig. 6.2B). This was likely caused by the significantly higher occluded air (R-MPC = 24.2 mL/100 g, RN-MPC = 36.3 mL/100 g) and porosity (R-MPC = 0.69, RN-MPC = 0.88) values reported by McSweeney et

al. (2021) for this powder. Analysis of water diffusion is most relevant to the wetting stage of powder rehydration and can demonstrate how quickly initial water transfer occurs.

Large differences were observed in effective diffusivity between agglomerated and regular MPC powders (Fig. 6.3). AN-MPC had the highest effective diffusivity value of 8.09^{-12} m^2/s compared to 4.09^{-12} m^2/s for A-MPC. Conversely, the movement

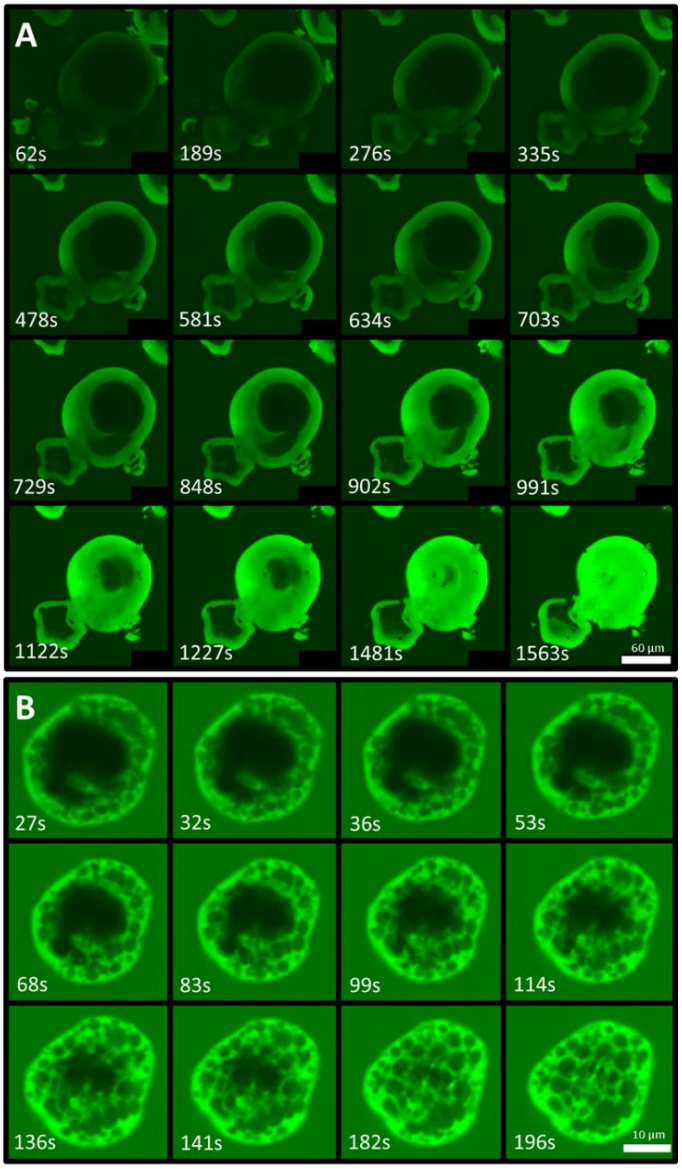

Fig. 6.2. Confocal laser scanning microscopy images showing the movement of rhodamine B dye into (A) regular and (B) regular with N_2 injection milk protein concentrate powders over time (s).

of rhodamine dye into R-MPC occurred at the slowest rate among all powders at 3.29^{-13} m^2/s, with RN-MPC slightly higher at 4.18^{-13} m^2/s. It is apparent that NI prior to spray drying assisted the transfer of the aqueous dye into the powder particles. The rate of diffusion was most likely higher for agglomerated MPC powders due to the larger powder particle size compared to regular MPC powders. The link between higher effective diffusivity and increasing powder particle size has been reported previously by Power et al. (2020) for enzymatically crosslinked MPC powders.

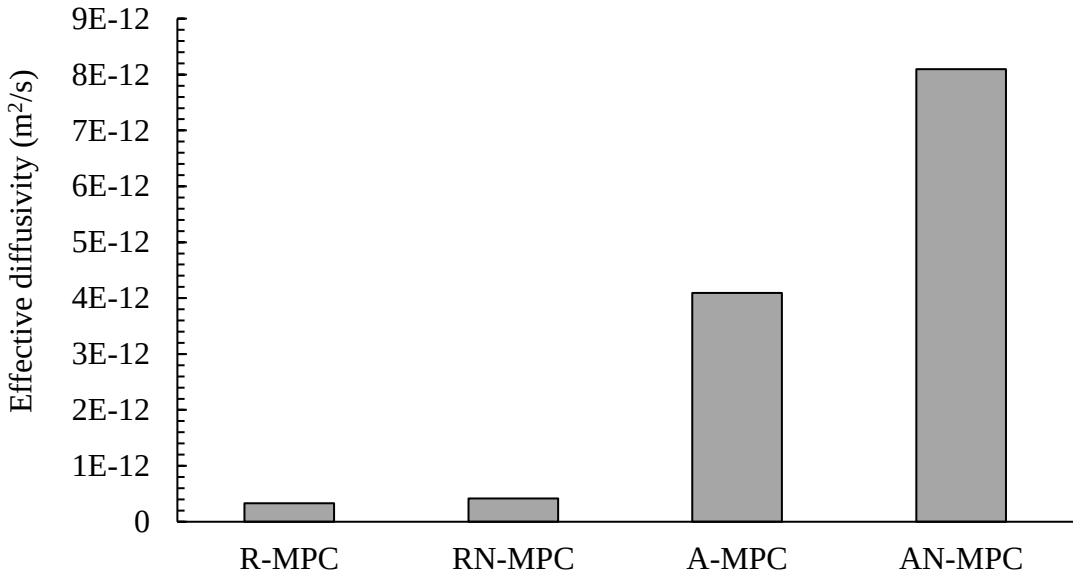

Fig. 6.3. Effective diffusivity (m^2/s) for regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

6.3.3. Water sorption isotherms

Water sorption profiles for MPC powders are displayed in Fig. 6.4. It is evident that water content and the time to reach equilibrium increased with increasing relative humidity (RH). Lactose crystallisation did not occur, as this is generally indicated by a sudden decrease in water content, while Kelly et al. (2015) also reported the absence of lactose crystallisation in MPC powders containing ~80% protein (w/w). NI

promoted a faster uptake of moisture during the early stages of RN-MPC powder storage compared to R-MPC powders (Fig. 6.4A and B). For example, after 8 h at 85% RH, R-MPC had a water content of 7.2 g/100 g compared to 9.5 g/100 g for RN-MPC. However, after 144 h, R-MPC (18.2 g/100 g) and RN-MPC (17.8 g/100 g) powders had similar water contents. Agglomerated powders (Fig. 6.4C and D) absorbed more water overall than their non-agglomerated counterparts. After 8 h at 85% RH, AN-MPC had a water content of 14.9 g/100 g compared to 11.8 g/100 g for A-MPC. This trend was also evident for the effective diffusivity analysis presented in Section 6.3.2., whereby larger powder agglomerates favoured the movement of water into the particles. Particle size distribution has been previously identified as an important determinant of a materials water sorption behaviour. Mathlouthi and Roge (2003) reported that smaller particles of sugar were capable of absorbing more water than larger particles, while Murrieta Pazos et al. (2014) observed a similar trend for durum wheat semolina. However, Ji et al. (2017) reported that MPI powders agglomerated using fluidised bed granulation showed similar water sorption, despite differences in particle size. In the current study, the surface composition of powders may have been a contributing factor as the surface of agglomerated powder particles was significantly higher in lactose than that of regular powders (McSweeney et al., 2021).

6.*3.4. Measurement of mineral release using conductivity*

The release of minerals from powder particles was complete by approximately 3000 s (Fig. 6.5). It is evident that R- and A-MPC released ions at a faster rate than both RN- and AN-MPC powders. The R- and A-MPC powders underwent wetting and sinking after approximately 600 s (time to reach steady state), which can be inferred from the beginning of the plateau on the graph. However, a surface barrier was evident

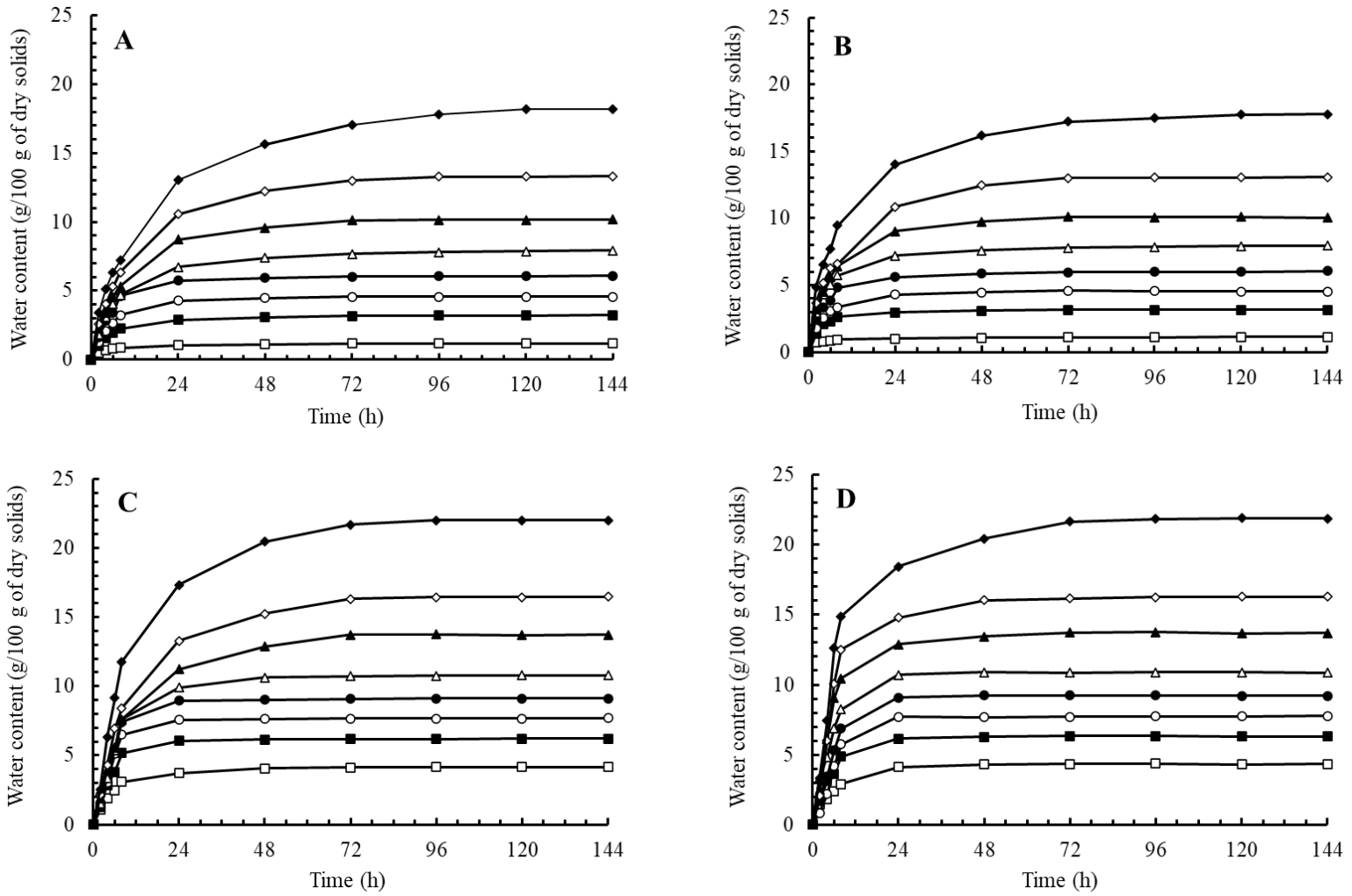

Fig. 6.4. Water sorption isotherms for (A) regular, (B) regular with N_2 injection, (C) agglomerated and (D) agglomerated with N_2 injection milk protein concentrate powders at relative humidity values of 11 (□), 23 (■), 33 (○), 44 (●), 55 (Δ), 65 (▲), 76 (◊) and 85% (♦) over 144 h.

during stirring for powders produced using NI and it took ~1400 s for this plateau to be reached. This result is likely related to the physical properties of the NI powders as they had lower bulk and particle density values and higher air contents. Masters (1985) reported that sinking of powder particles is supported by high particle density and low occluded air, while a low particle density will cause the powder to float on the surface of the water. Fitzpatrick et al. (2016) attributed the poor wettability (>1 h) of an MPI powder to its low apparent density (0.81 g/cm^3), which was similar to the particle density values for RN-MPC (0.96 g/cm^3) and AN-MPC (0.88 g/cm^3). It has been previously reported by Mimouni et al. (2010) that minerals (non-micellar material) are freely released during rehydration of MPC, but that protein dispersion is the rate-limiting stage.

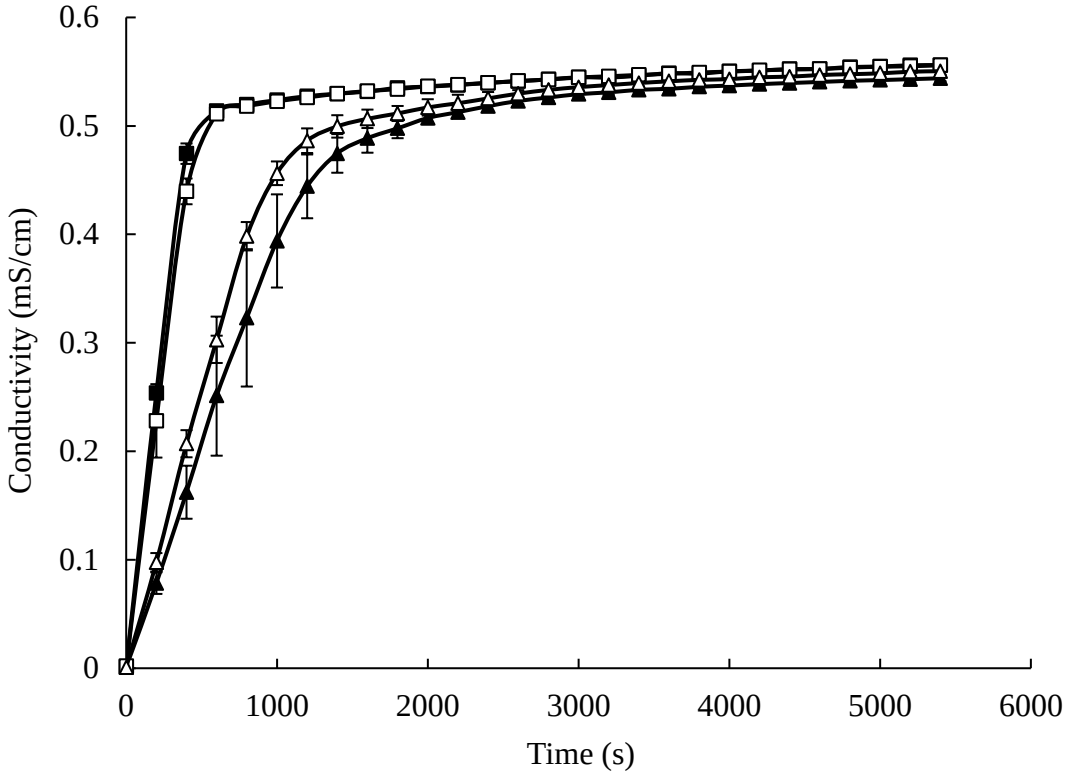

Fig. 6.5. Conductivity profiles of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders measured while stirring in ultrapure water (25 °C) for 90 min.

6.3.5. Particle size distribution and solubility of milk protein concentrate dispersions

NI significantly enhanced the dispersion of MPC powder particles following reconstitution (Table 6.1). When powders were mixed for 30 s at 23 °C, sub-micron particles were not present and all samples had monomodal volume-based distributions, suggesting that casein micelles were not released from primary powder particles (Fig. 6.6A); however, a significantly smaller particle size was observed for RN-MPC ($D_{[4.3]}$ = 32.6 µm) compared to R-MPC ($D_{[4.3]}$ = 79.6 µm). For agglomerated powders, the $D_{[4.3]}$ was significantly lower for AN-MPC (41.8 µm) in comparison to A-MPC (119 µm) under these conditions. The improvement in dispersion of RN and AN-MPC powder is likely accounted for by the higher porosity and interstitial space. The more porous structure of NI powder particles and the presence of large air voids between these particles would facilitate increased water transfer, while also increasing the physical space between casein micelles and reducing protein-protein interactions. This would appear to promote the structural collapse of powder particles when added to water, as interactions between poorly dispersible micellar casein, particularly at the particle surface, are considered to be responsible for the slow rehydration of MPC (Anema et al., 2006; Mimouni et al., 2009, 2010).

The water temperature used during reconstitution significantly affected the particle size distribution, with a higher temperature enhancing the fragmentation of MPC powder particles (Fig. 6.6B). When the temperature of the reconstitution water was 50 °C, the $D_{[4.3]}$ values were 18.4 µm and 1.59 µm for the R- and RN-MPC powders, respectively (Table 6.1). All powders had a bimodal volume-based distribution, with a peak <1 µm and a second peak in the size range of 8-300 µm. However, the volume of sub-micron particles was higher for RN- and AN-MPC compared to R- and A-MPC. This implies that a large quantity of particles present in

Table 6.1. Particle size distribution parameters for regular (R), regular with N_2 injection (RN), agglomerated (A), and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) dispersions after high-speed (HS) mixing at 23 °C and 50 °C for 30 s using a solubility index meter and after low-speed (LS) mixing at 23 °C for 1 h using a magnetic stirrer.

Rehydration conditions	MPC	D_{50}	D_{90}	$D_{[4,3]}$
			μm	
HS mixing 30 s at 23 °C	R	$68.4^{a} \pm 3.16$	$155^{a} \pm 6.83$	$79.6^{a} \pm 3.38$
	RN	$25.6^{b} \pm 0.85$	$66.0^{b} \pm 2.19$	$32.6^{b} \pm 0.81$
	A	$108^{c} \pm 4.09$	$224^{c} \pm 5.87$	$119^{c} \pm 3.67$
	AN	$35.5^{d} \pm 1.27$	$79.8^{d} \pm 1.75$	$41.8^{d} \pm 1.07$
HS mixing 30 s at 50 °C	R	$0.20^{a} \pm 0.07$	$66.0^{a} \pm 10.9$	$18.4^{a} \pm 4.69$
	RN	$0.09^{b} \pm 0.00$	$0.42^{b} \pm 0.02$	$1.59^{b} \pm 0.13$
	A	$0.33^{c} \pm 0.15$	$56.7^{c} \pm 5.29$	$19.6^{a} \pm 3.10$
	AN	$0.10^{b} \pm 0.00$	$0.52^{b} \pm 0.06$	$2.21^{b} \pm 0.36$
LS mixing 1 h at 23 °C	R	$76.6^{a} \pm 4.67$	$156^{a} \pm 6.46$	$83.7^{a} \pm 4.01$
	RN	$0.16^{b} \pm 0.05$	$51.4^{b} \pm 11.3$	$13.6^{b} \pm 3.97$
	A	$55.2^{c} \pm 7.67$	$129^{c} \pm 17.3$	$66.3^{c} \pm 8.44$
	AN	$0.19^{b} \pm 0.04$	$51.7^{b} \pm 5.78$	$14.4^{b} \pm 2.45$

[a-d] Values within columns not sharing common superscripts differ significantly ($P < 0.05$).

the NI powder dispersions were casein micelles, suggesting higher levels of dissolution were achieved. A-MPC powder had poorer dissolution properties as indicated by the larger particle size ($D_{[4.3]}$ = 119 μm) after reconstitution at 23 °C

compared to R-MPC ($D_{[4.3]}$ = 79.6 μm). Therefore, the agglomeration of high-protein powders during spray drying appears to be counter-productive for improving rehydration, unlike its use in the production of skim and whole milk powders (Pisecky, 2012). Gaiani et al. (2005, 2007) reported similar results, whereby agglomeration increased the overall rehydration time of NPC powders as it delayed the dispersion process. However, in the current study, reconstitution at 50 °C resulted in no significant differences between agglomerated and regular (non-agglomerated) powders, with $D_{[4.3]}$ values of 20 and 18.4 μm for A-MPC and R-MPC, respectively (Table 6.1). This suggests that increasing the water temperature may moderately alleviate this issue with A-MPC dispersion.

MPC powders were also analysed after magnetic stirring for 1 h in ultrapure water at 23 °C, with bimodal particle size distributions obtained for NI powders compared to monomodal size profiles for non-NI powders (Fig. 6.6C). This corresponded to $D_{[4.3]}$ values of 13.6 μm for RN-MPC and 14.4 μm for AN-MPC compared to 83.7 and 66.3 μm for R- and A-MPC, respectively (Table 6.1). This result further highlights the improved dispersibility of the NI powders, at a relatively low reconstitution temperature and agitation rate, compared to non-NI powders. Mimouni et al. (2009) reported that 480 min of stirring at 24 °C was required to fully solubilise a MPC powder (85%, w/w, protein). Similarly, Gaiani et al. (2007) reported that 807 and 572 min of stirring at 24 °C were required to dissolve agglomerated and non-agglomerated micellar casein powders, respectively.

Aside from the particle size data of reconstituted powders, solubility results confirmed the superior dissolution of NI powders, particularly at 23 °C (Table 6.2), with R- and RN-MPC having 83.6 and 96.2% solubility, respectively. The lower loose

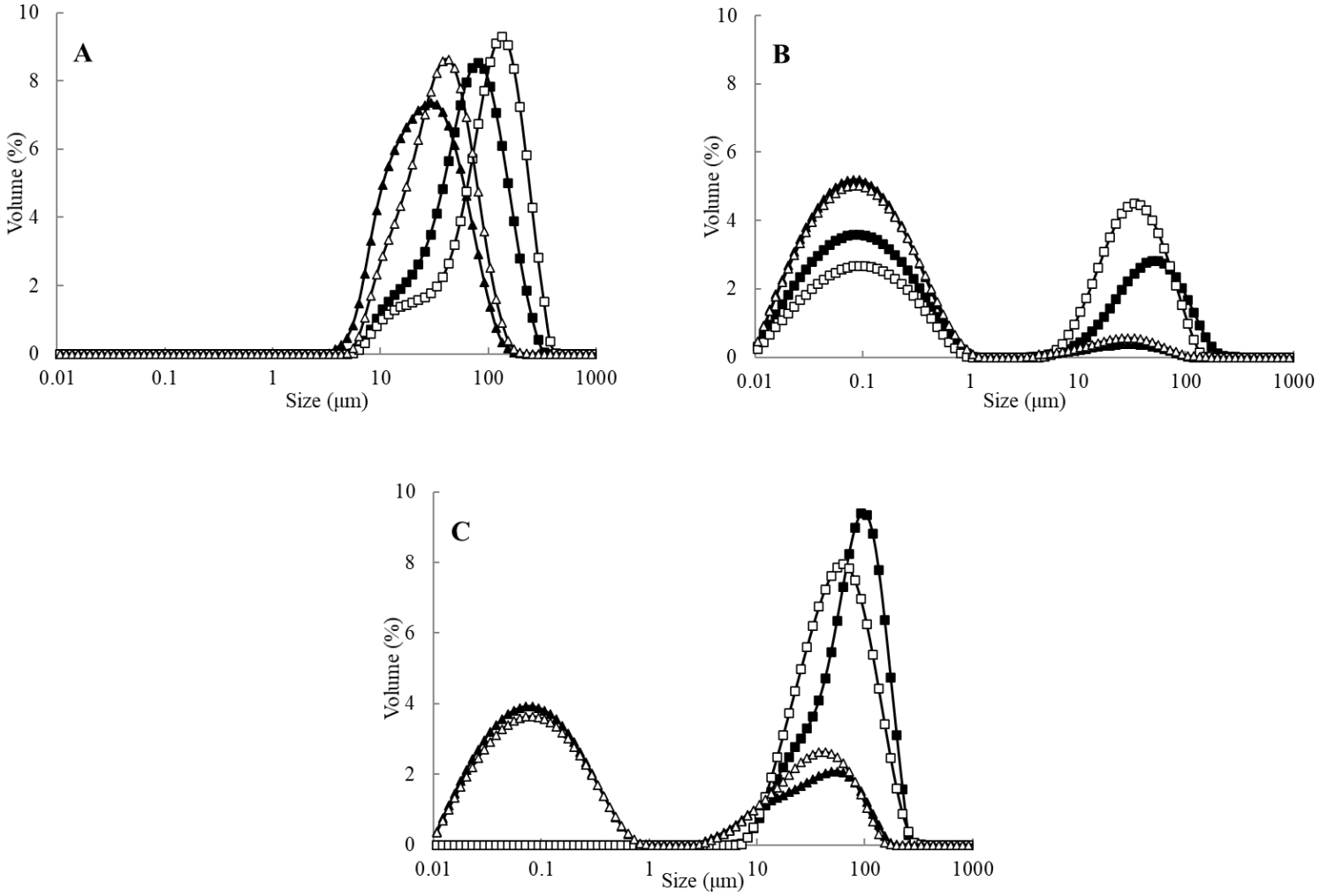

Fig. 6.6. Particle size distribution profiles of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders measured after reconstitution in ultrapure water for (A) 30 s at 23 °C, (B) 30 s at 50 °C and (C) 1 h at 23 °C.

bulk density and higher porosity for RN-MPC are also likely to be responsible for the higher levels of solubility (McSweeney et al., 2021). Similarly, A-MPC had a solubility value of 62.6% compared to 92.1% for the AN-MPC powder (Table 6.2). However, when powders were reconstituted at 50 °C, no significant differences in solubility were observed. It has been previously reported by Mimouni et al. (2009)

Table 6.2. Powder solubility (%) of regular (R), regular with N_2 injection (RN), agglomerated (A), and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) samples taken after mixing at 23 °C and 50 °C for 30 s using a solubility index meter and centrifuged at 3000*g* for 10 min.

MPC	Solubility (23 °C)	Solubility (50 °C)
R	$83.6^{a} \pm 1.9$	$96.7^{a} \pm 0.9$
RN	$96.2^{b} \pm 1.3$	$98.2^{a} \pm 1.1$
A	$62.6^{c} \pm 6.9$	$96.6^{a} \pm 0.7$
AN	$92.1^{a} \pm 3.1$	$98.1^{a} \pm 0.3$

[a-c] Values within columns not sharing common superscripts differ significantly ($P < 0.05$).

that an increase in solvent temperature accelerates the release of constituent materials from MPC powder particles into the aqueous phase. Overall, these results indicate that physical and structural properties (e.g., density, air content and porosity) play a significant role in the rehydration of high-protein MPC powders.

Following reconstitution for 1 h, it was evident that the samples with the smallest particle size and highest solubility (i.e., RN- and AN-MPC) had the lowest viscosity (Fig. 6.7). A-MPC had the lowest dispersibility and solubility but the highest viscosity. The general trend observed from the rehydration experiments (i.e., rehydration performance was highest for RN- and AN-MPC, followed by R- and A-

MPC) was also apparent for the viscosity data. Warncke and Kulozik (2020) reported that MPC powders (74%, w/w, protein) reconstituted in water at 50 °C had higher viscosity and lower dispersibility than micellar casein powders (67%, w/w, protein),

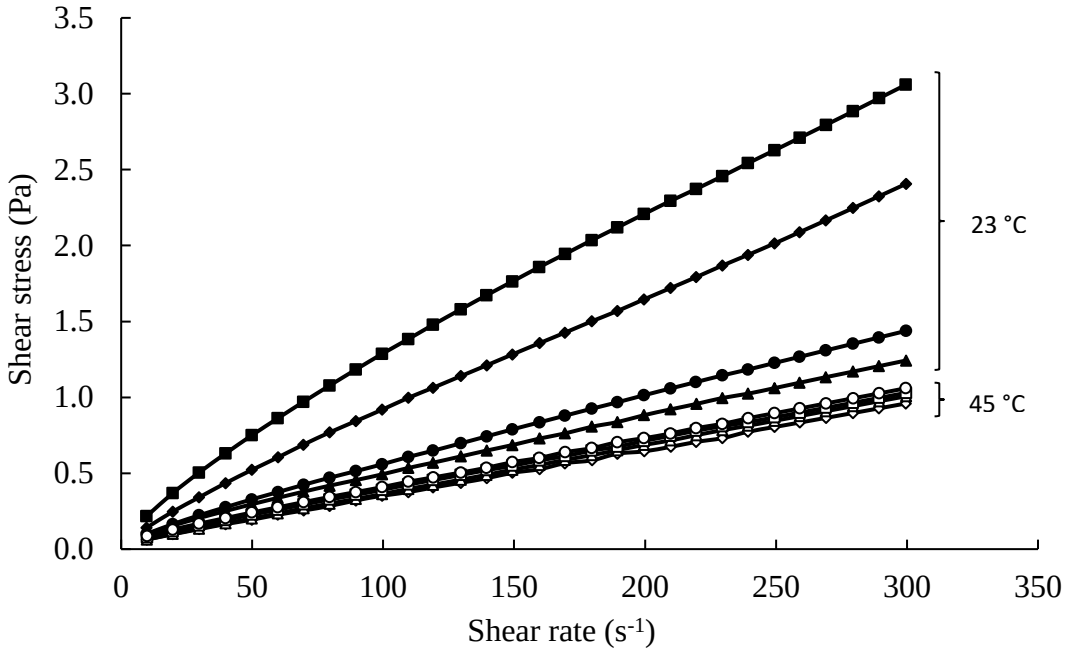

Fig. 6.7. Shear stress as a function of shear rate of MPC powders reconstituted for 1 h at 23 °C or 45 °C for 45 min followed by overnight stirring: R 1 h (♦), RN 1 h (▲), A 1 h (■), AN 1 h (●), R overnight (◊), RN overnight (Δ), A overnight (□) and AN overnight (○).

due to the presence of large particles. Similarly, Pathania et al. (2018) reported that MPC powder reconstituted using hydrodynamic cavitation had lower viscosity and a smaller average particle size compared to dispersions prepared using a conventional high-shear process.

The foam expansion index (FEI) values for MPC powders are shown in Fig. 6.8. It is evident that powder reconstitution at lower temperatures reduces the quantity of foam produced during mixing. For example, for R-MPC, the FEI was 54% at 23 °C but 112% at 50 °C. Furthermore, the FEI was slightly higher for powders produced

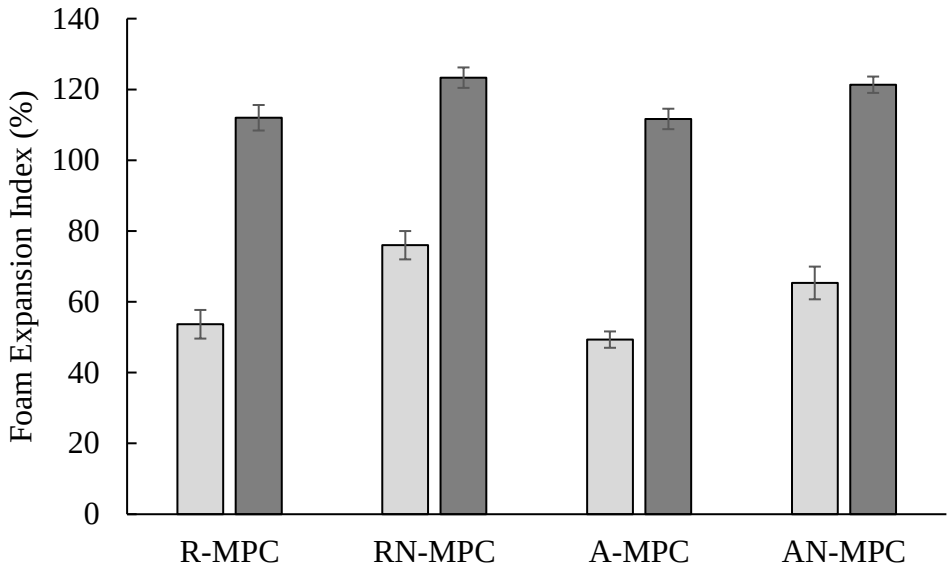

Fig. 6.8. Foam expansion index values (%) for milk protein concentrate (MPC) powders reconstituted in ultrapure water at 23 °C (light grey bar) and 50 °C (dark grey bar) for 1 min.

using NI compared to the controls, particularly at the lower temperature (e.g., 49% for A-MPC compared to 65% for AN-MPC when the temperature of reconstitution was 23 °C). This may have occurred due to the higher interstitial and occluded air values for these powders which would likely promote the formation of foam. The higher solubility of these powders may also have been an important factor as more protein would have been available to diffuse to the air-liquid interface, stabilise air bubbles and enhance foaming. A relationship between solubility and foaming capacity has been reported by Jambrak et al. (2008) for ultrasound-treated whey protein suspensions, whereby the whey protein isolate suspension with the highest solubility (85%) also had the higher foaming capacity (235%), and conversely, the sample with the lowest solubility had the lowest foaming capacity. Higher foaming capacity of MPC may be a useful characteristic for certain food applications (e.g., mousses, ice cream, instant coffee).

6.4. Conclusion

This study demonstrated that the dissolution of MPC powder is enhanced by the injection of N_2 into the concentrate prior to spray drying. It is proposed that higher powder particle porosity and interstitial space between particles are responsible for the improvement in rehydration performance. Water transfer during reconstitution was promoted by the presence of large air voids and pores throughout the powder particles, resulting in a large volume of small, dispersed particles (i.e., <1 μm). Agglomeration alone favoured powder wetting, water uptake and particle hydration; however, it had a negative impact on powder particle dissolution. Combining N_2 injection with agglomeration resulted in further improvements in diffusion and wetting behaviour but did not confer any additional improvement in dispersion and solubilisation of powder particles compared to N_2 injection alone. The injection of N_2 into high-protein concentrate prior to spray drying is a relatively simple and effective processing technology to enhance powder particle dispersibility and solubility, while avoiding the use of chemical additives which may disrupt casein micelle integrity. The impact of N_2 injection on bulk handling and other functional properties of MPC powders (e.g., density and dissolution) after storage and transport should be considered in future research.

6.5. References

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Chapter 7

Influence of milling on the physical and rehydration properties of milk protein concentrate powders manufactured using nitrogen gas injection and agglomeration

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Abstract

In this study, the physical, bulk handling and rehydration properties of regular and agglomerated milk protein concentrate (MPC) powders, prepared using nitrogen (N_2) gas injection as part of the spray drying process, were analysed following milling. Powder loose and tapped bulk density increased, while air content and powder particle size decreased, following milling. Differences in surface composition were observed, in particular a lower proportion of fat and a higher proportion of lactose at the surface of powder particles produced using N_2 injection, with the reverse trend apparent for regular MPC powder particles. Scanning electron microscopy images showed breakage and fragmentation of powders into fine and coarse particles. No change in conductivity was recorded following milling, as regular and agglomerated powders released minerals into solution at a higher rate than the corresponding powders produced using N_2 injection. The dispersion and solubilisation of all MPC powders were generally lower following the milling process, but still remained higher for those manufactured using N_2 injection directly prior to atomisation.

7.1. Introduction

The physical and rehydration properties of high-protein (>80%, w/w, protein), micellar casein-dominant dairy powders, such as milk protein concentrate (MPC), present the food and beverage industry with several processing challenges. Previous research has established that spray drying protein-enriched dairy concentrates, containing a high proportion of micellar casein, generates ingredients with poor powder particle and bulk characteristics (e.g., cohesive flow behaviour, low particle and bulk density) and a reduced capacity to readily rehydrate in water (McSweeney et al., 2020; Crowley et al., 2014, 2015). The poor physical and bulk handling properties of MPC powders have been primarily attributed to the low total solids contents of spray dryer feeds (Crowley et al., 2014; McSweeney et al., 2020) due to the increased viscosity of protein-enriched concentrates, subsequently leading to high occluded and interstitial air contents. In addition, the rehydration performance of such powders is often sub-optimal due to the high content of micellar casein in these ingredients, reducing water transfer (Schuck et al., 2002), and the low concentration of lactose which can typically act as a physical spacer and reduce interactions between casein proteins (Anema et al., 2006). As a result, the industrial use of MPC presents issues such as blockage of powder silos, sedimentation of insoluble material in reconstituted products (Pandalaneni et al., 2019) and decreased gel strength and firmness in yogurt applications (Karam et al., 2016a). The influence of nitrogen gas (N_2) injection on MPC powder particle structure and density was described in Chapter 5, while in Chapter 6, N_2 injection was shown to accelerate powder rehydration in water. Therefore, as these powders presented enhanced dissolution but relatively low density, the objective of this study was to mill these samples to increase powder density and subsequently elucidate the impact it would have on their physical and functional

properties (e.g., would the improvement in powder rehydration still be observed at higher bulk density). Although the focus was to increase the density of the powders produced using N_2 injection, agglomerated samples were also included in this study as they were part of the original sample set and had different structural, physical and functional properties. One application of milling agglomerated powders could be to control the particle size of powders produced by roller compaction agglomeration (Hazlett et al., 2021).

Comminution is a size-reduction unit operation (e.g., conventional dry grinding or milling) that uses forces such as compression, impact and attrition to induce the breakdown of food materials (Barbosa Canoas et al., 2005). Several different devices are used depending on the characteristics of the feed material (e.g., hammer mills for producing flour from cereal grains). Other current applications of standard milling include spice manufacture and converting dehydrated fruits and vegetables into powders for use as food ingredients (Baudelaire, 2013). Micronisation, or superfine grinding, is another size reduction process that reduces materials to less than 10 µm through particle-particle collisions or impact against solid surfaces (Karam et al., 2016b). Ball- and jet-milling are two commonly used micronisation techniques that have gained interest in the development of nanoscale food ingredients (Sanguansri and Augustin, 2006) and the impact of superfine grinding on the physicochemical properties of food powders has recently been reviewed by Gao et al. (2020). In addition, superfine milling is also utilised in the pharmaceutical industry to improve drug solubility and flowability (Naik and Chaudhuri, 2015), but few research studies have investigated the milling of dairy powders and what impact this process can have on powder functionality.

7.2. Materials and methods

7.2.1. Materials and manufacture of milk protein concentrate powders

The details of manufacture, and composition, of milk protein concentrate (MPC) powders used in this study are provided in Chapter 5.

7.2.2. Powder milling

Milling of MPC powders was carried out using a ZM 200 ultra-centrifugal mill (Retsch GmbH, Haan, Germany). Powder samples were introduced *via* a hopper situated at the top of the mill where they fell onto a 12-tooth rotor operating at a centrifugal speed of 8000 rpm. The powders subsequently passed through a fixed ring sieve with an average pore size of 80 μm and quickly into a collection cassette, with particles reduced in size by impact and shearing effects.

7.2.3. Bulk density, particle density, porosity, occluded and interstitial air

The bulk density, particle density, porosity, occluded air and interstitial air values were calculated, as described in Chapter 3

7.2.4. Colour

The colour space values and total colour difference of milled MPC powders were calculated, as described in Chapter 4.

7.2.5. Powder particle size distribution

The powder particle size distribution values for milled MPC powders were determined, as described in Chapter 3.

7.2.6. Specific surface area

Specific surface area values for milled MPC powders were calculated, as described in Chapter 5.

7.2.7. Scanning electron microscopy

Scanning electron microscopy images of milled MPC powders were obtained, as described in Chapter 3.

7.2.8. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy values for milled MPC powders were determined, as described in Chapter 5.

7.2.9. Measurement of mineral release using conductivity

The release of minerals from milled MPC powders was determined using conductivity, as described in Chapter 6.

7.2.10. Powder wettability

Powder wettability was recorded for milled MPC powders using the GEA Niro method (GEA Niro, 2009) with one modification; 4 g of each powder was added to the beaker of water (250 mL; 25 °C). Photographs were taken after 10 min to determine if a visual difference could be observed between samples.

7.2.11. Particle size distribution and solubility of milk protein concentrate dispersions

The particle size distribution and solubility values of milled MPC dispersions were obtained using the methods described in Chapter 3.

7.2.12. Statistical data analysis

Measurements of powder characteristics were performed in triplicate, with the exception of specific surface area and surface composition which were conducted in duplicate. Results were presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was carried out using IBM SPSS (Version 24; Armonk, New York, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

7.3. Results and discussion

7.3.1. Colour, density, porosity, interstitial and occluded air content

Centrifugal milling resulted in several physical changes to the milk protein concentrate (MPC) powders. The trend observed for the colour of the powders did not change following the milling process, i.e., regular with N_2 injection (RN) and agglomerated with N_2 injection (AN) MPC had more positive L* and a* values but less positive b* values compared to regular (R) and agglomerated (A) MPC (Table 7.1). The a* value became more positive, while the b* values became less positive, for all powders (i.e., they became more red and less yellow), likely due to the heat created during centrifugal milling. The ΔE was 5.76 and 5.42 for RN- and AN-MPC, which was considerably higher than the value for A-MPC of 0.18. Sun et al. (2015) reported that whey protein concentrate (WPC) powder was more red following superfine grinding for 2 h using a nano ball mill. The bulk, particle and tapped density increased for all samples as a result of milling (Table. 7.1). For example, the loose

Table 7.1. Density and air content of regular (R), regular with N_2 injection (RN), agglomerated (A), and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders after milling.

MPC	L*	a*	b*	ΔE	ρ_p	ρ_b	ρ_t	V_{ia}	V_{oa}	ε
						(g/cm^3)			mL/100 g	
R	92.9 ± 0.01	-1.88 ± 0.03	9.69 ± 0.01	-	1.18^{bc} ± 0.01	0.35^{b} ± 0.00	0.41^{b} ± 0.01	159^{c} ± 7.98	17.4^{a} ± 0.58	0.65^{c} ± 0.01
RN	95.2 ± 0.01	-0.79 ± 0.02	4.50 ± 0.02	5.76 ± 0.02	1.17^{b} ± 0.02	0.17^{d} ± 0.00	0.19^{d} ± 0.00	440^{a} ± 6.23	17.7^{a} ± 1.29	0.84^{a} ± 0.00
A	93.0 ± 0.01	-1.98 ± 0.02	9.80 ± 0.01	0.17 ± 0.02	1.20^{ac} ± 0.00	0.38^{a} ± 0.01	0.46^{a} ± 0.01	132^{d} ± 3.69	16.2^{ab} ± 0.27	0.61^{d} ± 0.01
AN	94.9 ± 0.01	-1.03 ± 0.03	4.73 ± 0.00	5.41 ± 0.01	1.22^{a} ± 0.01	0.22^{c} ± 0.01	0.24^{c} ± 0.00	329^{b} ± 7.54	15.0^{b} ± 0.56	0.80^{b} ± 0.01

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). L* = colour varying from black (0) to white (100), a* = colour varying from green (-) to red (+), b* = colour varying from blue (-) to yellow (+), ρ_p = particle density; ρ_b = loose bulk density; ρ_t = tapped bulk density; V_{ia} = volume of interstitial air; V_{oa} = volume of occluded air; ε = porosity.

bulk density increased from 0.09 to 0.17 mL/100 g for R-MPC, and from 0.07 to 0.22 mL/100 g for AN-MPC, while the highest increase in bulk density from milling occurred for A-MPC (from 0.18 to 0.38 mL/100 g). The particle density increased significantly (e.g., from 0.87 to 1.22 g/cm^3 for AN-MPC), with all milled powders having similar values (i.e., range of 1.17-1.22 g/cm^3). As expected, with this increase in density, there was a corresponding decrease in both the occluded and interstitialair contents of all powders. The interstitial air value remained significantly higher for RN- and AN-MPC (440 and 329 mL/100 g, respectively), compared to 159 and 132 mL/100 g for R- and A-MPC. There was no significant difference between the occluded air content of both R- and RN-MPC (17.4 and 17.7 mL/100 g) or between A- and AN-MPC (16.2 and 15.0 mL/100 g). Porosity decreased for all powders following milling, but still remained higher for those produced using N_2 injection (NI), e.g., decreased from 0.88 and 0.69 to 0.84 and 0.65 for RN- and R-MPC, respectively. The change in air content and porosity could have a significant impact on the rehydration properties of the powders, particularly RN- and AN-MPC.

7.3.2. Powder particle size and specific surface area

MPC powders produced using the NI process had higher decreases in powder particle size following milling, in comparison to R- and A-MPC (Table 7.2). The D_{90} (i.e., particle size below which 90% of the sample volume is represented by particles smaller than the size indicated) decreased from 134 to 79.4 μm for R-MPC, while it decreased from 148 to 36.7 μm for RN-MPC. This was likely caused by the presence of more interstitial and occluded space in RN-MPC, making the powder possibly more brittle and less strong mechanically when under substantial compression in the mill. However, it was shown to have similar friability to R-MPC in Chapter 5, but this is

not directly comparable with the milling process. The D_{90} was 110 and 38.4 μm for A- and AN-MPC, while it was previously 244 and 256 μm for these powders, respectively. It was expected that agglomerated powders would experience greater attrition during milling as the results in Chapter 5 highlighted that such powders were more friable than regular MPC powders. An increase in specific surface area (SSA) of MPC powders following milling was also recorded, with RN- and AN-MPC powders still having significantly higher SSA than R- and A-MPC (Table 7.2). This is likely accounted for by the reduction in the size of powder particles during milling, thereby increasing their surface area. The decrease in powder particle size, combined with the increase in SSA, would likely present challenges with respect to powder flow behaviour in an industrial powder plant.

Table. 7.2. Mean powder particle size and specific surface area (SSA) values of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders after milling.

MPC	D_{50}	D_{90}	$D_{[4,3]}$	SSA
		(μm)		(m^2/g)
R	$41.3^{b} \pm 0.42$	$79.4^{b} \pm 0.42$	$44.6^{b} \pm 0.32$	$0.64^{b} \pm 0.03$
RN	$13.8^{c} \pm 0.15$	$36.7^{c} \pm 0.20$	$17.4^{c} \pm 0.15$	$3.53^{a} \pm 0.10$
A	$56.7^{a} \pm 0.10$	$110^{a} \pm 1.00$	$60.6^{a} \pm 0.31$	$0.61^{b} \pm 0.01$
AN	$11.1^{d} \pm 0.21$	$38.4^{c} \pm 0.84$	$16.5^{d} \pm 0.36$	$3.38^{a} \pm 0.05$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). D_{10} = particle size below which 10% of sample volume exists; D_{50} = particle size below which 50% of sample volume exists; D_{90} = particle size below which 90% of sample volume exists; $D_{[4,3]}$ = volume-weighted mean particle diameter.

Herceg et al. (2005) investigated the effect of tribomechanical micronisation on the physical properties of WPC powders containing 60 and 80% protein (w/w).

This process involved passing the powder through two opposing metal discs, which contained rings of metal teeth on the surface, rotating at speeds of 16,000, 20,000 and 22,000 rpm to shear the powder. The median particle size (D_{50}) decreased from 76.7 to 46.6 µm for WPC80, while the SSA remained 0.2 m^2/g, at a rotational speed of 16,000 rpm. However, when WPC60 was subjected to the same treatment, the SSA increased from 0.2 to 1.3 m^2/g. In the current study, the D_{50} for R-MPC also decreased from an initial value of 65.3 to 41.3 µm, while the SSA remained unchanged with milling. Similarly, Steckel et al. (2006) investigated the influence of milling lactose monohydrate on subsequent powder functionality and reported a decrease in the D_{90} value from 235 to 177 µm after treatment at 6000 rpm and an increase in SSA values with increasing milling speed, while France et al. (2020) reported that micronised calcium citrate had significantly lower particle size and higher SSA than conventional calcium citrate powder. Banach et al. (2017) reported that the application of jet-milled MPC in nutritional bar formulations influenced the textural properties of the product during storage. Powder particle size was reduced from 86 to 22 and 6 µm with coarse (1000 rpm) and fine (2500 rpm) jet milling, respectively. Nutrition bars prepared using jet-milled MPC displayed greater textural stability (i.e., increased firmness and cohesiveness) compared to the control powder.

7.3.3. Surface composition and microstructure

Surface characterisation by x-ray photoelectron spectroscopy (XPS) demonstrated that the milling process altered the quantity of protein, lactose and fat at the powder particle surface (Table 7.3). In particular, the surface fat was higher for the control samples (e.g., 5.61 and 7.64% for R- and A-MPC, respectively) compared to the NI powders (e.g., 3.15 and 1.76% for RN- and AN-MPC, respectively). As a result

of the decrease in percentage fat at the surface of the NI powders (previously 11.1 and 11.4% for RN- and AN-MPC), they had slightly higher levels of surface protein and lactose. For R-MPC, milling increased the proportion of fat at the surface of the powder particles from 2.02 to 5.61%, while milled A-MPC had a slightly higher proportion of lactose at the powder particle surface (increased from 2.61 to 4.90%). However, as will be discussed in Section 7.3.4, these changes did not result in visible improvements in powder wettability.

Table 7.3. Surface composition (%) of regular (R), regular nitrogen injected (RN), agglomerated (A) and agglomerated nitrogen injected (AN) milk protein concentrate (MPC) powders after milling, as determined by x-ray photoelectron spectroscopy analysis.

MPC	Protein	Lactose	Fat
R	$91.05^{ab} \pm 2.20$	$3.23^{a} \pm 0.57$	$5.61^{a} \pm 1.39$
RN	$92.61^{a} \pm 0.00$	$4.95^{a} \pm 0.02$	$2.15^{b} \pm 0.09$
A	$87.16^{b} \pm 1.10$	$4.90^{a} \pm 0.87$	$7.64^{a} \pm 0.22$
AN	$93.00^{a} \pm 0.55$	$5.11^{a} \pm 0.58$	$1.76^{b} \pm 0.17$

[a-b] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

Microstructural analysis by scanning electron microscopy demonstrated the significant effects of milling on powder particle structure, shape and morphology (Fig. 7.1). It is evident that breakage had occurred for all powders, with fragments of powder particles adhering to each other (e.g., fines to larger particles). For example, in Fig. 7.1B, the internal structure of some powder particles was visible. However, some R- and A-MPC particles (Fig. 7.1A and C), with smooth, slightly dimpled surface structures, were still evident while spherical RN- and AN-MPC particles(Fig. 7.1B

and D) also remained.

7.3.4. Wetting behaviour and mineral release

The static wetting behaviour of the MPC powders before and after milling is shown in Fig. 7.2. None of the powders completely wetted within the time of analysis, which is similar to the powder wettability results reported in previous chapters. However, the water below the surface of R-MPC became more turbid after 10 min as some powder particles became wet and sank, possibly due to the higher bulk density

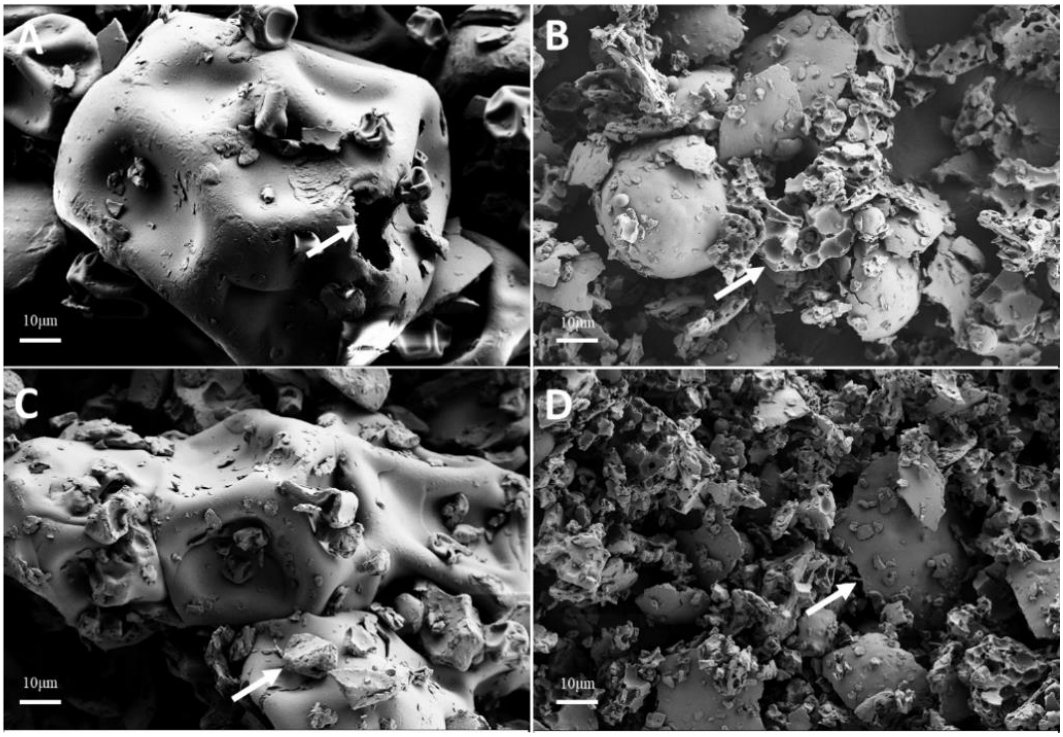

Fig. 7.1. Scanning electron microscopy images of (A) regular, (B) regular with N_2 injection, (C) agglomerated and (D) agglomerated with N_2 injection milk protein concentrate powders after milling. Arrows show where powder particle breakage occurred. Scale bar represents 10 µm.

of the powder. For RN-MPC, the water remained clear which suggests milling may have negatively impacted its wetting behaviour, despite the density increasing and

surface fat content decreasing for this powder. This could be explained by the decrease in occluded and interstitial air content and removal of voids, which were identified as playing an important role in water transfer and powder rehydration in Chapter 6. The wetting behaviour of A-MPC remained largely unchanged after milling, despite the increase in both powder density and the proportion of lactose at the particle surface, while some large fragments of AN-MPC powder underwent visible sinking, which was not recorded previously. It is possible that changes in surface composition following milling played a role in these observed differences, as the distribution of their components were altered (i.e., more protein and lactose but less fat was present at the surface). It is important to mention that the milled MPC powders were analysed

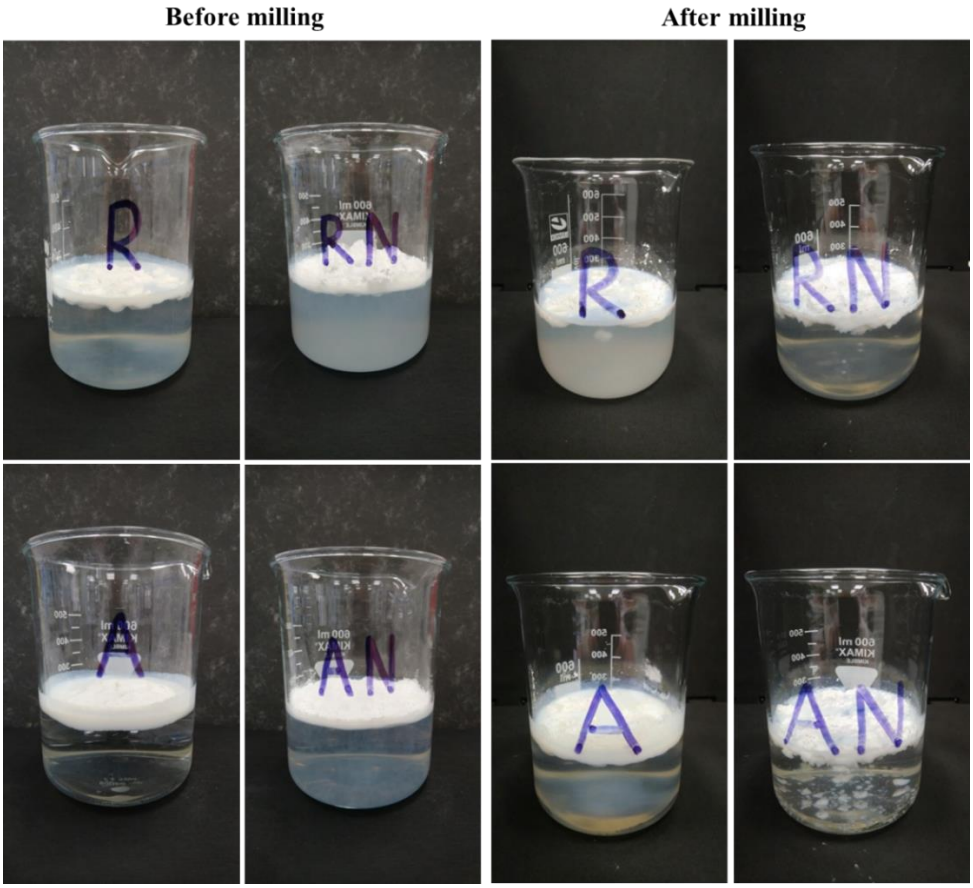

Fig. 7.2. Regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate, 10 min after addition of 4 g of powder to 250 mL of ultrapure water (23 °C).

several months after production so the age of the powders may have been a contributing factor. Furthermore, XPS results are limited by the small quantity of sample analysed and may not be entirely representative of the bulk powder sample, while the observations in a static wetting test may not carry over to the dynamic wetting of powder in an industrial vessel. Another influential factor in the wetting and sinking behaviour of the milled powder particles may have been their shape. Yekeler et al. (2004) reported that the shape and surface topography of milled mineral (talc) powders had an influence on wettability, whereby powders with higher surface roughness and roundness had higher wettability values.

Milling did not appear to have any effect on the conductivity of MPC powders (Fig. 7.3). The time to reach steady-state (i.e., the point where a plateau develops) took approximately 600 s for R- and A-MPC compared to 1400 s for RN- and AN-MPC. Therefore, R- and A-MPC powders still released minerals more rapidly than RN- and AN-MPC powders. This highlights the significance of powder bulk and particle density in the measurement of mineral release. As conductivity measurements are performed by adding powder to a beaker of water under agitation, it also provides a visual aid regarding powder wettability, and it was apparent that NI powders did not wet and sink into the water as quickly as the respective control powders.

7.3.5. Particle size distribution and solubility of dispersions

The particle size distribution profiles for milled MPC powders after reconstitution are given in Fig. 7.4. When powders were reconstituted for 30 s in 23 °C ultrapure water, the mean particle diameter ($D_{[4,3]}$) was significantly lower for RN-MPC (25.5 μm) compared to R-MPC (68.0 μm; Table 7.4). Similarly, AN-MPC had a significantly lower $D_{[4,3]}$ value (33.1 μm) than A-MPC (90.6 μm). However, all

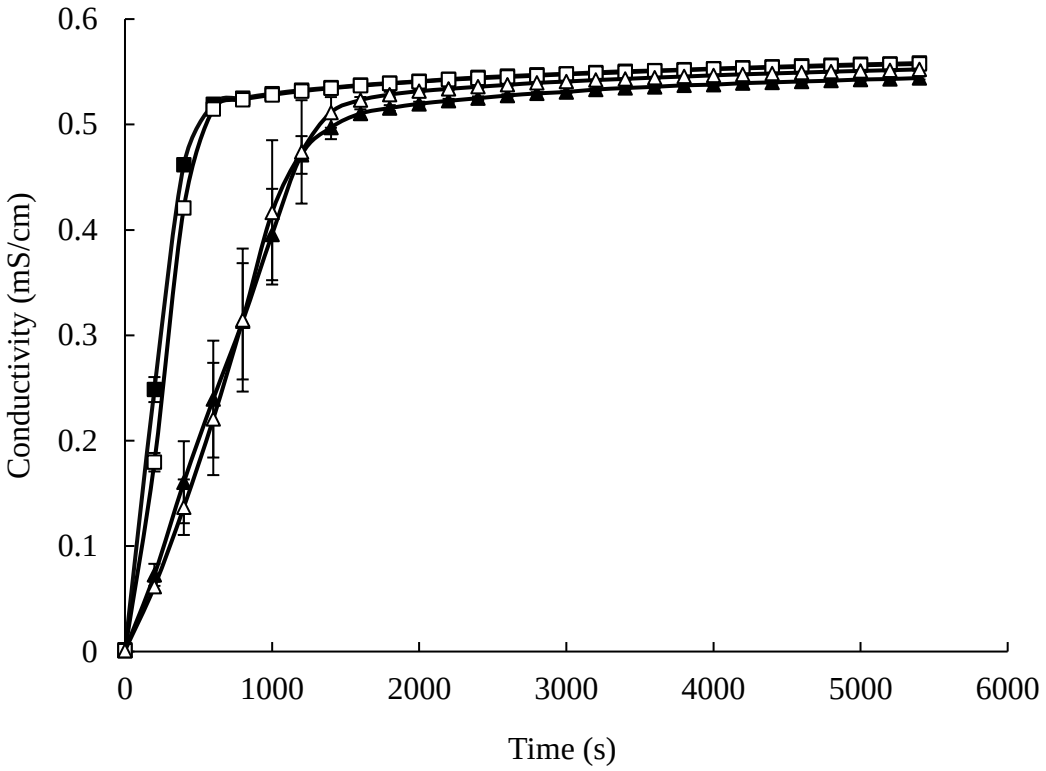

Fig. 7.3. Conductivity profiles of milled regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders measured for 90 min at 25 °C.

samples had a monomodal distribution in the size range of approximately 10-1000 μm. When the reconstitution temperature was increased to 50 °C, RN-MPC was the only sample to show particles in the 0.01-1 μm size range. The $D_{[4,3]}$ was significantly lower for both powders manufactured using NI; 9.96 and 28.2 μm for RN- and AN-MPC, respectively, while there was no difference between R- and A-MPC (49 μm) under these conditions.

The ability of these powders to disperse in water was also investigated over a longer time (1 h) but using a slower stirring speed (550 rpm). Similar to the previous particle size results, both RN- and AN-MPC powders had a significantly lower $D_{[4,3]}$ values as a result of the manufacturing process, but there was no difference between them. However, the $D_{[4,3]}$ was higher for R-MPC (73 μm) than A-MPC (59.5 μm),

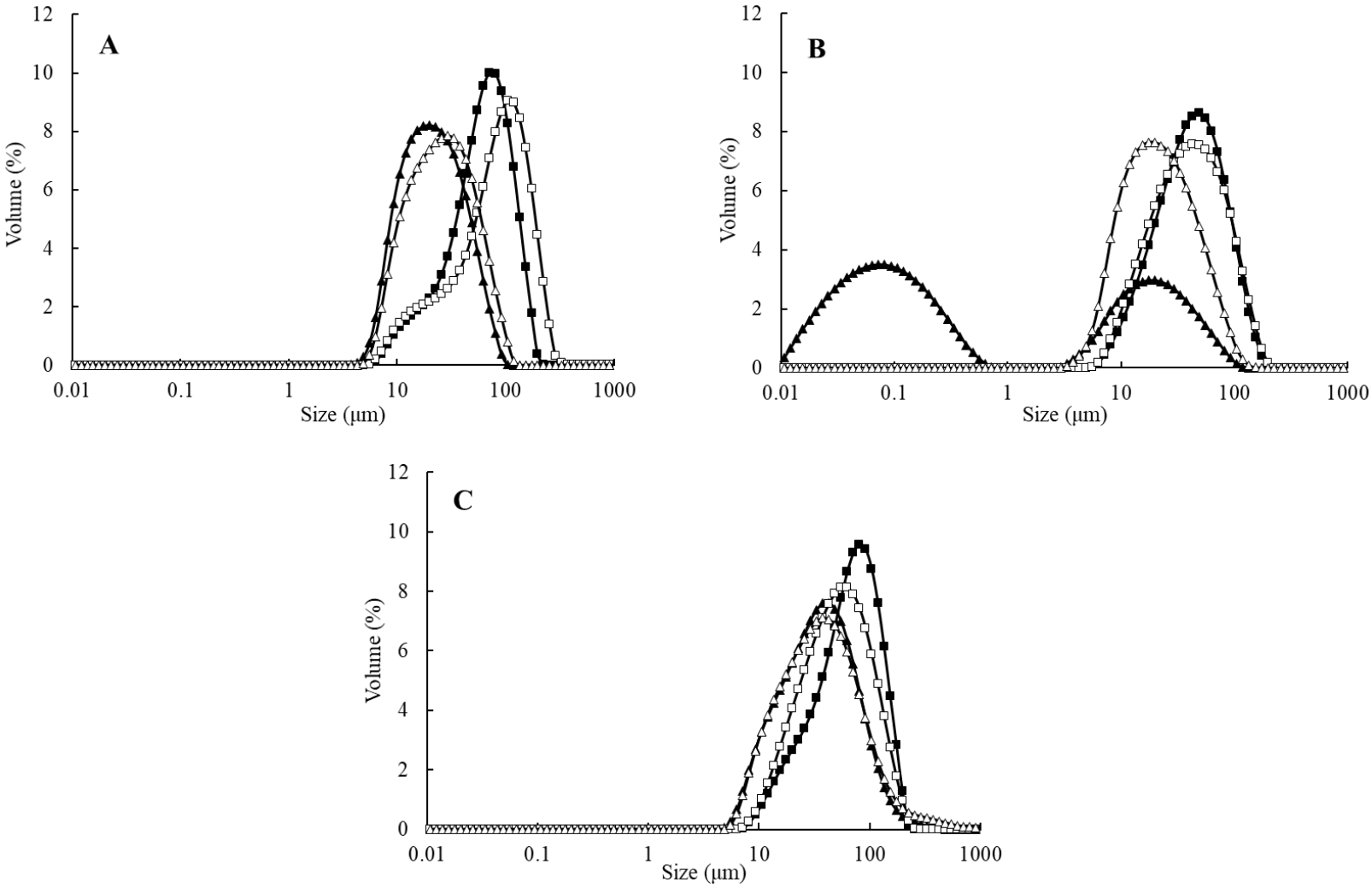

Fig. 7.4. Particle size distribution profiles of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders measured after reconstitution in ultrapure water for (A) 30 s at 23 °C, (B) 30 s at 50 °C and (C) 1 h at 23 °C.

which suggests that these rehydration conditions used are more favourable for agglomerate breakdown. Similar to the particle size distribution results, solubility remained higher for RN-MPC (94%) and AN-MPC (88%), compared to R-MPC

Table 7.4. Mean particle size distribution values of regular (R), regular with N_2 injection (RN), agglomerated (A), and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) dispersions after high-speed (HS) mixing in 23°C and 50 °C ultrapure water for 30 s using a solubility index meter and after low-speed (LS) mixing in 23 °C ultrapure water for 1 h using a magnetic stirrer.

Rehydration conditions	MPC	D_{50} (μm)	D_{90} (μm)	$D_{[4,3]}$ (μm)
HS mixing 23 °C for 30 s	R	$62.7^{b} \pm 1.97$	$123^{b} \pm 2.06$	$68.0^{b} \pm 1.74$
	RN	$20.7^{d} \pm 0.41$	$49.4^{d} \pm 0.98$	$25.5^{d} \pm 0.45$
	A	$83.2^{a} \pm 2.19$	$173^{a} \pm 4.91$	$90.6^{a} \pm 2.45$
	AN	$25.4^{c} \pm 1.12$	$60.9^{c} \pm 2.50$	$31.1^{c} \pm 1.22$
HS mixing 50 °C for 30 s	R	$42.0^{a} \pm 0.26$	$94.3^{b} \pm 2.31$	$49.5^{a} \pm 0.70$
	RN	$0.19^{d} \pm 0.01$	$33.1^{d} \pm 3.39$	$9.96^{c} \pm 0.94$
	A	$39.8^{b} \pm 0.89$	$98.1^{a} \pm 0.81$	$49.2^{a} \pm 0.61$
	AN	$21.6^{c} \pm 0.35$	$57.7^{c} \pm 1.09$	$28.2^{b} \pm 0.56$
LS mixing 23 °C for 1 h	R	$66.5^{a} \pm 2.70$	$134^{a} \pm 3.59$	$73.0^{a} \pm 2.35$
	RN	$35.0^{c} \pm 3.26$	$87.1^{d} \pm 8.12$	$45.3^{c} \pm 5.26$
	A	$49.9^{b} \pm 4.65$	$115^{b} \pm 9.76$	$59.5^{b} \pm 4.76$
	AN	$35.8^{c} \pm 2.13$	$103^{c} \pm 12.0$	$51.4^{c} \pm 6.16$

[a-d] Values within columns not sharing common superscripts differ significantly ($P < 0.05$).

(87%) and A-MPC (51%), when measured after reconstitution in 23 °C water. It is apparent that milling presents an opportunity to increase the density of powders produced using NI. It does not appear to offer a benefit to the subsequent wettability, dispersibility and solubility of the powder particles, but the greater rehydration performance of these powders compared to those manufactured without NI remains apparent. Comparing the original R-MPC (Chapter 6) with the milled RN-MPC in the current study, the $D_{[4,3]}$ values after stirring (1 h in 23 °C water) were 83.7 and 45.3 µm, respectively, but this difference may be accounted for by the smaller powder particle size of the milled MPC. Furthermore, solubility values were lower for RN-, A- and AN-MPC after milling (by 2.2, 12 and 3.6%, respectively), while it was 3% higher for R-MPC. The generation of heat during the milling process may have also contributed to the observed decreases in powder rehydration performance.

Gaiani et al. (2011) reported that dispersibility was higher for micellar casein powder when the powder particle size was 220 µm (39%) compared to 80 µm (19%). Herceg et al. (2005) reported a decrease in solubility of WPC from 85 to 78% after tribomechanical micronisation at 16,000 rpm, despite maintaining the temperature of the system at approximately 20-30 °C and attributed this to the exposure of hydrophobic amino acids. An increase in surface hydrophobicity has been reported by Hayakawa et al. (1993) following jet-mill grinding of casein, and after ball-milling of soy protein isolate powder by Liu et al. (2017). However, hydrophobicity does not always correlate with solubility as Mao et al. (2012) reported that an MPC powder produced with the addition of sodium chloride during diafiltration had both higher solubility and surface hydrophobicity than the control. Sun et al. (2015) investigated the effect of ball-milling for 8 h using a nano mill on the physicochemical properties of WPC, reporting that protein solubility increased slightly from 75.5 to 78.3%, while

surface hydrophobicity also increased. The results from the current study, and the related literature, demonstrate that differences in both particle size reduction methods and equipment (e.g., centrifugal, jet or ball-milling), as well as the composition of the material to be processed, play an important role in subsequent rehydration properties. Further studies involving the aforementioned milling techniques could elucidate if the functionality of high-protein dairy powders could be modified in a targeted manner.

7.4. Conclusion

This study demonstrated that the process of centrifugal milling induced several changes to the physical and rehydration properties of milk protein concentrate (MPC) powders. The bulk, particle and tapped density of all powders were increased due to reduction in powder particle size and the removal of air voids within and between particles, which would favour industrial powder packaging and transport operations. In addition, microscopy analysis showed significant breakdown and fracture of powder particles, which generated an increase in specific surface area of powder particles. The surface composition of MPC powders was altered by milling, whereby the surface fat content of N_2 injection (NI) powders was lower after milling. As a result of the change in powder structure, some of the rehydration properties of the powders were altered. The MPC powders which were produced using NI and subsequently milled did not display the same improvement in dispersibility as initially found, but still presented significantly lower mean particle size distribution values compared to powders manufactured without NI. Overall, the results presented in the study provide new information regarding the influence of milling post-drying on the physicochemical properties of high-protein MPC powders manufactured using NI and agglomeration. Further research investigating the effects of alternative particle size

reduction processes (e.g., jet milling) on dairy powder functionality is required.

7.5. Acknowledgments

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Chapter 8

General discussion

and

suggestions for future research

8.1. General discussion

Milk protein concentrate (MPC) has emerged as an important dairy ingredient powder for global food and beverage manufacturers seeking to enhance the nutritional profile of their products using high-quality proteins. Before these powders can be incorporated into value-added food (e.g., yogurt, follow-on infant formula and protein bars) or beverage (e.g., high-protein drinks for clinical and performance nutrition) formulations, it is essential for producers and end-users to establish the powder's physical, bulk handling and techno-functional properties to optimise processing conditions (e.g., minimise fouling of liquid concentrates, facilitate smooth transport of powder throughout the manufacturing plant, rehydrate powders efficiently) and ensure the final product is of suitable quality.

Powder functionality, such as flowability and solubility, is influenced by powder density, specific surface area, surface composition and particle size, while the chemical composition of the powder is also a factor, as outlined in the study of MPC powders with a range of protein contents (**Chapter 3**). This study provided insights into the changes that occur with increasing the protein content of MPC powders and the challenges and opportunities associated with their use, which may assist dairy processors with predictive processing and control strategies. Increasing protein content above 65% (w/w) had a detrimental impact on bulk powder properties (e.g., lower bulk density and poor flow behaviour). Additionally, these powders showed inadequate wetting behaviour, dispersion and overall solubility. Ultrafiltration (UF) permeate was added to UF retentate from an MPC80 process to standardise composition, and it was shown for the first time that such powders had similar properties to those produced using direct membrane concentration (Crowley et al., 2014, 2015; Kelly et al., 2015). Although MPC powders containing <65% protein have

more suitable physical and functional properties (e.g., higher powder density and solubility), their use is likely to be offset by the requirement for an ingredient with both a higher protein and lower lactose content (i.e., MPC80). Therefore, MPC containing approximately 80-85% protein (w/w) strategically formed the focus of the research in the remainder of the thesis. The subsequent chapters examined the behaviour and functionality of liquid and powdered MPC, as influenced by standard or new processing strategies.

One major processing step during the manufacture of MPC that can affect functionality is heat treatment. Previous studies have only investigated the effect of heat-treating skim milk, prior to membrane filtration, on the solubility of MPC powders, while it is common industrial practice to perform heat treatment of the liquid UF concentrate directly prior to evaporation and spray drying, to alter the functionality of the final powder (e.g., heat stability). Heat treatment (80, 100 and 120 °C for 30 s) of MPC after membrane filtration (i.e., UF and diafiltration) altered the physicochemical properties of the liquid concentrate, primarily an increase in calcium ion concentration, total colour difference and viscosity (**Chapter 4**). Although not the primary focus of this study, the higher viscosity in heat-treated MPC is important to acknowledge, as it may limit the total solids content achievable with evaporation, which will have important implications in relation to energy efficiency during spray drying. Heat treatment at temperatures ≥100 °C for 30 s significantly impaired powder dispersion and solubilisation, likely due to the formation of whey protein and whey-casein aggregates. Although rehydration performance diminished, one benefit which heat treatment presented was an improvement in heat stability at pH 6.9 and 7.0 for the high-heat treated (i.e., 120 °C for 30 s) MPC. Therefore, these findings present

dairy ingredient researchers with a new lever to modify the functionality of MPC powder, especially rehydration properties.

While the studies involving concentrate composition and heat treatment generated important findings for understanding how they can influence the processing and physicochemical properties of MPC, the next objective was to identify a processing strategy to specifically enhance powder rehydration. As discussed in Chapter 2, many strategies have investigated the influence of chemical modifications (e.g., calcium removal, addition of monovalent salts), but few have produced spray-dried MPC powders with different bulk handling, physical and functional properties. Research involving regular and agglomerated MPC powders demonstrated that the injection of nitrogen gas (N_2) into the spray dryer feed line, between the high-pressure pump and atomisers, could substantially alter bulk powder properties (**Chapter 5**) and significantly improve the dispersibility and solubility of these ingredients in water (**Chapter 6**). High-pressure N_2 injection (NI) induced a multitude of changes to the physical properties of regular MPC powders, whereby powder particles were slightly larger, had a more spherical shape with many pores and fractures, a higher specific surface area and lower friability or susceptibility to breakage. Furthermore, surface composition analysis indicated that a higher percentage of fat was present at the surface of the powder particles produced using NI. The bulk handling properties were also altered by NI, with these powders classified as cohesive after flow testing, while also having a lower bulk and particle density and higher compressibility. The presence of N_2 in the atomised droplets likely caused droplet expansion, which led to the formation of larger and more spherical powder particles compared to the smaller, deflated regular MPC particles. It was also apparent that the NI powder had higher porosity, with segments of these particles missing, likely due to the formation of foam

in the concentrate and removal of some N_2 from droplets in the drying chamber. It was expected that powder density would decrease with this process modification as the N_2 would have displaced more of the MPC from the droplets and also increased the air content. Although powder particle size increased slightly, a further significant result of the process was a reduction in powder flowability. The higher specific surface area of these powders suggests that more cohesive, particle-particle interactions could occur and thereby impair flow. Agglomeration by fines return was performed to generate further insights into powder particle structure and functionality. The agglomerated MPC powder particles were larger and had superior flow behaviour compared to the regular MPC powder. However, they were less dense and significantly more susceptible to particle breakage. Interestingly, agglomeration combined with NI produced a cohesive powder, suggesting that some aspect of the NI process negates the benefits provided by agglomeration; this is likely due to the higher surface fat and specific surface area of the initial powder particles, combined with their lower density. Therefore, the physical and bulk handling properties of the powders produced using NI would present challenges industrially in terms of powder transport and packaging, which could not be solved using agglomeration.

In relation to the rehydration performance of these powders produced, high-pressure NI did not significantly change the wetting behaviour of the MPC powders. Although some improvements in both immersional and capillary rise wetting were noted compared to the regular, non-NI powder, this did not occur within a practical timescale (i.e., <5 min) and it is unlikely to correspond to a meaningful difference at industrial scale. However, it was interesting to observe this trend, despite NI appearing to cause a greater migration of fat to the surface of powder particles. The use of lecithin, a common surfactant in the food industry, would have been interesting to

enhance the wetting behaviour of the MPC powders. Images captured during microscopy analysis highlighted the faster diffusion of liquid into powder particles produced using NI, which further supported the results generated from powder wetting experiments. The primary reason for applying the gas injection process was to increase powder porosity and interstitial space, with the goal of accelerating particle fragmentation when added to water. It was evident that the change in powder particle structure significantly enhanced water transfer and dissolution, as shown by the results from particle size and solubility measurements (e.g., mean particle diameters of 13.6 and 83.7 μm for R- and RN-MPC after stirring for 1 h in 23 °C water). This improvement in rehydration performance translated to improved rheological behaviour of these dispersions (i.e., those with the highest solubility had the lowest viscosity). It is apparent that NI directly prior to spray drying can enhance powder rehydration, but if implemented industrially, it would involve several trade-offs in relation to the density and flowability of the final powder. The powders produced using NI were subsequently milled to increase bulk density and determine its effect on other functional properties (**Chapter 7**). While rehydration performance of these powders decreased slightly after milling, the trends observed for powder dispersion and solubilisation remained (i.e., powders produced with NI showed better rehydration performance). Overall, this work constitutes an important development in our understanding of the complex relationship between composition, processing and powder functionality.

8.2. Suggestions for future research

The findings presented in this thesis highlight the complex interplay between processing operations, bulk powder behaviour and rehydration properties.

Recommendations for future studies involving high-protein, micellar casein-dominant dairy powders are as follows:

1. Assessment of the influence of both heat treatment and evaporation on the rehydration properties of micellar casein-dominant powders to elucidate the impact of thermal processing on techno-functional properties. The application of such powders in different food systems (e.g., protein bars and beverages) should be explored and would provide greater insight into how processing parameters can directly affect product quality. For example, studies involving the influence of different levels of whey protein denaturation in MPC powders could provide more clarity on the requirement for denatured whey proteins to create an appropriate gel strength, and how this relates to or influences the need for good powder solubility to prevent issues with yogurt texture such as graininess.
2. Investigation of injecting other inert gases (e.g., argon) or pH-modifying gases (e.g., carbon dioxide) into the high-protein concentrate prior to atomisation and its effects on subsequent powder functionality. Like nitrogen, argon is an inert gas, and therefore would not modify pH and calcium phosphate integrity. It is listed as generally recognised as safe by the FDA (Adhikari et al.. 2018) and has been used to create foamy beverages (Finley, 2014). However, there are no reports of its influence on dairy powder functionality. Carbon dioxide has been utilised during membrane filtration for enhancing subsequent powder rehydration via decreases in calcium content (Marella et al., 2015), but the physical and bulk-handling characteristics have not been reported in detail. Studies investigating different gas injection levels would provide useful information, potentially on the critical point at which rehydration could be

significantly improved without impairing the physical and bulk handling properties of the powder.

3. Investigation of the influence of pneumatic conveying and storage conditions on MPC powders produced using gas injection. This would provide ingredient manufacturers with further insights on powder behaviour during processing and transport to market (e.g., susceptibility of powders to breakage during movement along production line and powder compression during storage, thereby altering particle structure and functionality). This would support the results provided in Chapter 5 regarding powder friability. Furthermore, other approaches for increasing the density of powders produced using N_2 injection could provide useful insights, such as dry blending this powder with regular or agglomerated MPC produced by the conventional process to optimise bulk density.
4. Assessment of novel, low-temperature dehydration technologies such as spray freeze drying for the production of high-protein dairy powders. Freeze-drying alone can improve powder wettability, but it does not directly create a fine powder unless further processing occurs, similar to the microwave drying of MPC in trays discussed in the thesis appendix. However, atomising the feed into liquid nitrogen to create small, frozen particles before the conventional freeze-drying step may be a processing strategy to enhance powder dispersion. Rogers et al. (2008) reported an improvement in skim milk powder rehydration using this processing technology, but it would increase capital and operating costs for processors and may not be practical at a commercial scale.

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Additional Preliminary Experimental Studies

Part A: Vacuum-microwave drying of milk protein concentrate

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A.1. Introduction

Protein-enriched, spray-dried dairy powders (80-90%, w/w, protein) are used in the development of many premium food and beverage products globally. This category of ingredients, derived from membrane filtration of skim milk, includes milk protein concentrate (MPC), milk protein isolate and micellar casein concentrate. They represent a significant source of casein proteins and therefore have several important techno-functional and nutritional properties. However, these powders do not readily disperse and solubilise upon mixing with water, which remains a significant challenge to expressing and optimising their functionality.

Investigating novel dehydration technologies, other than spray and freeze drying, for processing high-protein dairy concentrates is a key area of academic and commercial scientific interest and relevance. A vacuum-microwave dryer, recently developed by EnWave, represents one potential option for developing innovative dairy ingredients and has been utilised for the creation of cheese snacks (Chudy et al. 2018; Anli. 2020). Microwaves use a type of electromagnetic radiation (Venkatesh and Raghavan., 2004) and generate heat by two mechanisms: friction between rotating molecules and ionic conduction whereby ions collide with other molecules (Nijhuis et al., 1998). This drying technique can quickly remove moisture from food and pharmaceutical products while operating at relatively low temperatures. Therefore, it represents a promising unit operation in terms of reducing operating costs and preserving the nutritional qualities of products (e.g., heat-labile nutrients). Therefore, the objective of this study was to produce a dried protein-enriched dairy product using vacuum-microwave drying and investigate its physicochemical properties.

A.2. Materials and methods

MPC powder (82.7%, w/w, protein) were supplied by a local ingredient manufacturer and reconstituted to 15% total solids (w/w; 50 L) using a high-shear mixer (Crepaco), followed by overnight stirring in a jacketed tank at 4 °C to ensure powder solubilisation. Evaporation of MPC to 28% total solids (w/w) was performed using a single effect, falling film vacuum evaporator operating at 65 °C in recirculation mode.

For each microwave drying trial, 1.2 kg of concentrate was poured into four trapezoidal trays (length and width of 68 and 22 cm, respectively) and dried to different total solids contents using a NutraREV® 10 kW pilot-scale, vacuum-microwave (EnWave Corporation, Delta, British Columbia, Canada). The vacuum pressure was set at a high of 23 bar and a low of 20 bar, the drum rotation was 30% and the critical temperature was 65 °C for all samples. The drying process was divided into 4 segments, with the first segment using 85% of the total energy, and the remaining three using 5% each. The power applied was 0.8 kW for segment 1 and 1 kW for segments 2-4. Four samples were produced in total, to give estimated total solids contents of 40, 55, 70 and 95% (w/w). The total solids content was determined by oven drying MPC samples at 102 °C overnight and calculating the percentage change in weight. The viscosity of evaporated MPC was measured using an AR-G2 controlled-stress rheometer (TA Instruments, Crawley, UK), equipped with a parallel plate geometry. Samples were pre-sheared at a shear rate of 100 s^{-1} for 10 s and a temperature ramp of 50 to 5 °C, at a shear rate of 100 s^{-1}, was carried out, with the temperature controlled using a Peltier system (± 0.1 °C). Solubility was determined the day following production by stirring vacuum-microwave dried MPC for 1 h in 50 °C ultrapure water using an overhead stirrer (450 rpm), followed by centrifugation at 3000g for 10 min, as described in Chapter 3.

A.3. Results and discussion

The vacuum-microwave instrument is shown in Fig. 1. Initial trials using this equipment involved identifying what drying conditions were most suitable for MPC.

Fig 1. Enwave pilot-scale vacuum-microwave system.

This mainly involved determining (A) quantity of product per tray (0.3 kg), (B) vacuum settings (0-350 mbar), (C) tray rotation speed (1-100%), (D) power level (e.g., 0.5-2.5 kW) and (E) the drying rate (0.7-1.1 kg/kWh). For these initial trials, MPC was reconstituted (50 °C for 1 h) to 20% total solids (w/w) using a Silverson high-shear mixer and held overnight at 4 °C. Although useful for quickly dispersing powders, this method generated a substantial amount of foam within the reconstituted product, which subsequently, combined with the relatively low total solids content, often led to the product foaming, and expanding over the sides of the trays upon heating. This loss of MPC from the trays prevented accurate determination of moisture removal and drying rates. Alternative trays with a higher wall were obtained to address this challenge but this proposal did not prove successful. Therefore, it was decided

that evaporation would be necessary in future trials to resolve this issue and retain the MPC in the trays.

The apparent viscosity profile of evaporated MPC over the temperature range 5-50 °C is shown in Fig. 2, with viscosity decreasing as temperature increased. After evaporation of MPC to 28% total solids (w/w), it was allowed to cool to room temperature and stored in the fridge (4 °C) for approximately 40 h. On the day of the

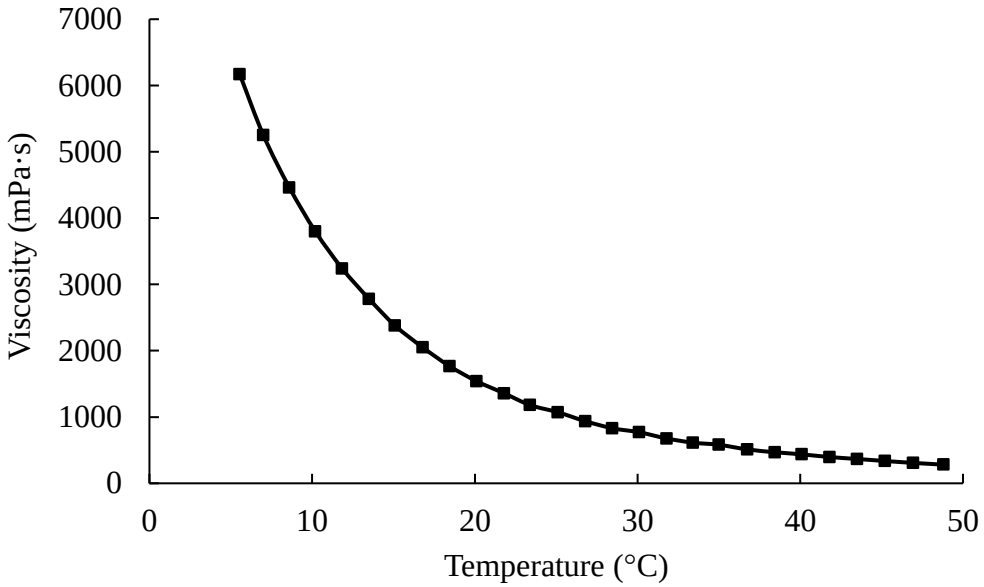

Fig. 2. Apparent viscosity profile (shear rate of 100 s^{-1}) of evaporated MPC (28%, w/w, total solids) over the temperature range 5-50 °C.

trial, the MPC was removed from the fridge and 300 g of this cold-set gel was placed on each tray (Fig. 3). The total solids contents achieved after vacuum-microwave drying for the four samples were 38.4, 54.2, 77.8 and 91% (w/w), with the amount of energy required for water removal shown in Fig. 4. The MPC dried to 77.8% total solids is shown in Fig. 5. The MPC failed to disperse after stirring for 1 h in 50 °C water, and solubility was very poor for all samples (10-20%; results not shown), possibly due to the association of micellar casein and gelation prior to drying.

Fig. 3. MPC (28% total solids; 300 g/tray) on trays prior to microwave-vacuum drying.

Therefore, the results generated from these trials suggest that vacuum-microwave drying would not be suitable for drying micellar casein-dominant concentrates that

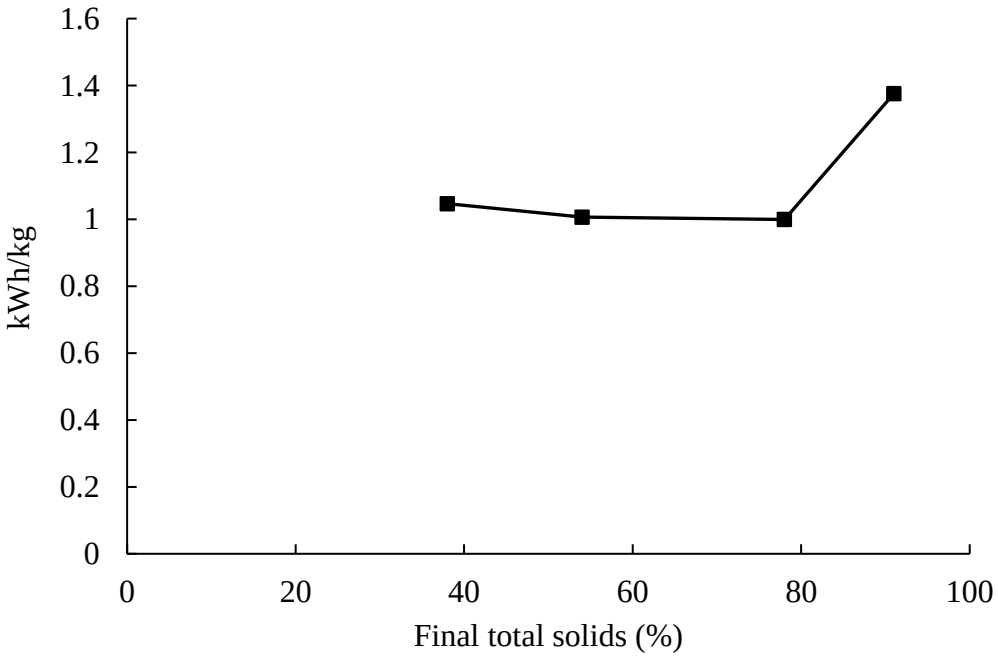

Fig. 4. Final total solids as a function of kilowatt-hour per kilogram of water removed for milk protein concentrate dried using the vacuum-microwave.

require subsequent rehydration. One possible use of MPC with this system could be the creation of high-protein snacks. When the concentrate was stored at 4 °C, it formed

Fig. 5. Vacuum-microwave dried milk protein concentrate at a total solids content of approximately 78% (w/w).

a cold-set gel and this enabled the creation of protein balls, which were subsequently dried using the Enwave (Fig. 6). This may offer a dairy processor with one option for

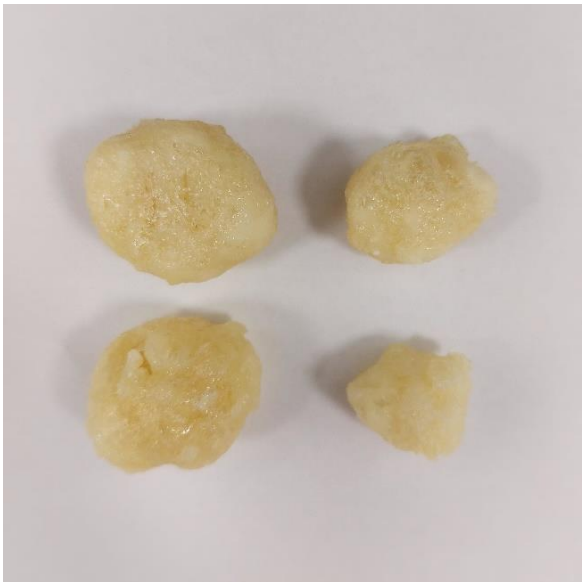

Fig. 6. Milk protein concentrate snack concept.

utilising MPC without the need for rehydration. As the concentrate contained mainly milk protein, the final product had a hard texture and would be difficult to chew. Therefore, significant product development would be required, and other ingredients would need to be added (e.g., fat) to the starting formulation to make the concept more consumer acceptable.

A.4. Conclusion

Vacuum-microwave drying does not appear suitable for the dehydration of liquid milk protein concentrate intended to be rehydrated. However, it may have potential in the development of high-protein snacks.

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Part B: Electrostatic spray drying of milk protein concentrate

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B.1. Introduction

Conventional spray drying of membrane-concentrated proteins has provided the dairy industry with an opportunity to create value-added, compact, shelf-stable ingredients that can be transported to international markets. However, a limitation of this system is the production of protein-enriched powders that exhibit suboptimal rehydration performance (e.g., incomplete dispersion). Processing modifications pre- and post-spray drying, to ameliorate this situation, have been reviewed in Chapter 2; however, few research studies have investigated changes to the dehydration step, specifically the investigation of novel, innovative drying technologies.

Electrostatic spray drying (ESD) is a very new area of research for producing dairy ingredients. This process differs from traditional spray drying as it uses a nozzle that is capable of applying a charge to the feed material. As a result, the solvent (i.e., water), which has a greater electric dipole moment and is more polar, will be brought to the surface of the atomised droplet, while the other components (e.g., proteins) are less polar and will remain at the core. Therefore, for ESD of milk protein concentrate (MPC), it is hypothesised that more of the hydrophobic casein proteins should remain at the centre and allow other milk components (e.g., lactose) to migrate to the outer surface. This system enables water evaporation at lower drying temperatures, preventing shell formation and reducing particle heat exposure. It is proposed that powder rehydration (e.g., wetting behaviour and dispersion of powder particles) would be positively influenced by this drying technique. With the exception of Johnson et al. (1996) who investigated the effect of electrostatic atomisation during spray drying (charge of 0-900 V) on the functional properties of whole milk powder, previous research involving ESD has predominantly explored its ability to encapsulate bioactive food ingredients (Mascaraque and Lopez-Rubio., 2016). The objective of

this research was to determine some properties of high-protein MPC powder produced using ESD.

B.2. Materials and methods

MPC powder (82.7%, w/w, protein) was obtained from a local ingredient manufacturer. MPC was dispersed in water (50 °C; 22%, w/w, total solids) using high-shear (2000 rpm) and stored at 4 °C overnight. Powder manufacture was performed by FluidAir (Nantes, France) using a laboratory-scale spray dryer (PolarDry®; model 001 and serial number 10501) with an electrostatic two-fluid nozzle (SUJ4B). The inlet gas temperature was set at 120 °C, the outlet temperature ranged from 51-55 °C, gas flow was 25 Nm^3/h, the atomising gas temperature was 45 °C and the pump speed was set at 6 rpm. The commercial MPC powder represents the starting material that was reconstituted for the trial. The 8 kV sample represents the powder produced when 8 kV was applied to the nozzle during spray drying, while the <1 kV sample represents a powder produced using the PolarDry system but with the lowest possible charge on the nozzle.

Particle density of the MPC powders was measured using a gas pycnometer, as described in Chapter 3. Powder wettability was visually observed by adding 2 g of MPC powder to a beaker of ultrapure water (150 mL; 23 °C) and photographs were taken after 1 and 5 min (Fig. 1). MPC produced using a standard laboratory-scale spray dryer (European Spray Dry Technologies, UK) was also included in wettability analysis. The particle size distribution of MPC dispersions was measured by static light scattering using a laser-light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens, as described in Chapter 3. The dispersions (4%, total solids, w/w) were prepared by stirring (450 rpm)

MPC powder in 50 °C ultrapure water for 1 h, followed by magnetic stirring (250 rpm) for 21 h at 4 °C. Solubility was also measured for these powders following reconstitution for 1 h, using the method described in Chapter 3.

B.3. Results and discussion

The moisture contents for commercial, <1 kV and 8 kV MPC powders were 3.69, 3.74 and 5.28% (w/w), respectively. The particle density values are shown in Table 1 and was highest for MPC produced with low nozzle charge (i.e., <1 kV), and decreased slightly when the charge of 8 kV was applied. Johnson et al. (1996) reported that a change in charging voltage did not have a significant impact on the particle density of whole milk powders. Bulk density was quite similar for all powders

Table. 1. Physical properties of milk protein concentrate (MPC) powders.

MPC	Particle density (g/cm^3)	Bulk density (g/cm^3)	D_{50} (μm)
Commercial	1.2672 ± 0.0080	0.273	35.8 ± 0.12
<1 kV	1.3069 ± 0.0044	0.266	8.21 ± 0.12
8 kV	1.2702 ± 0.0388	0.266	9.66 ± 0.37

D_{50} is the powder particle size below which 50% of the powder volume is represented by particles smaller than the size indicated.

analysed, with no difference recorded between powders produced using different nozzle charges. The median size of the commercial powder particles was higher than those produced using ESD. The difference in particle size would be expected as the commercial powder was spray dried at an industrial scale using a feed with a higher total solids (~30%). Powder wettability was visually determined by adding a sample

of each MPC powder to beakers of ultrapure water (23 °C; Fig 1). Although powders did not completely wet and sink within a practical timescale (e.g., 10 min), MPC powder produced using the standard laboratory spray dryer and electrostatic spray drying system did not appear to wet and sink into the water to a greater extent than the commercial powder, and the water below the surface became more turbid after 1 and

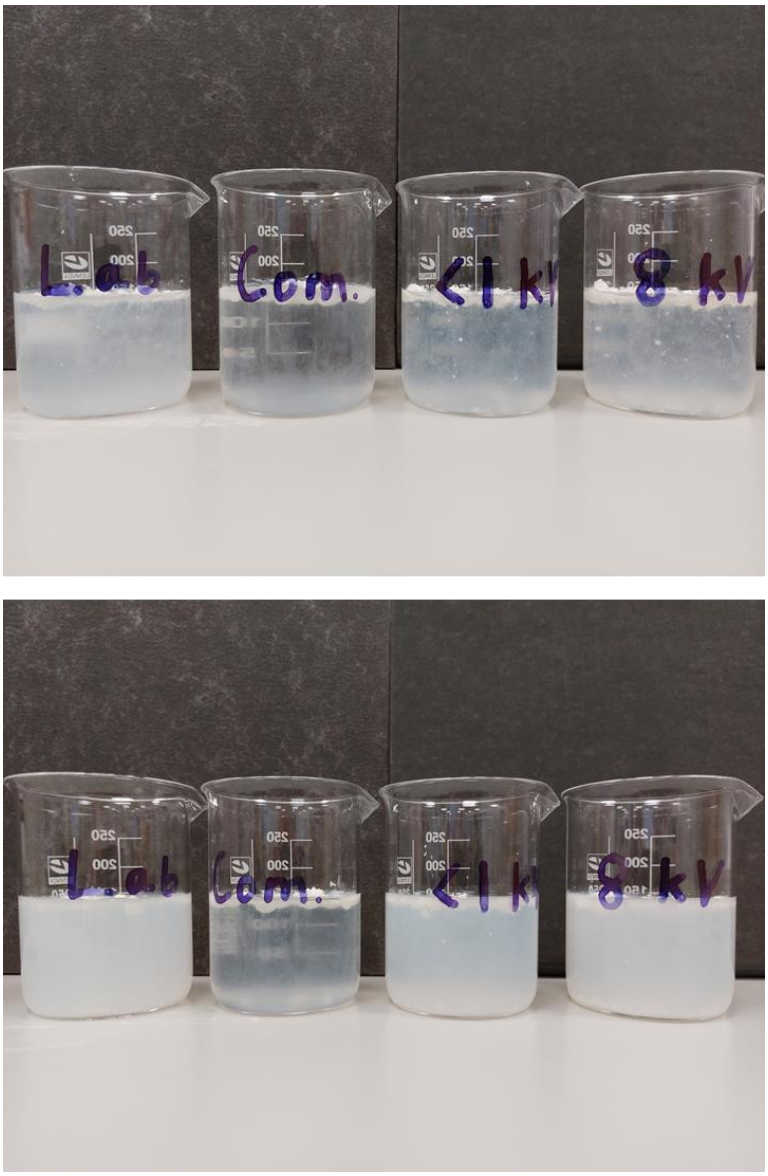

Fig. 1. Milk protein concentrate powders produced by (i) standard laboratory spray dryer, (ii) commercial spray dryer, (iii) electrostatic spray dryer with nozzle charge of <1 kV and (iv) electrostatic spray dryer with nozzle charge of 8 kV; 1 min (top image) and 5 min (bottom image) after addition to 150 mL ultrapure water (23 °C).

5 min. This could be due to differences in the surface composition, particle size or morphology of the powders.

The volume-based particle size distribution profiles, recorded following reconstitution of MPC powders, are shown in Fig. 2. The commercial MPC powder

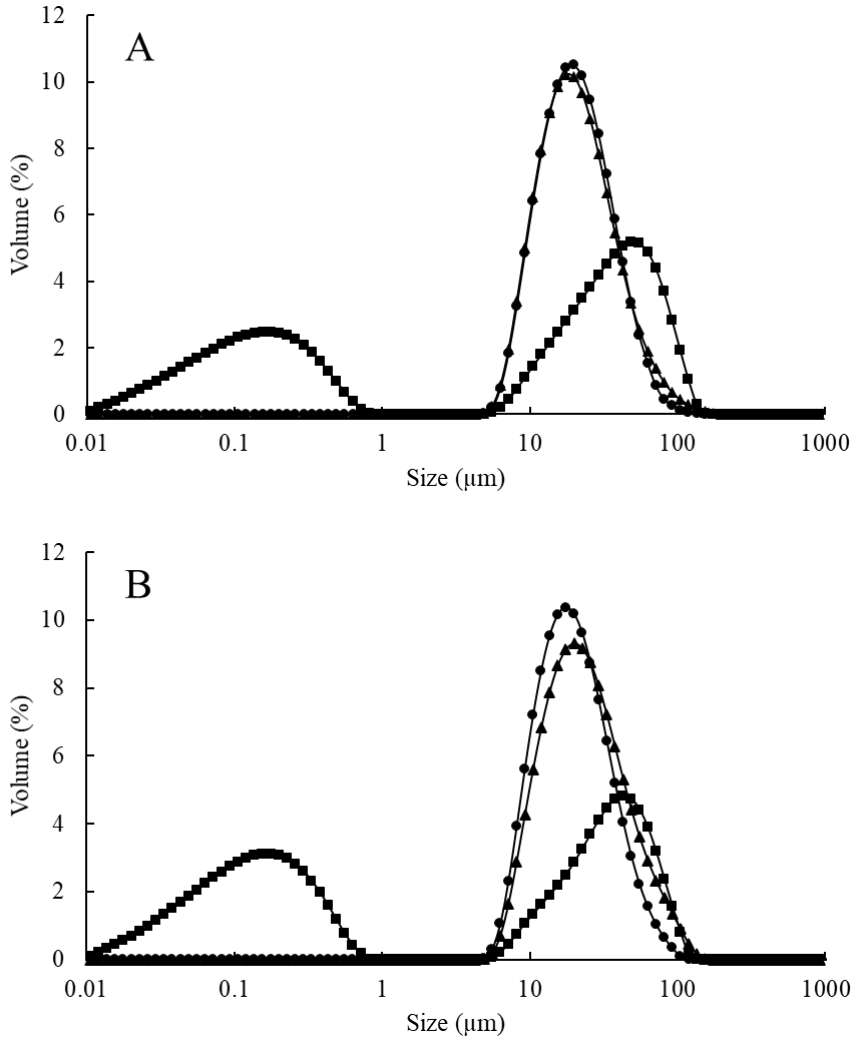

Fig. 2. Particle size distribution of commercial (■), <1 kV (●) and 8 kV (▲) milk protein concentrate powders after reconstitution in (A) ultrapure water (50 °C) for 1 h, followed by (B) stirring for 21 h at 4 °C.

had peaks in both the 0.01-1 µm and 8-300 µm size ranges, while the ESD powders had a larger single peak between 8 and 300 µm (Fig. 2A). This can be observed in the particle size values shown in Table 2, as the D_{10} was in the casein micelle range for

Table 2. Particle size distribution parameters for commercial, <1 kV and 8 kV milk protein concentrate (MPC) dispersions after stirring (450 rpm) in 50 °C ultrapure water for 1 h, followed by magnetic stirring for 21 h at 4 °C, and solubility values after stirring in 50 °C ultrapure water for 1 h.

Rehydration conditions	MPC	D_{10}	D_{50}	D_{90}	$D_{[4,3]}$	Solubility
		μm				%
1 h at 50 °C	Commercial	0.06	17.5	71.4	26.5	98.1
	<1 kV	10.1	19.9	42.1	23.6	90.9
	8 kV	9.99	19.8	45.8	24.8	88.0
21 h at 4 °C	Commercial	0.05	6.85	58.7	20.2	-
	<1 kV	9.61	18.9	41.4	22.9	-
	8 kV	10.4	22.1	54.7	28.2	-

the commercial MPC, but not the other two powders, suggesting that ESD did not improve powder dispersion. These dispersions were stirred for a further 21 h at 4 °C, but no considerable differences in rehydration performance were observed (Fig. 2B). The particle size for the commercial sample did decrease slightly, while it generally did not change for the ESD powders. Solubility was slightly lower for the powders produced by ESD compared to the starting material (Table 2), which does support the trend observed for particle size distribution results. However, the solubility values were high overall (88-98%) despite no particle size peak in the casein micelle range for ESD powder dispersions, possibly due to the small size of the particles which subsequently did not sediment during centrifugation.

B.4. Conclusion

The results in this preliminary study suggest that electrostatic spray drying does not improve the dispersibility of milk protein concentrate. Future research should investigate if a higher nozzle charge (e.g., 12 kV) could have a significant effect on powder properties, the effect of electrostatic spray drying on the properties of other dairy products containing a diverse composition (e.g., infant milk formula), and whether the increased presence of hydrophilic components at the powder particle surface could reduce the storage-induced solubility loss of powders. Furthermore, as this study was performed on a laboratory-scale dryer, research investigating the properties of MPC powder produced by pilot- and industrial-scale electrostatic spray dryers are required.

B.5. References

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Review

Strategies to enhance the rehydration performance of micellar casein-dominant dairy powders

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ABSTRACT

Due to their excellent nutritional (e.g., high calcium) and functional (e.g., heat stability and gelation) properties, the use of protein-enriched, micellar casein-dominant dairy powders, including milk protein concentrate/isolate and micellar casein concentrate, has increased considerably among food and beverage manufacturers. However, the poor and often inconsistent rehydration properties of these powders in water, specifically their low dispersibility and solubility (attributed to protein–protein interactions related to the high proportion of micellar casein), remains a significant challenge. This review provides a detailed analysis of the main physical (e.g., injection of gas, ultrasonication) and chemical (e.g., ion exchange, pH adjustment) processing strategies that have been applied, at both laboratory and pilot-scale, to enhance the rehydration performance of high-protein, micellar casein-dominant dairy powders. The information provided will support the advancement of dairy ingredient research and the technological development of nutritional powders that can be used across several industrial applications.

Contents

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1. Introduction

Milk protein concentrate (MPC), milk protein isolate (MPI), micellar casein concentrate (MCC) and sodium caseinate (NaCas) are some of the many casein-dominant powders currently available from the dairy industry. Two of the largest global producers of casein ingredients are the New Zealand and Irish dairy industries, producing 57,000 and 55,000 tonnes in 2019, respectively (Bord Bia, 2020). MPC and MPI powders are produced by ultrafiltration (UF) and diafiltration (DF) of skim milk, followed by evaporation and spray drying, while microfiltration (MF) is used in the production of MCC, by partially removing whey proteins. The final products normally contain at least 80% protein (w/w) and are extensively depleted in lactose and mineral salts. Applications of such micellar casein-dominant powders include medical nutritional beverages for individuals with disease-related malnutrition, performance nutrition bars for athletes, follow-on infant formulas, as well as cheese, yoghurt and ice cream (Agarwal, Beausire, Patel, & Patel, 2015).

For many applications, rehydration of a powder in water or an aqueous medium is required for complete expression of protein functionality (Fang, Selomulya, Ainsworth, Palmer, & Chen, 2011); therefore, achieving efficient dissolution of high-protein powders is normally essential for ingredient users (Freudig, Hogekamp, & Schubert, 1999). For example, Karam, Gaiani, Hosri, Hussain, and Scher (2016) reported that the rehydration state of micellar casein (MC) powder influenced the textural and rheological properties of acid milk gels, whereby graininess decreased and gel firmness increased as the MC ingredient became more soluble with rehydration time. Furthermore, for the consumer, complete rehydration of powdered ingredients is a key quality indicator.

Rehydration of micellar casein-dominant powder is a complex process influenced by several factors (e.g., powder composition, powder density and structure, solvent composition and temperature) but generally constitutes five stages: (i) wetting, (ii) sinking, (iii) swelling, (iv) dispersion and (v) solubility or dissolution, as described by Crowley, Kelly, Schuck, Jeantet, and O'Mahony (2016). The most commonly reported techniques in the literature to characterise these stages of rehydration include, but are not limited to, wetting behaviour using contact angle (Crowley et al., 2015, 2018), capillary rise and immersional wetting (Ji, Cronin, Fitzpatrick, Fenelon, & Miao, 2015; Selomulya & Fang, 2013); dispersion by particle size analysis (static light scattering) following stirring (Gaiani, Banon, Scher, Schuck, & Hardy, 2005; Jeantet, Schuck, Six, Andre, & Delaplace, 2010) and solubility by determining changes in total solids or protein content of a powder dispersion before and after centrifugation (Bansal, Truong, & Bhandari, 2017; Eshpari, Tong, & Corredig, 2014). However, it is evident that substantial variation exists with respect to the experimental parameters used for many of these techniques (e.g., for solubility determination, there are differences in the concentration of the dispersions, temperature of powder reconstitution and centrifugation conditions), which can make the comparison of results challenging. Furthermore, the authors are aware that in industrial settings, a glass slide is often used as an indicator of rehydration state by submerging it in a reconstituted product to observe the presence of insoluble material or flecks. Although this is a rapid method, it is highly subjective, and further demonstrates the uncertainty and discrepancy in how the rehydration properties of high-protein dairy powders are assessed. Furthermore, off-line techniques such as particle size analysis may not always be available to dairy processors with limited resources.

Previous reviews by Crowley et al. (2016) and Felix da Silva, Ahrné, Ipsen, and Hougaard (2018) have mainly focused on the manufacture, characteristics and stages involved in the rehydration of high-protein dairy powders, as well as advanced analytical techniques (e.g., nuclear magnetic resonance relaxometry) used for monitoring rehydration. However, the objective of this review is to specifically provide an overview of the main processing and formulation strategies that have been investigated to modify the rehydration properties of high-protein, micellar casein-dominant dairy powders.

2. Scientific basis for poor and inconsistent rehydration properties

Research investigating why high-protein, micellar casein-dominant dairy powders express poor rehydration performance, both after spray drying and during storage, has presented several mechanisms responsible for the development of insolubility (Fig. 1). Anema, Pinder, Hunter, and Hemar (2006) suggested that a network of casein micelles at the powder particle surface, formed by non-covalent bonding (e.g., hydrophobic interactions and/or hydrogen bonds), was responsible for the low solubility of MPC, with increasing storage time and temperature accelerating this deterioration in solubility. The low lactose content of MPC also facilitates protein–protein interactions as lactose would provide spatial separation of the casein micelles. This was supported by Havea (2006) who reported that the constituents of the insoluble material in MPC were linked together by non-covalent interactions.

Le, Bhandari, and Deeth (2011) reported a correlation between the development of Maillard reaction products during MPC powder storage and a decrease in solubility. A subsequent study by Le, Holland, Bhandari, Alewood, and Deeth (2013) identified α_{S1}-casein as the predominant component of the insoluble fraction in MPC following storage and reported that methylglyoxal, formed in the advanced stages of the Maillard reaction, was capable of inducing non-disulphide, covalent cross-linking of the proteins. However, Nasser et al. (2018) reported that lactose, expected to be a key reactant in the Maillard reaction, did not play a significant role in the loss of solubility of MPC powder during storage. Indeed, Nasser et al. (2017) established a relationship between loss of α-helix protein structure and a decrease in solubility of MC powder during storage. Mimouni, Deeth, Whittaker, Gidley, and Bhandari (2009) reported that structural collapse and fragmentation of MPC powder particles during rehydration was restricted by the presence of a network of micellar casein at the surface of powder particles. Mimouni, Deeth, Whittaker, Gidley, and Bhandari (2010a) suggested that the loss of solubility of MPC powder during storage was caused by altered rehydration kinetics (i.e., impaired dispersion), due to the persistence of a closely-packed skin of casein micelles at the powder particle surface, while a study by Mimouni, Deeth, Whittaker, Gidley, and Bhandari (2010b) demonstrated that rehydration of MPC was characterised by distinct populations of slow (casein and colloidal mineral) and fast (whey protein and lactose) dissolving components, and that incomplete dispersion was not directly due to the formation of insoluble material during storage or reduced water penetration. Research by Schuck et al. (1998, 2002), has suggested that the high micellar casein content of native phosphocaseinate (NPC) reduces the transfer of water and subsequent rehydration of powder particles. Finally, despite high-protein dairy powders containing a low quantity of fat, this component is often over-represented at the surface of spray dried powder particles and Gaiani et al. (2009) reported that lipids also migrated from the bulk to the surface of NPC powder particles during storage, thereby increasing wetting times.

Several physical and chemical processing strategies have been investigated in an effort to resolve the aforementioned challenges. An overview of these approaches are given in Tables 1 and 2, while a schematic representation of the stages in the manufacturing process where some of these strategies may be implemented is

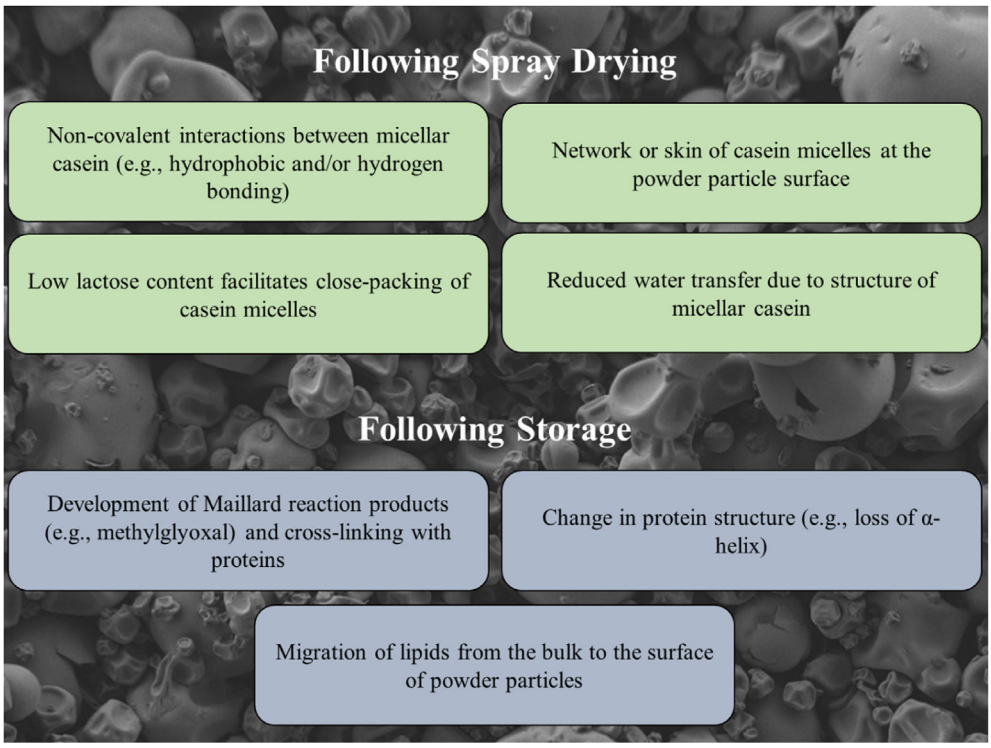

Fig. 1. Summary of research regarding the reasons for impaired rehydration of micellar casein-dominant powders following spray drying and on subsequent storage of spray dried powders.

given in Fig. 2. It is important to consider that many of the approaches discussed are applied for the purpose of creating a spray dried powder with enhanced rehydration properties, while other strategies are examined in the context of aiding powder solubilisation after spray drying.

3. Physical processing strategies to enhance powder rehydration

3.1. Addition of gas to the concentrate before spray drying

The addition of gases to dairy concentrates prior to spray drying has been investigated as an approach for modifying the physical and rehydration properties of powders. Marella, Salunke, Biswas, Kommineni, and Metzger (2015) injected carbon dioxide (CO_2) into skim milk before and throughout UF to modify the subsequent rehydration properties of MPC powder, with an improvement in powder solubility attributed to the solubilisation of calcium phosphate, caused by a reduction in pH due to the formation of carbonic acid (the effect of decreasing concentrate pH on subsequent powder rehydration is further discussed in Section 4.1.). Aside from altering the chemical composition of the powder (i.e., lower calcium content), gas injection has been used to improve rehydration performance by modifying the structure of powder particles. Bell, Hanrahan, and Webb (1963) produced skim milk powder with higher dispersibility by injecting compressed air into the product feed line of the spray dryer, between the high-pressure pump and atomisation nozzle. Recent studies by McSweeney, Maidannyk, O'Mahony, and McCarthy (2021a,b) demonstrated that nitrogen (N_2) gas injection prior to spray drying (i.e., between the high-pressure pump and atomisation nozzles) can improve the rehydration characteristics, particularly the dispersion and solubility, of MPC80 (i.e., 80%, w/w, protein). This improvement in water transfer was attributed to higher powder porosity and interstitial space, combined with lower powder density. Particle size distribution (PSD) analysis showed that the mean D_{90} value (i.e., the size of particles below which 90% of the sample lies), following reconstitution in ultrapure water (50 °C), was significantly lower for MPC powder produced using N_2 injection (0.4 μm) compared with the control (66 μm).

Bouvier, Collado, Gardiner, Scott, and Schuck (2013) used a novel technology called extrusion-porosification to produce MPC powders with a high dispersibility index (96%) compared with a conventionally spray dried MPC powder (38%). This process involved the incorporation of CO_2 into a high-total solids (38%, w/w) concentrate using a twin-screw extrusion-aeration system, followed by spray drying of a high-solids foam; after 2 h of rehydration, only sub-micron sized particles were present in the sample produced using extrusion-porosification, indicating complete dissolution. The formation of numerous pores within the powder particles and the partial dissociation of casein micelles were responsible for the improvements in water transfer and rehydration. It is evident that using gases such as N_2 and CO_2 during dairy processing can enhance the dispersion of dairy protein powders via changes in composition (e.g., reduced calcium content following the incorporation of CO_2 into the liquid concentrate), micellar casein structure and/or powder particle structure, depending on where in the process it is applied. However, an important consideration is the altered physical and bulk handling properties of such ingredients produced using gas injection (McSweeney et al., 2021a); for example, the injection of N_2 gas directly prior to spray drying can lower the particle and bulk density and produce cohesive powders that do not flow easily, thereby potentially presenting challenges in industrial powder handling processes.

Table 1
Overview of literature regarding physical processing strategies to enhance powder rehydration.[a]

Strategy	Powder	Measurement techniques	Results	Reference
Addition of gases				
CO_2 injection during membrane filtration	MPC80	Dispersion: Particle size distribution (PSD)	↑ dispersion	Marella et al. (2015)
		Solubility: Total solids (TS) before & after centrifugation (700×*g*, 10 min)	↑ solubility	
N_2 gas injection before drying	SMP	Solubility: TS before & after filtration (100 and 150 mesh funnel)	↑ dispersion	Bell et al. (1963)
	MPC80	Dispersion: PSD	↑ dispersion	McSweeney et al. (2021b)
		Solubility: TS before & after centrifugation (3000×*g*, 10 min)	↑ solubility	
Extrusion-porosification	MPC80	Dispersion: PSD and dispersibility index	↑ dispersion	Bouvier et al. (2013)
		Solubility: TS before & after centrifugation (160×*g*, 5 min)	↑ solubility	
High-shear treatment				
Microfluidisation before drying	MPC80 & 90	Solubility: Protein content before & after centrifugation (3000×*g*, 10 min)	↑ solubility MPC80	Augustin et al. (2012)
		Insolubility index: Sediment height after centrifugation (160×*g*, 10 min)	↔ solubility MPC90	
Homogenisation before drying	MPC80		↑ solubility	Augustin et al. (2012)
Hydrodynamic cavitation before drying	MPC80	Solubility: TS before & after centrifugation (700×*g*, 10 min)	↔ solubility	Li et al. (2018)
Homogenisation after drying	MPC80	Solubility: TS before & after centrifugation (700×*g*, 10 min)	↑ solubility	Sikand et al. (2012)
	MPC55 & 80	Dispersion: PSD	↑ dispersion	Warncke and Kulozik (2020)
	MPC80 & MC	Dispersion: PSD	↑ dispersion	Chandrapala et al. (2014a)
		Solubility: TS before & after centrifugation (4400 rpm for 5 min)	↑ solubility	
Hydrodynamic cavitation after drying	MPC80	Dispersion: PSD & analytical centrifugation (670×*g*, 3 h)	↑ dispersion	Pathania et al. (2018)
High-pressure processing				
Before drying	MPC85	Solubility: Protein content before & after centrifugation (3000×*g*, 10 min)	↑ solubility	Udabage et al. (2012)
Ultrasonication				
Before drying	MPC80		↑ solubility	Augustin et al. (2012)
	MPC80 & CaCas	Dispersion: PSD	↑ MPC solubility	Chandrapala et al. (2014b)
		Solubility: TS before & after centrifugation (2125×*g*, 5 min)	↔ CaCas solubility	
	MPC80	Dispersion: PSD	↑ dispersion	Yanjun et al. (2014)
		Solubility: TS before & after centrifugation (4400×*g*, 10 min)	↑ solubility	
After drying	MPC80 & MC		↑ dispersion ↑ solubility	Chandrapala et al. (2014a)
	MPC80	Dispersion: PSD	↑ dispersion	McCarthy et al. (2014)

Table 1 (*continued*)

Strategy	Powder	Measurement techniques	Results	Reference
		Solubility: TS before & after centrifugation (700×*g*, 10 min)	↑ solubility	
Membrane filtration				
Cold (4 °C) microfiltration	MCC75	Wettability: Contact angle	↔ wettability	Crowley et al. (2018)
		Dispersion: PSD	↑ dispersion	
Microfiltration and acidification	MCC85	Insolubility index: Sediment height after centrifugation (700×*g*, 10 min)	↑ solubility	Schäfer et al. (2021)
Feed concentration using nanofiltration	MPC60	Insolubility index: Sediment height after centrifugation (900×*g*, 5 min)	↑ solubility	Cao et al. (2015, 2016)
Agglomeration and granulation				
Fluidised bed granulation with binders (lactose, sucrose or water)	MPI	Wettability: Washburn method	↑ wettability	Ji et al. (2015)
		Dispersion: PSD	↔ dispersion	
Addition of lecithin or tween 80 during fluidised bed granulation	MPI	Wettability: Wetting time & contact angle Dispersion: PSD Solubility: Analytical centrifugation	↑ wettability ↔ dispersion & solubility	Wu et al. (2020)
Agglomeration using fines return during co-drying	NPC & WPI	Turbidity sensor	↑ wettability ↓ rehydration time	Gaiani et al. (2007)
Agglomeration using fines return	MPC80	Wettability: Capillary rise	↑ wettability	McSweeney et al. (2021b)
		Dispersion: PSD	↓ dispersion	
		Solubility: TS content before & after centrifugation (3000×*g*, 10 min)	↓ solubility	
Rehydration conditions				
Influence of temperature, stirring speed & solid concentration	MCI	Dispersion: PSD	↓ rehydration time with ↑ in temperature	Jeantet et al. (2010)
Influence of temperature, agitator & stirring speed	NPC	Dispersion: PSD	↓ rehydration time with ↑ in stirring rate	Richard et al. (2013)

[a] Abbreviations are: MPC, milk protein concentrate; SMP, skim milk powder; MC, micellar casein; CaCas, calcium caseinate; MCC, micellar casein concentrate; MPI, milk protein isolate; NPC, native phosphocaseinate; MCI, micellar casein isolate. The number following the powder abbreviation denotes the approximate protein content (%, w/w).

3.2. High shear: homogenisation, microfluidisation and hydrodynamic cavitation

High-shear treatments, including homogenisation, microfluidisation and hydrodynamic cavitation (HC), have been investigated as processing technologies that could be used to improve powder rehydration, without altering the ingredients chemical composition. Microfluidisation is a form of homogenisation which operates on the principle that the liquid is divided into two or more microstreams which are directed towards each other using a high-pressure pump (McCrae, 1994), whereby a combination of turbulent flow, cavitation and shear reduce droplet size (Maa & Hsu, 1999). Augustin, Sanguansri, Williams, and Andrews (2012) reported the effect of homogenisation or microfluidisation of the liquid concentrate before spray drying on the solubility of high-protein MPC powders after production and subsequent storage at 22 °C for eight months. The solubility of the MPC powder produced following microfluidisation of the concentrate (800 bar) was 89.5% after manufacture and 68.7% after eight months of storage, while in comparison, concentrates homogenised at first- and second-stage pressures of 350 and 100 bar had solubility values of 74.5 and 58.7% after production and eight months of storage, respectively. The solubility of the control powder (i.e., no treatment) was 70.1 and 51.1% at these respective time points, but statistical significance was not provided. In a separate investigation within this study, microfluidisation was applied at three different pressures (400, 800 and 1200 bar) to liquid MPC before spray drying and it was reported that solubility of the MPC powders was not significantly different from the non-microfluidised powders after manufacture and 2 months of storage, suggesting its use before spray drying may not be worthwhile. Another study involving high-shear treatment of dairy concentrate, performed by Li, Woo, Patel, Metzger, and Selomulya (2018), investigated the use of HC prior to spray drying and reported that concentrate viscosity decreased but powder solubility was not noticeably changed by the HC process. This technology involves the generation and collapse of bubbles due to changes in pressure, with the accompanying release of energy, causing a powerful mixing effect, which reduces particle size (Gogate, 2011).

An alternative option of using high-shear to enhance powder solubilisation after a standard spray drying process has also been reported by Sikand, Tong, Vink, and Walker (2012), whereby powder reconstitution in 37 °C water, followed by homogenisation (138 bar), improved the solubility of MPC powder. The mean solubility index, which represented the quantity of sedimented material present following centrifugation, was significantly lower

Table 2
Overview of literature regarding chemical modification and formulation strategies to enhance powder rehydration.[a]

Strategy	Powder	Measurement techniques	Results	Reference
Adjustment of pH before, during or after membrane filtration				
Acidification (pH 6.7, 6.0, 5.7, 5.4)	MPC85	Dispersion: Particle size distribution (PSD)	↑ dispersion	Liu et al. (2019)
		Solubility: Total solids (TS) before & after centrifugation (700×*g*, 10 min)	↑ solubility	
Acidification (pH 6.7, 6.3, 5.9, 5.5)	MPC55	Solubility: Protein content before & after centrifugation (12,000×*g*, 25 min)	↑ solubility (pH restoration)	Luo et al. (2016)
Acidification (pH 6.6, 6)	MPC65 & 80	Dispersion: PSD	↔ dispersion	Eshpari et al. (2014)
		Solubility: TS before & after centrifugation (700×*g*, 10 min)	↑ solubility	
Alkalinisation (pH 6.9, 7.3, 7.6)	MCC75	Wettability: Contact angle	↓ wettability	Panthi et al. (2021)
		Dispersion: PSD	↑ dispersion	
Ion exchange				
Before drying	MPI	Solubility: TS before & after centrifugation (700×*g*, 10 min)	↑ solubility	Bhaskar et al. (2001)
Addition of calcium-binding agents				
Sodium phosphate (SP), trisodium citrate (TSC) or sodium pyrophosphate before membrane filtration	MPC80	Dispersion: PSD	↑ dispersion	Sun et al. (2017)
		Solubility: TS before & after centrifugation (4400×*g*, 10 min)	↑ solubility	
SP or TSC via co-drying, bi-drying & dry-mixing	NPC	Insolubility index	↑ solubility	Schuck et al. (2002)
		Nuclear magnetic resonance (NMR)	↓ rehydration time	
Citrate before drying	MC85	Dispersion: PSD	↑ solubility	Schokker et al. (2011)
		Solubility: TS before & after centrifugation (750×*g*, 15 min)		
SP, TSC or sodium hexametaphosphate after drying	MPC80	Dispersion: PSD	↑ dispersion	McCarthy et al. (2017)
		Solubility: TS before and after centrifugation (3000×*g*, 10 min)	↑ solubility ↓ turbidity	
Addition of monovalent or divalent salts				
KCl or NaCl during diafiltration	MPC80	Solubility: Protein content before & after centrifugation (20,000×*g*, 30 min)	↑ solubility	Sikand et al. (2013)
NaCl during diafiltration	MPC80	Solubility: TS before & after centrifugation (700×*g*, 10 min)	↑ solubility	Mao et al. (2012)
NaCl or $CaCl_2$ before drying	NPC		↑ solubility (NaCl)	Schuck et al. (2002)
NaCl or $CaCl_2$ before drying	MC85		↑ solubility (NaCl)	Schokker et al. (2011)
NaCl before dying	NPC	Insolubility index	↑ solubility	Davenel et al. (2002)
		Rehydration time: NMR		
NaCl or $CaCl_2$ after drying	NMC	Rehydration time: Turbidity sensor	↓ rehydration time	Hussain et al. (2011)
Enzymatic or chemical modifications of protein				
Crosslinking using transglutaminase before drying	MPC80	Wettability: Washburn method	↑ wettability	Power et al. (2020)
		Diffusion: Confocal laser scanning microscopy	↑ diffusion	
Chymotrypsin, trypsin and papain after drying	MPC80	Solubility: Protein content before & after centrifugation (10,000×*g*, 10 min)	↑ solubility (pH 4.6–7)	Banach et al. (2013)
Flavourzyme™, Neutrase™ and Protamex™ after drying	MPI	Solubility: Protein content before &	↑ solubility (pH 6.5)	Ryan et al. (2018)

Table 2 (*continued*)

Strategy	Powder	Measurement techniques	Results	Reference
		after centrifugation (3000×*g*, 10 min)		
Succinylation after drying	MPC85	Dispersion: PSD Solubility: Protein content before & after centrifugation (1200×*g*, 20 min)	↑ dispersion ↑ solubility	Shilpashree et al. (2015)
Addition of dairy proteins				
NaCas before diafiltration, before drying or dry-blending with MC	MC85		↑ solubility	Schokker et al. (2011)
NaCas via wet- or dry-blending	MPI	Dispersion: PSD	↑ dispersion	Bot et al. (2020)
Whey protein before drying	NPC	Rehydration time: NMR	↓ rehydration time	Davenel et al. (2002)
Whey protein-rich peptide hydrolysate before drying	MPC80	Protein solubility assay	↑ solubility	Torres-Hernandez et al. (2018)
Addition of molecular spacers				
Addition of lecithin nanovesicles before drying using microfluidisation	MPC80	Solubility: TS before & after centrifugation (1000×*g*, 10 min)	↑ solubility	Bansal et al. (2017)

[a] Abbreviations are: MPC, milk protein concentrate; MPI, milk protein isolate; NPC, native phosphocaseinate; NMC, native micellar casein; MC, micellar casein; MCC, micellar casein concentrate; NaCas, sodium caseinate. The number following the powder abbreviation denotes the approximate protein content (%, w/w).

(1.02 mL) when homogenisation was applied compared with non-homogenised MPC (1.79 mL). Similarly, Warncke and Kulozik (2020) investigated the effect of high-pressure homogenisation (HPH; 100–500 bar) on the solubility of reconstituted (45 min at 50 °C) MPC55, MPC80 and MC powders. MPC55 already had a monomodal PSD in the casein micelle size range (i.e., 150–200 nm) after stirring and further treatment using HPH did not alter solubility. However, for MPC85, a monomodal PSD in this range was obtained after HPH at 200 bar, while a pressure of 500 bar was required to dissolve the MC powder. Furthermore, HC has also been investigated as a physical processing strategy for accelerating rehydration of spray dried powders. Pathania, Ho, Hogan, McCarthy, and Tobin (2018) demonstrated that HC was more effective in rapidly rehydrating MPC powders in comparison with conventional high-shear treatment. The volume-weighted mean particle diameter ($D_{[4,3]}$) value was significantly lower for the HC dispersion (0.19 μm) compared with the sample prepared using conventional high-shear mixing (5.62 μm).

It has been suggested by Augustin et al. (2012) that when high-shear treatments are applied to the concentrate prior to spray drying, these technologies may decrease viscosity and/or alter protein structure, thereby improving solubility of the subsequent powder. However, the exact mechanism by which this occurs has not been elucidated and some studies have found no beneficial effect on powder solubility using this specific approach (Li et al., 2018). Alternatively, when these physical processing strategies are used to reconstitute spray dried powders, enhancement of solubility is generally attributed to energy input, which accelerates the breakdown of large powder particles and disrupts protein–protein interactions; however, their use may incur high capital and operating costs. Overall, these technologies do not address the challenge encountered by ingredient manufacturers in creating high quality,

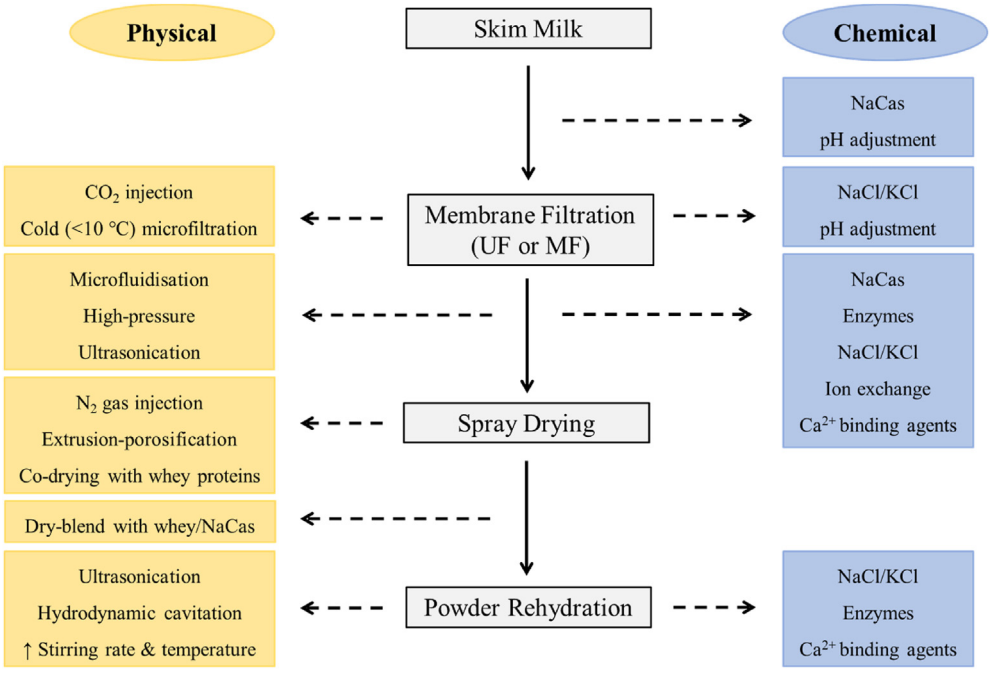

Fig. 2. Schematic representation of the stages during processing where physical and chemical modifications may be implemented to alter powder rehydration properties.

soluble powders for customers but would be useful for end-users who need to quickly reconstitute spray dried dairy powders for use in various applications.

3.3. High-pressure processing

The use of high-pressure (HP) treatment in dairy processing has been reviewed by Huppertz, Fox, de Kruif, and Kelly (2006) and Huppertz, Kelly, and Fox (2002), with some of the reported effects including whey protein denaturation and a change in casein micelle size, and the magnitude of these effects dependent on factors such as pressure and temperature. The potential use of HP treatment to enhance the rehydration characteristics of high-protein, micellar casein-dominant powders has been investigated by Udabage, Puvanenthiran, Yoo, Versteeg, and Augustin (2012). A range of pressures (100–400 MPa) and temperatures (10–60 °C) were applied to liquid MPC and the subsequent solubility of the MPC powder investigated after spray drying. The most significant improvement in solubility of the MPC was obtained when a pressure and temperature of 200 MPa and 40 °C, respectively, were applied to the concentrate, with the powder solubility value after this treatment being 85% compared with 66% for the MPC which received no HP treatment at 40 °C, and this was attributed to the partial dissociation of casein micelles to their non-micellar form. The authors also found that a high-protein powder produced by dry blending NaCas and whey protein isolate (WPI) had higher solubility than MPC, showing that micellar casein hinders the reconstitution process of these powders. Furthermore, it is important to note that MPC powders could not be produced when the concentrates were subjected to 200 MPa at 10 and 25 °C, or 400 MPa at 25, 40 and 60 °C, due to gelation caused by whey protein denaturation and dissociation of casein micelles. Cadesky, Walkling-Ribeiro, Kriner, Karwe, and Moraru (2017) also reported that HP processing (150–450 MPa) altered the physicochemical properties of liquid MPC and MCC, prepared at 2.5 and 10% protein (w/v). Dissociation of the casein micelles took place after the concentrates were subjected to a pressure of 150 MPa, while a gel formed after treatment at 450 MPa due to destabilisation and aggregation of casein micelles, with the denaturation of serum proteins also likely contributing. Therefore, gelation of concentrates would be an important factor to consider if HP were to be applied industrially for improving solubility of casein-dominant dairy powders. HP processing may be a useful strategy for partially dissociating casein micelles without altering the composition of the product or requiring the addition of other chemicals or ingredients; however, similar to high-shear treatments such as microfluidisation and HC, it may not be an economically feasible approach in terms of capital and operating costs.

3.4. Ultrasonication

There are two forms of ultrasonication (US) generally used in food processing: (i) low frequency (16–100 kHz), high intensity (10–1000 W cm^{-2}) and (ii) high frequency (100 kHz–1 MHz), low intensity (<1 W cm^{-2}) ultrasound (O'Sullivan, Park, Beevers, Greenwood, & Norton, 2017). Ultrasonic waves of high intensity induce changes to food systems through cavitation, capable of generating large increases in temperature and shear (O'Brien, 2007; O'Donnell, Tiwari, Bourke, Cullen, 2010). Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar (2014b) performed US (frequency of 20 kHz, power of 31 W and amplitude of 50%) on reconstituted (i.e., stirred for 1 h at 22 °C followed by overnight storage at 4 °C) MPC and calcium caseinate (CaCas) dispersions prior to spray drying, and measured solubility initially and after storage (30 and 60 d at 25 °C) at a relative humidity (RH) of 23 and 76%. Powders had similar solubility values after manufacture; however, following 30 d of storage at 23% RH, US-MPC samples displayed higher solubility (~97%) than the MPC control (83%). After 60 d of storage, this trend persisted, with solubility values of ~88 and 63% for US and control MPC powders, respectively; in contrast, US did not alter the solubility of CaCas, remaining at ~90% throughout the study. The higher solubility of MPC powders after storage was attributed to the breakdown of whey protein-casein micelle aggregates during US. It is possible that the dispersions prepared for spray drying were not completely solubilised beforehand given the short reconstitution time, which may have contributed to the presence of large particles in the powder. Similarly, Augustin et al. (2012) performed US (24 kHz, 160 mL min^{-1} at 600 W) on UF retentate prior to spray drying and reported that the solubility of the MPC powder was only slightly improved, with the measured solubility for US and control MPC powders after manufacture being 74.7 and 70.1%, respectively, while after eight months of storage, solubility remained marginally higher (55.1%) for US-MPC compared with the control (51.1%).

It appears that the application of US prior to spray drying does not significantly alter powder solubility initially, but provides some protection against storage-induced loss of solubility. However, Yanjun et al. (2014) also investigated the relationship between the application of US (20 kHz, 12.5 W and 50% amplitude) to UF concentrates before spray drying and the solubility of the MPC powder. Solubility was significantly higher for the MPC which received 5 min of US pre-treatment (88.3%) compared with the control (35.8%). The authors attributed the increase in solubility to a change in protein structure and an increase in the presence of charged groups (e.g., COO^{-}), although this was not specifically measured. Similar to the results involving high-shear treatment of concentrates in Section 3.2., it is apparent that the exact mechanism by which US prior to spray drying could confer enhanced solubility to powders remains unclear.

US has also been investigated for its potential in accelerating powder solubilisation after the spray drying process. Chandrapala, Martin, Kentish, and Ashokkumar (2014a) compared the solubilisation of spray dried MPC and MC powders using US (20 kHz, 31 W, amplitude of 50%), HPH (single stage at 80 or 200 bar) or high-shear rotor-stator mixing (HSRSM; 17,500 rpm). The $D_{[4,3]}$ values for MPC and MC were considerably lower after US for 5 min (1.1 and 0.8 μm, respectively) compared with 5 min of HSRSM (25 and 52 μm, respectively). HPH performed similarly to US in reducing particle size as the $D_{[4,3]}$ was 1.2 and 0.3 μm for MPC and MC, respectively. Each of these three approaches provided an improvement in solubilisation of micellar casein-dominant powders as they accelerated the structural collapse of powder particles and the release of their constituents (e.g., caseins, minerals). McCarthy, Kelly, Maher, and Fenelon (2014) investigated the effect of US (20 kHz, 70.2 W and amplitude of 100%) and overhead stirring (450 rpm) on rehydration of MPC powder. PSD analysis showed that after 10 min of overhead stirring, the D_{90} of the MPC dispersion was 76.6 μm, compared with 0.41 μm after US for 1 min. Furthermore, the solubility of MPC dispersions after 10 min of overhead stirring in water at 25 and 50 °C was 45.8 and 89.7%, respectively, while solubility was 99.6% following US for 1 min. Similar to high-shear treatments described previously, US appears to be a useful technology in facilitating the rehydration of spray dried, high-protein dairy powders, but it could also present several challenges with implementation at an industrial scale. For example, the installation of an US system would involve significant capital costs, be difficult to incorporate into a continuous industrial process, it generally provides a localised effect and the probe could erode over time and contaminate the product with metal fragments.

3.5. Membrane filtration: micro-, ultra- and nanofiltration

As membrane filtration is the technological enabler in the production of high-protein products, it seems logical that interventions offering potential to improve subsequent powder solubility would be considered at this stage in the process, with several recent studies reporting the impact of membrane filtration unit operations and processing conditions on the physicochemical properties of the derived streams and subsequent spray dried powders. Crowley et al. (2018) produced MCC powders using MF and DF of skim milk at both cold (<10 °C) and warm (50 °C) temperatures, followed by spray drying. No differences were recorded between powders in their wetting behaviour or contact angle, as measured using optical tensiometry. PSD analysis demonstrated that MCC powders produced using cold MF had higher dispersibility than powders produced using warm MF; for example, after rehydration in water (50 °C) for 90 min, 48% of the particles had diameters <1 μm for MCC powders produced using cold MF, compared with 7.5% for powders produced using warm MF. This suggests that a higher proportion of casein micelles were present in solution (i.e., released from dissolved powder particles) following reconstitution of the cold MF powders. The superior dispersibility of MCC powder produced using cold MF was likely a result of several factors, including lower calcium, lower β-casein and higher whey protein content in such powders. Schäfer, Hinrichs, Kohlus, Huppertz, and Atamer (2021) used membrane filtration and pH adjustment to produce calcium-reduced MCC powders. This was achieved by concentrating the skim milk at pH 6.2 using MF, followed by acidification of the MF retentate to pH 5.6 and performing both MF and DF prior to spray drying. Powders depleted in calcium by approximately 50% had significantly higher solubility compared with the control powder, as they formed 3.1 and 4.7 mL of insoluble material, respectively.

France, Kelly, Crowley, and O'Mahony (2021) recently investigated the impact of temperature (4, 8, 12, 16 and 20 °C) and transmembrane pressure (0.05 or 0.30 bar) on membrane filtration performance and the physicochemical properties of the streams produced from the MF of skim milk. Concentrate viscosity was higher and membrane flux was lower when MF was performed at 4 °C, while protein retention by the membrane increased as the temperature and transmembrane pressure were increased. The effect of temperature (5, 20 and 50 °C) during UF of skim milk, the initial step in MPC manufacture, has been reported by Puri, Singh, and O'Mahony (2020). Similar to the previous study, permeate flux was lower at lower temperature, most likely due to increased viscosity, resulting in membrane fouling and the blockage of pores. The retentates produced at 5 and 20 °C had a significantly lower content of total calcium and phosphorus compared with that produced at 50 °C, suggesting that some colloidal calcium phosphate (CCP) was solubilised at the lower processing temperature. The effect of cold UF on the rehydration properties of MPC powders has not been established in the literature but would likely generate improvements in powder dispersion due to lower total calcium content. The industrial application of cold membrane filtration to manufacture high-protein, micellar casein-dominant powders would possibly be limited by the operating costs to maintain a low processing temperature, higher pressures to pass components of the viscous feed through the membrane and longer operating times to achieve the desired protein content in the retentate.

Cao et al. (2015) compared the use of nanofiltration (NF) or evaporation (EP) for concentration of UF retentate before spray drying on the physicochemical properties of MPC powders. The insolubility index (ISI) was significantly lower for NF-MPC (0.32 mL) compared with EP-MPC (0.90 mL), while the free sulfydryl group content of NF-MPC powder was significantly higher than that of EP-MPC. It is possible that the heat treatment received by the concentrate during EP may have caused the formation of protein aggregates which subsequently sedimented during centrifugation. A follow-up study by Cao et al. (2016) investigated the influence of storage on these powders over 24 weeks at 25, 35 and 45 °C. NF-MPC had better solubility compared with EP-MPC after storage; for example, after 24 weeks at 25 °C, the ISI was approximately 2.4 and 4.8 mL for NF- and EP-MPC, respectively. It is apparent that membrane filtration conditions and concentration processes applied prior to spray drying play a crucial role in manipulating the rehydration properties of micellar casein-dominant powders.

3.6. Agglomeration during spray drying and fluidised bed granulation

Agglomeration is generally used to improve the physical (e.g., flowability) and rehydration (e.g., wettability) characteristics of low-protein dairy powders such as whole milk and fat-filled powders (Písecký, 2012), but has recently been investigated as a strategy to modify the functionality of high-protein powders. Gaiani, Schuck, Scher, Desobry, and Banon (2007) spray dried WPI, NPC and NPC plus WPI concentrates, and produced agglomerated and non-agglomerated variants of the powders to investigate the influence of protein type and agglomeration on powder rehydration, with agglomeration performed by returning fine particles to the top of the drying chamber and bringing them into contact with the atomised feed. The wetting behaviour of agglomerated, casein-dominant powders was improved compared with the non-agglomerated powders, but dissolution was impaired. McSweeney et al. (2021b) produced agglomerated MPC powders using fines return during spray drying and reported greater capillary rise wetting and water diffusion, but impaired dispersion and solubility, for the agglomerated powders compared with non-agglomerated MPC.

When agglomeration is performed in a fluidised bed towards the end of the spray drying process, the term granulation is often used to describe this process of joining powder particles together using binding agents. Ji et al. (2015) granulated MPI powders in a fluidised bed system using water or binders (i.e., sucrose or lactose solutions). Wettability was higher for MPI agglomerated using lactose, while it was lowest for the non-agglomerated MPI. The quantity of water absorbed increased with increasing powder particle size for all samples. However, PSD analysis demonstrated that granulation and the use of hydrophilic binders did not result in any improvement in the dispersion and solubilisation of the MPI powders. Wu, Fitzpatrick, Cronin, Maidannyk, and Miao (2020) sprayed surfactants (Tween 80 and lecithin) onto MPI powder during granulation in a fluidised bed and reported that wetting times were lower for Tween 80 and lecithin coated powders in comparison with the MPI powder with no added surfactant (e.g., 15−50 s for MPI coated with Tween 80 compared with 36 min for the MPI control), most likely due to reduced surface tension on inclusion of surfactant. However, dispersion and solubility were not significantly improved by the use of these surfactants. Therefore, agglomeration during spray drying and the use of surfactants or binders in fluidised bed granulation can improve the instant properties of micellar casein-dominant powders but are generally ineffective in improving the key subsequent stages of rehydration (i.e., dispersion and dissolution).

3.7. Rehydration conditions

The selection of appropriate rehydration conditions (e.g., solvent temperature, total solids content, stirring rate, impeller

design) can play an important role in optimising the dissolution of casein-dominant powders and thereby increase process efficiency for manufacturers. Jeantet et al. (2010) investigated the effect of temperature (26–30 °C), total solids concentration (4.8–12%, w/w) and stirring rate (400–1000 rpm) on the rehydration characteristics of MC powder. Temperature played a significant role in the process as it was shown that a 4 °C increase in temperature had the same effect on rehydration kinetics as doubling the stirring rate from 400 to 800 rpm. Increasing the concentration of solids significantly increased the stirring rate required but did not affect rehydration time to the same extent as temperature. Therefore, it was suggested that temperature is a crucial parameter to consider when rehydrating casein-dominant dairy powders. Richard et al. (2013) monitored how temperature (25 and 30 °C), stirring speed (500–900 rpm) and agitator design (six-pitched-blade impeller or two impellers with right angled arrangement) influenced the rehydration behaviour of granulated and non-granulated NPC, WPI, NPC plus WPI and NPC plus lactose powders. Increasing stirring speed from 700 to 900 rpm reduced rehydration time by 25% on average; however, similar to previous work by Jeantet et al. (2010), rehydration was more sensitive to changes in temperature than stirring rate. Granulated powders required longer rehydration times, particularly for NPC powders, e.g., 380 min for granulated NPC compared with 220 min for non-granulated NPC at 900 rpm. The choice of impeller design impacted the rehydration of NPC powder in particular; the 6-pitched blade design resulted in greater particle breakdown due to greater energy dissipation, while the dual propeller design instead created more particle circulation. It is evident that higher temperatures and stirring rates are advantageous in accelerating the rehydration of micellar casein-dominant powders but would result in greater energy consumption.

4. Chemical modification and formulation strategies to enhance powder rehydration

4.1. Adjustment of pH before, during or after membrane filtration

Several studies have investigated the effect of reducing the pH of skim milk during membrane filtration and the subsequent solubility of the MPC powders produced. Liu et al. (2019) acidified skim milk (pH 6.7, 6.0, 5.7 and 5.4) using glucono-delta-lactone (GDL) before membrane filtration, followed by pH restoration of the retentate directly prior to spray drying. The amount of total calcium present in the reconstituted MPC powder was lowest for the sample pre-acidified to pH 5.4, which can be attributed to the passage of serum calcium through the membrane into the permeate following solubilisation of CCP. PSD analysis showed a decrease in particle size of MPC dispersions with decreasing pH from 6.7 to 5.4. Solubility values for the MPC dispersions increased with decreasing pH of pre-acidification and were slightly higher when the retentate pH was re-adjusted prior to spray drying compared with samples which were acidified only. The pH 6.7 sample had an initial solubility of 89% but this was just 19% after 84 d of storage at 40 °C; however, the pH 5.7 sample prepared from pH restored retentate had a solubility of 97 and 91% at these time points, respectively. Importantly, this demonstrates that storage-induced solubility loss can also be reduced when skim milk is acidified prior to membrane filtration and spray drying. Luo, Vasiljevic, and Ramchandran (2016) acidified skim milk (pH 6.7, 6.3, 5.9 or 5.5) prior to UF and freeze drying. Lowering the pH of the skim milk feed from 6.7 to 5.5 before membrane filtration resulted in a significant decrease in solubility of the reconstituted MPC powders from 77 to 32%. However, upon restoration of the MPC dispersion to pH 6.7, this trend was reversed, e.g., ~90 and 73% solubility for pH 5.5 and 6.7 samples, respectively. In addition to the effects on powder solubility, lowering the pH of the feed to 5.5 significantly reduced membrane flux as pores became blocked, and the factors contributing to this included changes in casein micelle size, solubilisation of salts from the micelle and increased viscosity. Eshpari et al. (2014) acidified skim milk to pH 6 using GDL prior to UF alone or UF combined with DF, and reported that acidification caused a significant decrease in the calcium content of MPC from 1.84 to 1.59 g 100 g^{-1} powder. Solubility was higher for the MPC which was acidified using GDL (~82%) before UF and DF compared with the control which received no GDL treatment (~72%). However, the PSD profiles following reconstitution of control and acidified MPC powders were similar, with monomodal peaks in the size range 10–300 μm. Thus, some disparities are apparent in the rehydration data available from experiments involving pH adjustment before membrane filtration and further work is required to ascertain the effects on both powder dispersibility and solubility. Alternatively, Panthi et al. (2021) increased the pH of MF retentates (pH 6.9 to pH 7.3 and 7.6) prior to freeze drying and reported that MCC powders had lower wettability but higher dispersibility with increasing retentate pH. The powder derived from the retentate that was re-adjusted from pH 7.6 to pH 6.9 had the highest dispersibility and this was attributed to changes in the ionic environment of the serum phase (e.g., higher calcium concentration resulting from partial solubilisation of CCP). This supports the positive effect of pH re-adjustment on powder rehydration performance that was reported in previous studies by Liu et al. (2019) and Luo et al. (2016).

The pH adjustment of dairy concentrate enables the mineral profile of the powder to be altered via a reduction in the CCP content, and this appears to enhance solubility of resultant powders. However, casein-dominant powders with reduced levels of micellar casein and calcium phosphate may not be suitable for applications such as cheese manufacture. Lucey and Fox (1993) discussed the significant role played by calcium and phosphate in the production of several cheeses, including their impact on rennet coagulation and gel strength, while Lin, Kelly, O'Mahony, and Guinee (2017) reported that an increased presence of non-micellar casein, generated by the addition of NaCas to skim milk, can adversely affect rennet gelation as it impairs the formation of a gel network. Another consequence of concentrate acidification to consider is that the permeate generated from such a process will contain higher levels of calcium and phosphorus, which may present challenges in down-stream processing (e.g., higher levels of demineralisation may be required).

4.2. Use of ion exchange and calcium-binding agents

Reducing the calcium content of micellar casein-dominant dairy concentrates before spray drying has proven to be an effective approach for increasing solubility of resultant powders. Bhaskar, Singh, and Blazey (2001) described a process for producing a calcium-depleted MPI with improved solubility in water (20 °C); briefly, the retentate from UF of skim milk was acidified from pH 6.8 to 5.9 using citric acid and removal of calcium was performed using a strong cation exchange resin in the sodium form. After 1, 6, 15, 22 and 36 d of storage at 20 °C, the calcium-depleted powders (33, 50 and 83% calcium depletion) all showed 100% solubility. In comparison, control MPI powders had 70–80% solubility after storage for 1–6 d, and this was reduced to 50% after 15, 22 and 36 d.

In addition to ion exchange resins, calcium-binding agents have been used to reduce calcium contents and modify the functional properties of casein-dominant powders. Sun et al. (2017) added trisodium citrate (TSC), sodium pyrophosphate (SPP) and sodium phosphate (SP) to skim milk (0.3% of total solids) before membrane filtration. Calcium content was reduced significantly by the

addition of each calcium-binding agent. After stirring for 30 min, the median particle size was 40 μm for the control MPC, compared with 25, 20 and 25 μm for powders spray dried containing TSC, SP and SPP, respectively, while the solubility was 40, 67, 59 and 51% for control, TSC, SP and SPP powders, respectively. The sample with the highest solubility (83%) at that time point was one which contained a mixture of TSC and SPP (50:50). Schuck et al. (2002) produced NPC powders with added TSC or SP using three different manufacturing approaches: (i) co-drying (CD): calcium-binding agents added to NPC before spray drying, (ii) bi-drying (BD): mineral salt solution and NPC suspension spray dried together, and (iii) dry-mixing (DM): powders physically blended together after spray drying. NPC manufactured without additional calcium-binding agents had an ISI of 14.4 mL compared with <0.2 mL when SP (12 g 100 g^{-1} solids) and TSC (30 g 100 g^{-1} solids) were added before spray drying. Insolubility values were similar when SP and TSC were added using BD (1.8 and < 0.2 mL, respectively) but higher when SP and TSC were added via DM (13.9 and 7.5 mL, respectively). This suggests that the addition of calcium-binding agents should be performed prior to spray drying. TSC was more effective than SP at increasing solubilisation, as measured using a nuclear magnetic resonance (NMR) relaxometry technique; however, it is important to note this powder had lower protein content as greater amounts of this mineral salt were added. Similarly, Schokker et al. (2011) added citrate to the concentrate before drying and produced an MC powder with solubility of 79.5%.

Calcium-binding agents have also been used to promote powder dissolution after spray drying. McCarthy et al. (2017) added sodium hexametaphosphate (SHMP), SP or TSC (0–150 mEq L^{-1}) to MPC solutions prepared from reconstituted powder. PSD analysis showed that TSC and SHMP significantly improved the dispersion of MPC powders, particularly with increasing concentration of SHMP, while SP did not have a significant effect. Powder solubility was lower for the MPC control (89.7%) compared with 96.1 and 99.5% following the addition of 15 mEq/L of TSC and SHMP, respectively, with the changes in solubility attributed to the dissociation of casein micelles. Similarly, Nogueira et al. (2020) investigated the behaviour of demineralised and native casein micelle powders during rehydration, with calcium contents of 2.7 and 2.1 g 100 g^{-1} powder for control and demineralised samples, respectively. Following stirring at 50 °C for 1 h, large particles (>10 μm) were present in both samples and further analysis using electrophoresis demonstrated that non-covalent interactions played an important role in the formation of these aggregates. However, it is not possible to fully elucidate the reason for this as the type of calcium-binding agent used to manufacture the demineralised powder was not given.

Despite the reports of ion exchange and calcium-binding agents generally improving powder rehydration, it would be important to consider the limitations of their use. With the removal of calcium using ion exchange, the composition, technological (e.g., gelation) and nutritional properties of the powder would be altered and this should be carefully considered before their use in specific applications that require this micronutrient (e.g., clinical nutrition beverages). Moreover, the use of calcium-binding agents may alter ingredient listings, which may be undesirable in the food industry considering the increased consumer demand for more “clean label” products (Asioli et al., 2017).

4.3. Addition of monovalent or divalent salts

The incorporation of monovalent or divalent salts such as potassium chloride (KCl), sodium chloride (NaCl) and calcium chloride ($CaCl_2$) into dairy concentrates is a strategy that has been reported to modify powder dissolution. In a study by Sikand, Tong, and Walker (2013), the addition of NaCl or KCl (150 mM) to UF retentate during DF improved the solubility of MPC powder, whereby NaCl and KCl treated MPC powders had 100% solubility compared with 53% when no salt was added. The higher solubility of these MPC powders was likely related to the significantly lower calcium content of the powders with salt added during DF, suggesting that some solubilisation of CCP may have occurred during membrane filtration. Mao, Tong, Gualco, and Vink (2012) added increasing concentrations of NaCl (0–150 mM) to the retentate at the DF step during the manufacture of MPC, with solubility increasing with increasing concentration of NaCl added, e.g., after reconstitution for 30 min, solubility was approximately 95% with the addition of 150 mM NaCl, compared with only 33% for 0 mM NaCl. The number of exposed hydrophobic regions on the MPC proteins increased significantly, while average particle size and disulphide bond formation decreased significantly, with the addition of 50, 100 and 150 mM NaCl. The change in surface hydrophobicity suggests that NaCl caused a change in protein structure, while the decrease in the number of disulphide bonds could possibly account for the measured improvements in powder rehydration. In the study by Schuck et al. (2002), NPC powders with added NaCl and $CaCl_2$ were also produced. The ISI was 0.9 mL when NaCl was added (12 g 100 g^{-1} solids) by CD compared with 14.6 mL with $CaCl_2$ addition (11 g 100 g^{-1} solids). The positive impact of NaCl addition on NPC rehydration was related to the hygroscopic strength of salt rather than its effect on casein micelle hydration and structure. Schokker et al. (2011) reported that an MC powder which was manufactured by adding NaCl before DF had a solubility of 82.8%. Davenel, Schuck, Mariette, and Brulé (2002) also produced NPC powders containing additional NaCl. The reconstitution time, measured using NMR, and ISI values were 22 min and 14.4 mL for the NPC control, compared with 9.5 min and 9 mL when NaCl was added (12 g 100 g^{-1} solids) prior to spray drying, respectively. Carr, Bhaskar, and Ram (2004) also reported a process whereby NaCl added to UF retentate prior to spray drying was shown to improve powder solubility.

Hussain, Gaiani, Aberkane, and Scher (2011) used NaCl and $CaCl_2$ solutions, ranging in concentration from 0 to 12% (w/v), to reconstitute native micellar casein (NMC) powder and turbidity measurements were used to provide rehydration times for each solution. NMC alone had a rehydration time of 467 min, as indicated by turbidity stabilisation, but this was reduced to 238 and 192 min when the concentration of NaCl and $CaCl_2$ was 6% (1034 mM), respectively. The shorter rehydration time for the sample containing $CaCl_2$ appears to contradict a previous report of this salt not enhancing solubility when added before spray drying (Schuck et al., 2002), possibly due to differences in the stage of addition, concentration and measurement techniques. When salt concentrations of 6% were used, no swelling stage was observed, possibly due to changes in micellar structure, and it has been reported by Famelart, Le Graet, and Raulot (1999) that NaCl induced solubilisation of calcium and phosphorus when added to casein micelle suspensions but the addition of $CaCl_2$ did not cause any applicable modification. Similar to the removal of calcium as mentioned in Section 4.2., the addition of NaCl would negatively affect the nutritional content of the powder, particularly given its influence on cardiovascular health (Aaron and Sanders, 2013). However, KCl appears to be equally as effective for altering powder rehydration when added before spray drying, and may represent a more consumer-friendly and health-conscious alternative for powder end-users.

4.4. Enzymatic or chemical modifications of protein

Enzymes are used to perform several functions in the dairy industry, most notably the role of chymosin in cheese curd formation and proteinases to decrease allergenicity and improve the digestibility of infant formula (Nongonierma and FitzGerald, 2011). Modifying dairy protein structure and functionality using enzymes has also been explored as a strategy to enhance the rehydration of high-protein powders. Power, Fenelon, O'Mahony, and McCarthy (2020) produced MPC powders which were enzymatically cross-linked using transglutaminase (TGase) prior to spray drying to maintain micellar structure and control viscosity, as well as depleted in calcium using SHMP (0–25 mM) to improve rehydration performance of resultant powders. Capillary rise wetting and water sorption values were higher for TGase treated than control powders, which suggests this enzymatic treatment had a positive effect on water absorption. Diffusion was higher for TGase treated powders compared with control powders, which increased with increasing concentration of SHMP. Alternatively, Banach, Lin, and Lamsal (2013) performed enzymatic hydrolysis of reconstituted MPC using three digestive enzymes (chymotrypsin, trypsin and papain) and one cysteine protease (papain). All enzyme treated samples displayed increased protein solubility in the pH range 4.6–7.0 compared with the control powder. Similarly, Ryan, Nongonierma, O'Regan, and FitzGerald (2018) investigated the influence of enzymatic modification on the functional properties of reconstituted MPI powders. The enzymes used were Flavourzyme™, Neutrase™ and Protamex™ and the solubility index of the MPI hydrolysates was measured over the pH range 2–8. At pH 6.5, the MPI control had ~35% solubility; however, after incubation for 180 min, the solubility was 90, 97 and 88% for the MPI samples enzymatically treated with Flavourzyme™, Neutrase™ and Protamex™, respectively. The authors attributed the increase in solubility to the formation of low molecular weight, hydrophilic peptides, while a limitation of protein hydrolysis in this case would be that it changes the product to an extent to which it may no longer retain its original ingredient identification.

Aside from the use of enzymes to alter the chemistry of dairy proteins, Shilpashree, Arora, Chawla, and Tomar (2015) chemically modified the dairy proteins in MPC powder using succinylation, whereby succinyl groups were transferred to the ε-amino group of lysine residues, resulting in a change in amino acid charge from positive to negative. MPC proteins subjected to succinylation (90%) using succinic anhydride had a solubility of ~78% at pH 6 compared with 30% for the control. In addition, the average particle diameter was 200 and 720 nm for modified (i.e., 90% succinylation) and control MPC proteins, respectively. The improvements in solubility were attributed to changes in protein charge and a decrease in protein–protein interactions. Further research on the use of enzymatic or chemical modifications of dairy protein concentrates or powder dispersions, their feasibility and behaviour during pilot or industrial-scale processing (e.g., evaporation and spray drying) and their impact on other techno-functional and sensory properties of powders are required.

4.5. Addition of dairy proteins

The addition of whey or non-micellar casein proteins to high-protein, casein-dominant powders may appear counterintuitive but is based on the concept that lowering the concentration of micellar casein or partially dissociating casein micelles can promote solubilisation without reducing the total protein content of the powder. Schokker et al. (2011) added NaCas to the concentrate at different stages of the process and investigated the subsequent powder rehydration properties initially and after storage. The MC powder produced when NaCas was added before DF (1.5%) had a solubility of 79.0% compared with 69.7% for the control. The solubility was higher when NaCas was added directly before drying compared to when NaCas was dry-blended with the spray dried powder. The improvement in MC reconstitution was attributed to increased levels of non-micellar casein and the two mechanisms proposed to explain this observation were: (1) non-micellar casein could preferentially adsorb at the air–water interface instead of casein micelles during spray drying which would prevent the formation of a network of casein micelles at the surface of the powder, and (2) non-micellar casein may act as a physical spacer molecule and prevent the association of casein micelles with each other. Bot, Crowley, and O'Mahony (2020) compared the addition of NaCas to MPI powder, by wet- or dry-blending, on dispersion and solubility. The MPI control (i.e., no NaCas added) had a solubility of 89.6% but this was 92.3 and 97.5% when NaCas was added (15% of total protein) via the wet- and dry-blending approaches, respectively. The PSD profile for the MPI control and MPI plus NaCas wet-blended samples were similar, with both having a monomodal peak in the size range 6–100 μm. However, the MPI plus NaCas dry-blended powders all had bimodal distributions, with a peak <1 μm and a second peak between 6 and 100 μm. This suggests that dispersibility increased as the proportions of NaCas dry-blended with MPI powder increased.

Davenel et al. (2002) added whey proteins to NPC before freeze drying and measured its rehydration performance using NMR. Freeze dried NPC had a reconstitution time of 32 min but this was 13 min for the sample enriched with whey proteins (i.e., 12% of total solids). Torres-Hernandez, Howell, and Bennett (2018) reported that adding a whey proline-rich peptide hydrolysate (DISSEP), produced from enzymatic hydrolysis of WPI, to reconstituted MPC could improve protein solubility following storage at 4 °C. The addition of dairy proteins provides dairy manufacturers with a practical and convenient approach for improving powder rehydration and may add further value to the incorporated ingredients. Nevertheless, this approach alters the original composition, physical state and often the protein profile of the powder (i.e., lower proportion of micellar casein) and does not resolve the fundamental issue of solubilising a micellar casein-dominant powder.

4.6. Addition of molecular spacers

The introduction of other food ingredients (e.g., soy lecithin) into high-protein concentrates to spatially separate micellar casein and reduce protein–protein interactions has recently been investigated by Bansal et al. (2017). Microfluidisation was applied to soy lecithin dispersions (5%, w/w) to create nanovesicles with an average hydrodynamic diameter of 82 nm. These dispersions were then added (1, 5 and 10% of milk solids, w/w) to the concentrate (11% total solids, w/w) prior to spray drying. The MPC powders containing 5% lecithin had significantly higher solubility at the beginning of the study and after 30 d of storage at 25 °C than the MPC powders containing 0 and 1% lecithin. Furthermore, after 90 d of storage at 25 °C, all powders containing lecithin nanovesicles had significantly higher solubility than the control MPC. However, after 180 d, no significant difference in solubility was observed between samples, while MPC powders containing 5 and 10% lecithin did not differ significantly during the study. Although this presents an interesting approach for modifying powder solubility, it alters the powders chemical composition which may limit its use in certain applications. The concept of adding molecular spacers or fillers such as Sephadex beads (Barden, Osborne, McMahon, & Foegeding, 2015) or glass beads (Thionnet, Havea, Gillies, Lad, & Golding, 2017) to cheese has also been reported, whereby they were used to replace milk fat and investigate the subsequent rheological

properties of low-fat cheese. Futher research is required to evaluate if other molecular spacers (e.g., whey protein nanoparticles) could be used to design innovative dairy product structures and enhance the rehydration properties of micellar casein-dominant powders.

5. Conclusion and perspectives for the future

Improving the rehydration performance of high-protein, micellar casein-dominant dairy powders remains a significant challenge and the selection of suitable processing strategies by manufacturers thereof is influenced by numerous, inter-related factors (e.g., capital and operating costs, bulk powder properties and end-user applications). Furthermore, any chemical or formulation changes made to the existing micellar casein-dominant powders available industrially need to be considered with respect to regulatory compliance and maintenance of established standards of identity, in addition to any potential changes to taste perception and consumer acceptance.

Although not the rate-limiting stage of rehydration, the wettability of these powders can be improved using food-grade surfactants (e.g., lecithin) or agglomeration. Altering dairy concentrate composition and physical state (e.g., dissociation of micellar casein and reduction of calcium content using ion exchange) or injecting gas directly prior to spray drying to influence powder particle structure, appear to be the most effective strategies at enhancing the dispersibility and solubility of micellar casein-dominant dairy powders. However, a strategy that successfully accelerates powder rehydration, without altering the chemical composition or physical properties of these types of powders, has not yet been developed. When the end-user needs to solubilise and rehydrate powders prior to their inclusion in food and beverage products, the use of high-shear or turbulence-inducing equipment (e.g., hydrodynamic cavitation) is essential. Further research is required to advance our knowledge of high-protein, micellar casein-dominant dairy powders, such as exploring additional or alternative drying technologies (e.g., electrostatic spray drying and spray freeze drying), developing universal analytical techniques for characterising the stages of powder rehydration, creating an international system for categorising or grading powder dispersibility and solubility, and establishing a fundamental and comprehensive understanding of insolubility development during dehydration and storage (e.g., the mechanisms and nature of casein micelle interactions).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review paper.

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foods

MDPI

Article

The Influence of Composition and Manufacturing Approach on the Physical and Rehydration Properties of Milk Protein Concentrate Powders

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Abstract: This study investigated the physical and rehydration properties of milk protein concentrate (MPC) powders with five different protein contents (i.e., 38.9, 53.7, 63.6, 74.1, and 84.7%, *w*/*w*) prepared by recombining the ultrafiltration (UF) retentate and UF permeate of skim milk. Powder density and flowability increased, while the powder particle size decreased with decreasing powder protein content. The amount of non-wetting MPC powder decreased with decreasing protein content, demonstrating greater wettability for lower protein powders. At protein contents >65% (*w*/*w*), the dispersibility and solubility of the powders decreased significantly, likely due to the greater hydrophobic interactions between casein proteins and a lower concentration of lactose. Therefore, as the protein content of the MPC powders was decreased, their rehydration properties improved. The results obtained in this study provide novel insights into the relationship between the composition of recombined UF retentate and UF permeate streams on the subsequent powder particle size, density, and rehydration properties, and demonstrate that such powders possess similar properties to those prepared using conventional direct membrane filtration.

Keywords: milk protein concentrate powder; spray drying; rehydration; solubility

1. Introduction

The global demand for milk protein ingredients has increased greatly in recent years due to increased consumer awareness of the health benefits and importance of dietary protein as well as the economic development of countries in Europe and Asia [1]. Milk protein concentrate (MPC) ingredients are produced through the ultrafiltration (UF) of skim milk, followed by diafiltration to remove additional lactose and other low molecular weight material (i.e., to increase the protein content) before water removal through the use of evaporation and spray drying [2–4]. MPC generally contains 40–80% protein [5] and possesses the same ratio of casein to whey as found in skim milk (i.e., ~80:20). The quantity of lactose, minerals, and water in the skim milk decreases as the protein content increases during membrane filtration [6]. The permeate stream generated from this process (i.e., the milk components that pass through the membrane) is collectively referred to as milk permeate.

The applications of MPC include infant milk formula, cheese, yogurt, and products designed for sports and medical nutrition; however, its uses are often limited by its inherent poor solubility [7,8]. This is associated with the presence of insoluble material formed by non-covalent (hydrophobic) protein–protein interactions that occur during the powder manufacturing process and subsequent

storage. Therefore, hydration and dissolution of MPC powders is usually conducted in water at approximately 50 °C [9], whereby the increase in solvent temperature accelerates the release of material from the powder particles into the aqueous phase [10]. In order to ensure complete protein hydration, solutions may need to be cooled to 4 °C in order to reduce hydrophobic interactions between casein micelles and allow full hydration and swelling to occur. Furthermore, other high protein dairy powders such as micellar casein concentrate, which is produced by the microfiltration of defatted milk and consists predominantly of casein proteins, also exhibits poor reconstitution properties [11,12]. Such rehydration challenges are compounded when powders are exposed to unfavourable environmental conditions such as high temperature and high relative humidity [13–16]. The deterioration in solubility over time has been attributed to the presence of cross-linked casein micelles at the surface of the powder particles, which can reduce the transfer of water and thus inhibit dissolution [9,17]. Rehydration of casein-dominant powder is characterised by five stages: (a) wetting, (b) swelling, (c) sinking, (d) dispersion, and (e) dissolution [18]. These steps can be influenced by several factors: (i) pre-treatment of the concentrate (e.g., using high shear) [19], (ii) processing conditions such as spray drying temperatures [20], and (iii) the relative humidity and temperature at which the powder is stored [21]. Furthermore, the powder surface composition (e.g., presence of fat), particle structure (e.g., porosity), and rehydration conditions (e.g., stirring rate and solvent temperature) also play important roles in powder dissolution [22,23].

The standardisation of high protein dairy concentrates through the addition of milk permeate to UF retentate could allow for a precise and efficient approach to manufacture targeted MPC ingredients with a wide range of compositions. Therefore, the aim of this study was to first determine the influence of the protein content of MPC powders, prepared from blends of UF retentate and UF permeate, on the powder density, air content, particle size, flowability, microstructural properties, and subsequent powder rehydration. Second, these results were compared to previous studies from the literature that assessed high protein dairy (mainly MPC) powders produced via conventional direct UF, without the addition of milk permeate, to determine whether or not this novel manufacturing approach would produce powders with the same properties.

2. Materials and Methods

2.1. Manufacture of Milk Protein Concentrate Powders

Milk protein concentrate (MPC) powders were produced in the Bio-functional Food Engineering Facility at Teagasc Food Research Centre (Moorepark, Fermoy, Co. Cork, Ireland) using a similar method as that described by Maidannyk [24]. Liquid MPC (19.5 and 16.6% *w*/*w*, total solids, and protein, respectively; i.e., MPC85) and concentrated milk permeate (24% *w*/*w*, total solids) were obtained from a local dairy supplier directly after ultrafiltration (UF) and reverse osmosis, respectively. Milk permeate was then combined with the UF retentate to dilute the protein content to ~75, 65, 55, and 40% *w*/*w*, protein. The subsequent five (i.e., MPC85, 75, 65, 55, and 40) MPC batches were stored overnight at 4 °C under gentle agitation. MPC batches were then pre-heated to 45 °C and spray dried using a single-stage spray dryer (Anhydro F1 Lab Dryer; Copenhagen, Denmark) equipped with a two-fluid nozzle atomisation system (Type 1/8 JAC 316ss) under counter-flow drying conditions. The atomisation pressure was set at ~2–3 bar. Air inlet and outlet temperatures were maintained at 185 and 85 °C, respectively. After spray drying, powders were stored in polyethylene plastic bags at 4 °C for the duration of the study.

2.2. Compositional Analysis of Milk Protein Concentrate Powders

The free moisture and ash content of the MPC powders was determined using a TGA701 thermogravimetric analyser (LECO Corporation, St Joseph, MI, USA). The protein nitrogen values of the MPC powders were obtained by the Dumas method using a LECO FP628 nitrogen analyser (LECO Corporation, St Joseph, MI, USA); the protein content was determined by multiplying the nitrogen

concentration by a nitrogen-to-milk protein conversion factor of 6.38. The fat content of the MPC powders was analysed using the Rose Gottlieb method [25]. The lactose contents were calculated by difference. All analysis was carried out in triplicate, except for fat determination, which was performed in duplicate.

2.3. Bulk Density, Particle Density, Occluded, and Interstitial Air

The loose and tapped (100 taps) bulk density of the MPC powders were measured as per GEA Niro [26] using a jolting volumeter STAV II (Funke Gerber, Berlin, Germany). Particle density of MPC powders was measured using an AccuPyc II 1340 gas pycnometer (Micromeritics Instrument Corporation, Norcross, GA, USA), according to the air pycnometer method of GEA Niro [27]. The volume of interstitial air and occluded air was calculated as outlined in the GEA Niro method [27].

2.4. Powder Particle Size Distribution

The particle size of the MPC powders was determined using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with an Aero S dry dispersion unit. The refractive index was set at 1.45. The air pressure was set at 2 bar for all samples, and the feed rate was adjusted (from 25–100%), depending on the cohesiveness of the sample. Size measurements were recorded as the median diameter (D_{50}) and cumulative diameters (D_{90} and D_{10}) whereby 50, 90, and 10% of the powder volume is represented by powder particles smaller than the size indicated. The volume weighted mean particle diameter ($D_{[4,3]}$) was also calculated.

2.5. Powder Flowability and Compressibility

A Powder Flow Tester (PFT; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to measure the flowability, bulk density, and compressibility of the MPC powders. Samples were prepared for analysis by filling each into an aluminium trough (volume of 230 cm^3, 15.2 cm internal diameter). A curved blade was then used to bring the powder into the required conformation for flow function testing and a vane lid was attached to the compression plate before testing. Samples were analysed in triplicate.

A flow function (FF) test was carried out to determine the flowability of the MPC powders. This involved applying five normal stresses (1.0, 1.9, 2.9, 3.9, and 4.8 kPa) and three over-consolidation stresses at each normal stress. A FF graph was obtained by plotting major principal consolidating stress (MPCS) as a function of unconfined failure strength (UFS). This corresponds to the strength that develops within a powder when consolidated, which must be overcome to enable powder flow [28]. Flow index (i) values were calculated from the inverse of the slope of the FF curve. Loose bulk density (p_b) and tapped bulk density (p_t) were recorded at minimum and maximum MPCS, respectively. The Hausner ratio was calculated by dividing the tapped or compressed bulk density by the loose bulk density. The compressibility index (Equation (1)) was calculated as the percentage increase from the loose bulk density to tapped bulk density [29]:

$$C = \frac{p_t - p_b}{p_t} \times 100 \quad (1)$$

2.6. Scanning Electron Microscopy

Samples of each MPC powder were attached to double-sided adhesive carbon tabs mounted on scanning electron microscope stubs, and then coated with chromium (K550X, Emitech, Ashford, UK). Scanning electron microscopy images were collected using a Zeiss Supra 40P field emission SEM (Carl Zeiss SMT Ltd., Cambridge, UK) at 2.00 kV. Representative micrographs were taken at 5000× magnification

2.7. Wettability of Milk Protein Concentrate Powders

Wettability was first measured using the method of GEA Niro [30] with a slight modification; 4 g of each sample was added to a beaker of water (25 °C) instead of 10 g. Wettability was also assessed using the method of Fitzpatrick [31] with some modifications; briefly, 10 g of powder was placed onto the surface of 250 mL of water (25 °C) in a 600 mL volume glass beaker. After 20 min, the remaining surface powder was carefully removed using a spatula. This powder was dried in an oven (102 °C) and its original water content was determined. Wettability (%; Equation (2)) was defined as:

$$100 \times \frac{mass\ of\ powder\ disappeared}{mass\ of\ initial\ powder} \quad (2)$$

2.8. Particle Size Distribution of Milk Protein Concentrate Dispersions

The particle size distribution of the MPC dispersions were measured using static light scattering (SLS) with a laser-light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens. Particle and dispersant (i.e., water) refractive indices were set at 1.45 and 1.33, respectively. MPC powders were rehydrated (4% total solids, *w*/*w*) in ultrapure water under two different conditions: (a) high speed mixing for 30 s at 23 °C and (b) high speed mixing for 30 s at 50 °C. High speed mixing (3600 ± 100 rpm) was carried out using a solubility index meter (Labinco-BV, Breda, the Netherlands). Each sample was introduced into ultrapure water re-circulating at 20 °C in the dispersion unit (Hydro MV) at 1750 rpm. Size measurements were recorded as the median diameter (D_{50}) and cumulative diameters (D_{90} and D_{10}), whereby 50, 90, and 10% of the volume was smaller than the size indicated. Size distributions were obtained using polydisperse analysis. Measurements were recorded at a laser obscuration of 3–4% and all particle size measurements were performed in triplicate.

2.9. Powder Solubility

MPC powders were dispersed in ultrapure water (23 °C; 4%, *w*/*w*, total solids) for 30 s using a solubility index meter (Labinco BV, Breda, the Netherlands). Aliquots (30 mL) of these solutions were then centrifuged at 3000× *g* for 10 min (23 °C) and the total solids content of the supernatant was then determined using a moisture analyser (CEM Smart System5™, 3100 Smith Farm Road, Matthews, NC, USA). The solubility of the powders was given by the total solids content of the supernatant expressed as a percentage of the total solids content of the initial dispersion.

2.10. Statistical Analysis

Measurements of the powder physical and rehydration characteristics were performed in triplicate. Analysis of variance (ANOVA; Tukey's HSD) was carried out using the IBM SPSS (version 24, Armonk, NY, USA) statistical analysis package. The level of significance was determined at $p < 0.05$.

3. Results

3.1. Composition of Milk Protein Concentrate Powders

A process flow diagram comparing conventional milk protein concentrate (MPC) production with the novel approach used in this study is displayed in Figure 1, with the composition of the resultant MPC powders shown in Table 1. The recombination of the milk permeate with UF retentate resulted in a progressive decrease in the protein concentration of the MPC powders, with the powder moisture content tending to decrease with decreasing protein content. This was due to the higher viscosity of the feed prior to drying because of the higher protein content [32,33]. A high viscosity feed can result in larger spray droplets being produced during atomisation with reduced surface area available for the removal of moisture. Crowley [34] reported a moisture content of 4.6% (*w*/*w*) for MPC80 powder, compared to 3.4% (*w*/*w*) for MPC35. In the present study, significant ($p < 0.05$) differences in ash

content were measured for the MPC powders, with the values ranging from 6.88% for MPC85 to 7.82% for MPC40 (Table 1). Deeth and Hartanto [35] reported similar ash results of 7.5 and 7.1% (*w*/*w*) for MPC42 and MPC85, respectively. In the present study, there was an increase in ash:protein with decreasing protein content, whereby the ash:protein ratio increased from 0.08 for MPC85 to 0.20 for MPC40 (Table 1). In a similar manner, Crowley [8] reported an ash:protein ratio of 0.23 for MPC35 compared to 0.10 for MPC85.

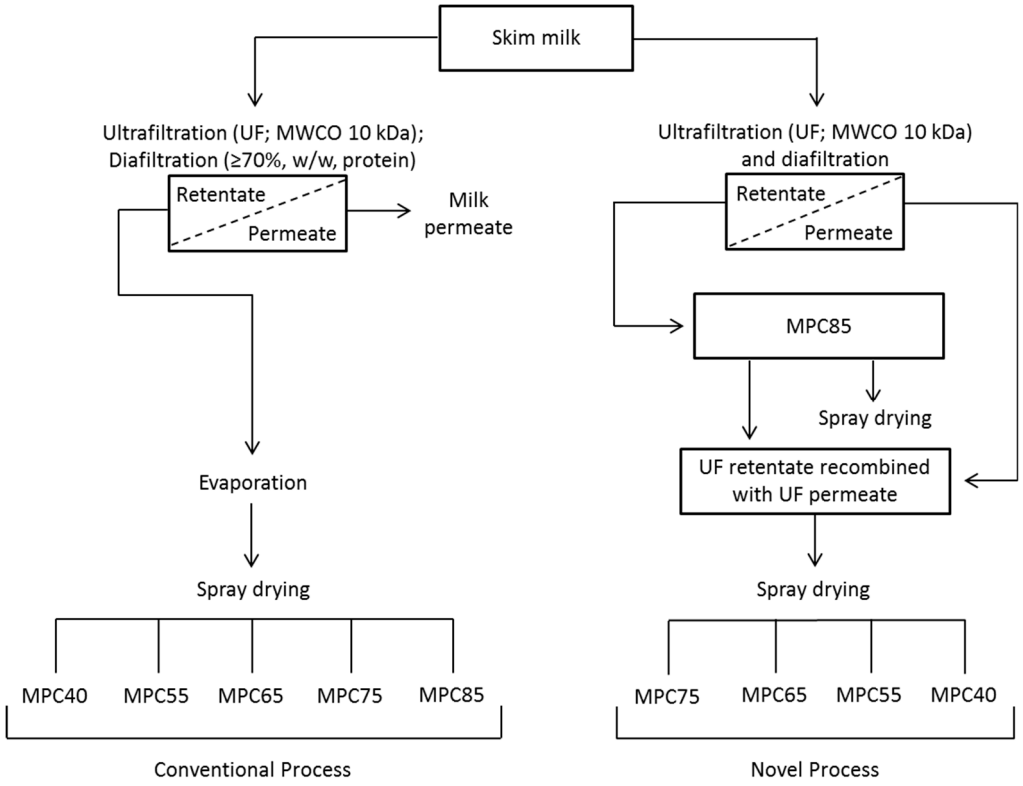

Figure 1. Process flow diagram of conventional and novel approaches for the production of milk protein concentrate (MPC) powders.

Table 1. Composition of milk protein concentrate (MPC) powders.

MPC	Protein	Lactose	Fat	Ash	Moisture	Ash:Protein
			(%, *w*/*w*)			
MPC85	84.7 ± 0.9	1.37	2.07	6.88 ^{a} ± 0.1	6.68 ^{a} ± 0.3	0.08
MPC75	74.1 ± 0.8	12.6	1.59	6.99 ^{b} ± 0.0	5.19 ^{b} ± 0.1	0.09
MPC65	63.6 ± 0.7	22.8	1.34	7.17 ^{c} ± 0.0	5.49 ^{b} ± 0.1	0.11
MPC55	53.7 ± 1.3	33.4	1.17	7.43 ^{d} ± 0.0	5.09 ^{b} ± 0.0	0.14
MPC40	38.9 ± 0.6	48.2	0.87	7.82 ^{e} ± 0.0	4.59 ^{c} ± 0.0	0.20

$^{a–e}$ Values within a column not sharing common superscripts differ significantly ($p < 0.05$).

3.2. *Physical Properties of Milk Protein Concentrate Powders*

3.2.1. Powder Particle Size

Powder particle size distribution analysis displayed a significant decrease in particle size with decreasing protein content (Figure 2); MPC85 had a $D_{[4,3]}$ of 57.3 µm compared to 18.9 µm for MPC40 (Table 2). This is most likely caused by differences in the protein content of the concentrates prior to spray drying (as mentioned in Section 3.1), with high protein concentrates possessing a higher viscosity, thereby generating larger droplets during the atomisation step of spray drying [36]. Rupp [37] reported

that the $D_{[4,3]}$ of the MPC powder increased significantly from 31 to 50 µm with an increase in the protein content of the concentrate from 19 to 23% (*w*/*w*). Crowley [34] reported D_{90} values of 64.6 µm for MPC35 and 51.9 µm for MPC80 spray dried under similar conditions to the present study; however, this difference may be explained by the large differences in the concentrate total solids before spray drying (i.e., 35.5% *w*/*w* for MPC35 and 14.7% *w*/*w* for MPC85).

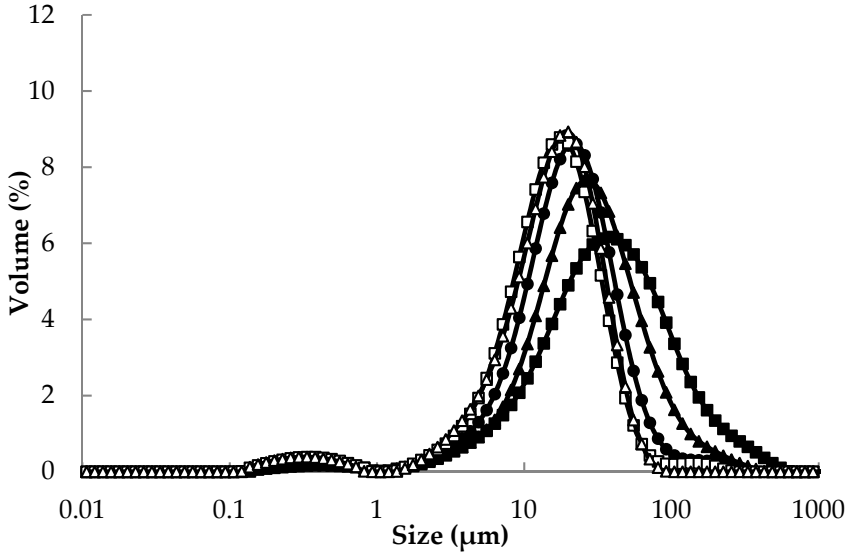

Figure 2. Particle size distribution of milk protein concentrate (MPC) 85 (■), MPC75 (▲), MPC65 (●), MPC55 (□), and MPC40 (Δ) powders.

Table 2. Particle density (p_p), loose bulk density (p_b), tapped bulk density (p_t), volume of interstitial air (V_{ia}), volume of occluded air (V_{oa}), particle size below which 90% of material volume exists (D_{90}), and the volume weighted mean particle diameter ($D_{[4,3]}$) values for milk protein concentrate (MPC) powders.

MPC	p_p	p_b	p_t	V_{ia}	V_{oa}	D_{90}	$D_{[4,3]}$
	(g/cm^3)			mL/100 g		µm	
MPC85	1.00 ^{a} ± 0.0	0.29 ^{a} ± 0.0	0.35 ^{a} ± 0.0	190 ^{a} ± 7.8	32.2 ^{a} ± 0.1	127 ^{a} ± 4.5	57.3 ^{a} ± 2.9
MPC75	1.08 ^{b} ± 0.0	0.32 ^{b} ± 0.0	0.38 ^{b} ± 0.0	173 ^{a} ± 5.6	25.5 ^{b} ± 0.4	76.1 ^{b} ± 1.4	37.5 ^{b} ± 0.7
MPC65	1.14 ^{c} ± 0.0	0.34 ^{c} ± 0.0	0.41 ^{c} ± 0.0	155 ^{b} ± 3.1	20.5 ^{c} ± 0.8	47.4 ^{c} ± 1.0	25.5 ^{c} ± 0.4
MPC55	1.18 ^{d} ± 0.0	0.39 ^{d} ± 0.0	0.44 ^{d} ± 0.0	141 ^{b} ± 10	17.5 ^{d} ± 1.1	36.3 ^{d} ± 0.8	19.9 ^{d} ± 0.6
MPC40	1.14 ^{c} ± 0.0	0.40 ^{d} ± 0.0	0.43 ^{cd} ± 0.0	143 ^{b} ± 0.8	21.1 ^{c} ± 0.7	35.9 ^{d} ± 0.3	18.8 ^{d} ± 0.2

$^{a–d}$ Values within a column not sharing common superscripts differ significantly ($p < 0.05$).

3.2.2. Density

Particle, loose and tapped bulk density values for the MPC powders increased with decreasing protein content (Table 2). For instance, the particle density increased from 1.00 g/cm^3 for MPC85 to 1.18 g/cm^3 for MPC55, while tapped bulk density increased from 0.35 to 0.44 g/cm^3, respectively. This finding is supported by the results of Crowley [34], who reported that particle density increased from 0.84 g/cm^3 for MPC85 to 1.25 g/cm^3 for MPC50, while tapped bulk density increased from 0.29 g/cm^3 for MPC85 to 0.59 g/cm^3 for MPC50. Eshpari [38] reported similar results to the present study with a particle density value of 1.07 g/cm^3 for the MPC80 powder. There was a corresponding increase in both the interstitial and occluded air content of the powders as the density decreased. MPC85 powder had the lowest density (i.e., particle, loose, and tapped) and the highest interstitial (190 mL/100 g) and occluded (32.2 mL/100 g) air content, which may be accounted for by the greater powder particle size of this sample [39]. The increase in particle density with a decrease in the protein content could be accounted for by the concomitant increase in lactose in the powders. Furthermore, the MPC40

in the current study had a loose bulk density value of 0.40 g/cm^3, which is lower than the value of 0.65 g/cm^3 recorded by Fitzpatrick [28] for a commercial skim milk powder. This difference in bulk density may be due to the difference in the total solids content of the concentrate between the MPC40 sample (21.7%) and a typical commercial skim milk concentrate (e.g., 50%).

3.2.3. Flowability

The flow index values obtained were similar for all powders (Table 3). For example, the flow index value for MPC65–85 was approximately 2.1. MPC40 had the highest flow index value of 2.6. However, as these values were all less than 4, the powders were categorised as cohesive according to the Jenike classification system for powder flowability. The poor flowability of the low-protein MPC sample (i.e., MPC40) is possibly related to the use of a two-fluid nozzle during spray drying, or the drying of this concentrate at a relatively lower total solids content than would be used for a typical commercial product with a similar protein content (e.g., skim milk). Crowley [34] reported that the flow index was reduced from 13.4 for MPC35 to 3.5 for MPC85, while Fitzpatrick [28] reported a flow index value of 6.1 for a commercial skim milk powder. The Hausner ratio (HR) values correlated with the flowability results, which demonstrated that high protein powders had poorer flowability than low protein powders. According to Turchiuli [40], a HR greater than 1.4 corresponds to a non-free flowing powder. Furthermore, the compressibility of MPC65-85 was significantly greater than that for both the MPC40 and MPC55 powders. This is most likely caused by the greater interstitial air content of the higher protein powders as these voids between powder particles would have been reduced considerably during compaction, resulting in a greater change in density.

Table 3. Flow and rehydration (wettability and solubility) properties of milk protein concentrate (MPC) powders.

MPC	i	JC	CI (%)	HR	Wettability (%)	Solubility (%)
MPC85	2.1 ± 0.1	Cohesive	41.2 ^{a} ± 1.5	1.71	14.7 ^{a} ± 1.8	83.0 ^{a} ± 2.2
MPC75	2.1 ± 0.0	Cohesive	42.1 ^{a} ± 0.7	1.73	17.5 ^{a} ± 2.0	92.9 ^{b} ± 1.6
MPC65	2.0 ± 0.3	Cohesive	41.9 ^{a} ± 2.6	1.73	49.3 ^{b} ± 1.1	98.0 ^{c} ± 1.3
MPC55	2.2 ± 0.2	Cohesive	35.0 ^{b} ± 1.3	1.55	48.3 ^{b} ± 1.1	98.5 ^{c} ± 1.1
MPC40	2.6 ± 0.2	Cohesive	32.4 ^{b} ± 1.8	1.50	48.3 ^{b} ± 0.9	98.1 ^{c} ± 0.8

$^{a–d}$ Values within a column not sharing common superscripts differ significantly ($p < 0.05$). i = flow index, JC = Jenike classification, CI = compressibility index, HR = Hausner ratio.

3.2.4. Microstructure

Scanning electron microscopy images of each MPC powder are shown in Figure 3. Low protein powders (e.g., MPC40) had a collapsed structure with wrinkled, concaved surfaces. However, for MPC75 and MPC85, the surface morphology changed significantly, with the surfaces of these powder particles appearing smoother and more dimpled. These results are supported by the findings of Kelly [41], who observed similar differences between the microstructures of spray-dried MPC powders (MPC35–90). The distinct differences in the microstructure of low and high protein MPC powders may be caused by several factors. Crowley [34] stated that lower protein MPC powders (i.e., MPC40) contained a lower volume of occluded air in comparison to higher protein MPC (i.e., MPC85), similar to the results of the current study, and likely accounts for the collapsed appearance of the particles. The smooth surface of high protein powders possibly arises from the compaction of casein micelles during the spray drying process [42]. Moreover, Sadek [43] and Tan [44] showed that protein type also plays an important role in powder particle morphology, with casein-dominant powder particles appearing more wrinkled compared to whey protein powders that possessed a spherical shape. Furthermore, spray drying temperatures can also affect particle morphology, with Tan [45] showing that an increase in drying inlet temperature could produce particles with wrinkled surfaces, while lower drying temperatures produced more spherical particles.

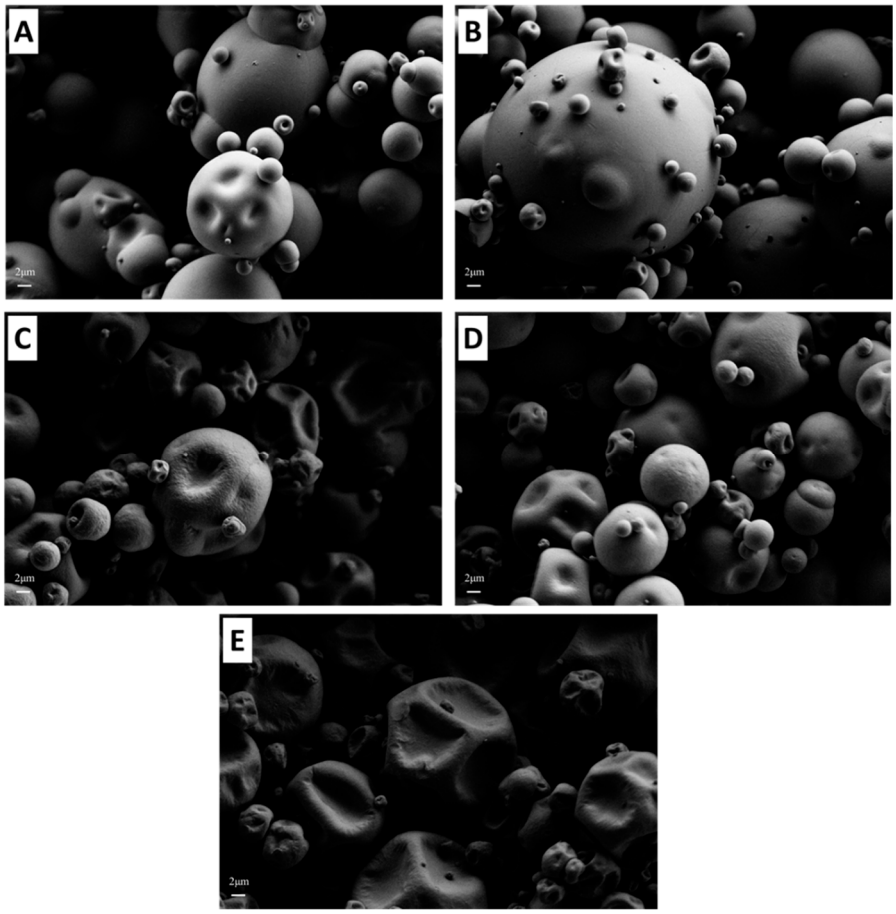

Figure 3. Scanning electron microscopy images of milk protein concentrate (MPC) 85 (**A**), MPC75 (**B**), MPC65 (**C**), MPC55 (**D**), and MPC40 (**E**) powders at 5000× magnification.

3.3. Wettability of Milk Protein Concentrate Powders

Wettability analysis showed that MPC85 and MPC75 had the lowest wettability at 14.7% and 17.5% after 20 min, respectively, compared to approximately 47% for MPC40–65 (Table 3). Poor wetting behaviour of the MPC powders has previously been attributed to the hydrophobic, protein-rich surface of these ingredient powders [8,13]. Despite possessing similar protein content to skim milk powder, the MPC40 in the current study displayed poor wetting behaviour. Fitzpatrick [31] found that a skim milk powder completely wetted after 55 s at 20 °C, likely due to its large D_{50} value (132 μm) and a tapped bulk density of 0.55 g/cm^3. MPC powders did not completely wet and sink within the time period measured; however, a visual difference was observed between samples (results not shown) with a smaller quantity of the low protein powders (i.e., MPC40 and MPC55) remaining on the surface of the water, with the water becoming more turbid, compared to the high protein powders (i.e., MPC75 and MPC85) that remained on the surface of the water and formed a surface film layer. This may also be accounted for by the differences in carbohydrate content between powders, with powders containing ≥22.8% lactose (*w*/*w*) likely being more hydrophilic, resulting in greater water transfer into and between proteins.

3.4. Dissolution and Solubility of Milk Protein Concentrate Powders

The particle size distribution data indicated the presence of large, poorly dispersible particles in high protein MPC powders (Figure 4). This was most apparent for MPC85 and MPC75 when dispersed in water at 23 °C as they exhibited monomodal size distribution in the range 5–100 μm (Figure 4A). Dispersion of powder particles is considered the rate limiting stage in the rehydration of MPC [7], and

this is most likely caused by protein–protein (e.g., hydrophobic) interactions between casein micelles in close proximity and the low concentration of lactose facilitating close packing [17,46]. On the other hand, bimodal distributions were observed for MPC40–65, which suggests the presence of both casein micelles (<1 μm) and primary powder particles (>1 μm).

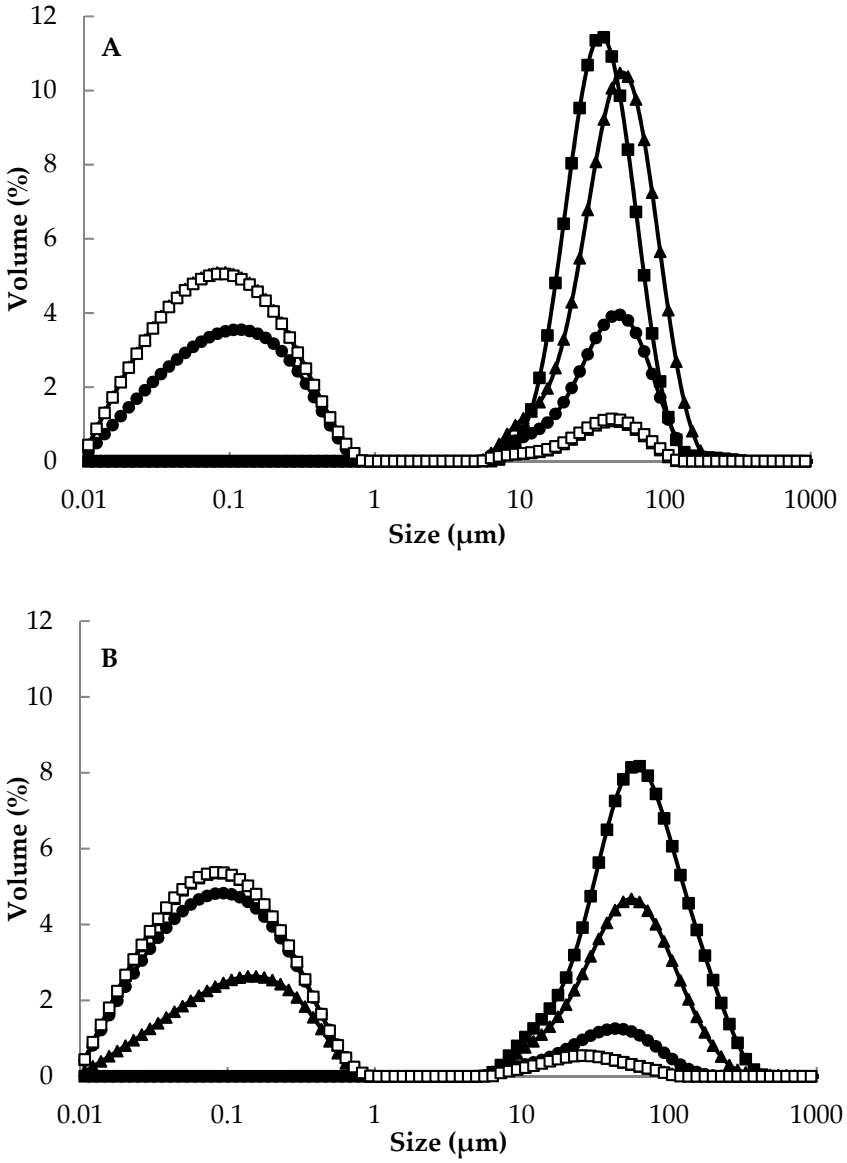

Figure 4. Particle size distribution of milk protein concentrate (MPC) 85 (■), MPC75 (▲), MPC65 (●), MPC55 (□), and MPC40 (Δ) powders after reconstitution in ultrapure water at (**A**) 23 °C and (**B**) 50 °C.

The volume of primary particles generally decreased with the reducing protein content of the powders. MPC55 and MPC40 displayed the highest dispersibility, which corresponded to a small volume of large particles in the range of 5–100 μm, and a larger volume of sub-micron (<1 μm) particles. Additionally, the $D_{[4,3]}$ value generally decreased as the protein content of the powders was reduced, e.g., 51.7 μm for MPC75 compared with 4.25 μm for MPC40 when the samples were reconstituted at 23 °C (Table 4). The target particle size profile for a rehydrated MPC would be a monomodal distribution in the size range of casein micelles, (i.e., <1 μm). It has been reported that a mean particle size of 0.08–0.2 μm represents the presence of casein micelles, providing evidence that the hydration of powder particles has taken place [10,47].

Table 4. Mean particle size of milk protein concentrate (MPC) dispersions after high speed mixing at 23 °C and 50 °C.

MPC	D_{90} (μm)		$D_{[4,3]}$ (μm)	
	23 °C	50 °C	23 °C	50 °C
MPC85	68.9 ^{a} ± 5.4	156 ^{a} ± 11	40.7 ^{a} ± 2.9	76.4 ^{a} ± 4.3
MPC75	92.6 ^{b} ± 4.2	98.2 ^{b} ± 2.2	51.7 ^{b} ± 1.9	36.7 ^{a} ± 3.5
MPC65	59.7 ^{c} ± 2.1	25.6 ^{c} ± 11	18.3 ^{c} ± 1.6	6.68 ^{a} ± 1.9
MPC55	13.1 ^{d} ± 4.6	0.39 ^{d} ± 0.0	4.57 ^{d} ± 0.3	1.98 ^{b} ± 0.2
MPC40	6.30 ^{e} ± 5.8	0.41 ^{d} ± 0.1	4.25 ^{d} ± 0.3	2.06 ^{b} ± 0.4

$^{a–d}$ Values within a column not sharing common superscripts differ significantly ($p < 0.05$). D_{90} = the size of particles below which 90% of the sample lies. $D_{[4,3]}$ = volume weighted mean diameter.

Reconstitution of MPC85 and MPC75 powder in water at 50 °C reduced the volume of primary powder particles, but resulted in the occurrence of some particles with a size >100 μm (Figure 4B). This may be accounted for by powder particle swelling caused by greater water uptake and hydration at 50 °C than at 23 °C; however, even though hydration occurred, it is suggested that complete particle dissociation did not occur as a large volume of particles remained in the 10–500 μm size range. The swelling stage of powder rehydration had previously been observed by Gaiani [12] during the rehydration of micellar casein powder, whereby swelling was recorded as a peak in particle size following powder wetting. The short period of reconstitution (30 s) in 50 °C water appears to have been sufficient to allow wetting of high protein powders to occur, but insufficient to enable complete dispersion of powder particles. Conversely, MPC40–65 powders had lower $D_{[4,3]}$ values when dispersed at 50 °C, compared to at 23 °C, indicating that after water sorption, the powder particles began to dissociate. The solubility was greater for the low protein powders, (i.e., MPC40 and MPC55) in comparison to the higher protein powder (i.e., MPC85; Table 3). The MPC40–65 powders all displayed solubility of approximately 98%, compared with just 83% for MPC85. These results support those recorded during the particle size distribution analysis; high protein MPC powders (75–85%, *w/w*) displayed poor dispersion and solubility properties in water. (Note: Lactose crystallisation, which is an important factor to consider in relation to the solubility of the MPC powders, did not occur in the current study (results not shown). Maidannyk [24] reported that MPC powders, ranging in protein content from 40–80% (*w/w*), did not show lactose crystallisation in their amorphous state following spray drying, but this process did occur for MPC40, 50, and 60 powders stored at high relative humidity).

4. Conclusions

This study provided new information on the physical properties of milk protein concentrate powders prepared through the novel combination of milk permeate and high protein UF retentate to create MPC powders at different protein contents, but with comparable physical and rehydration characteristics to those produced by conventional direct UF concentration and drying. Powder particle size decreased with a decrease in the protein content of the concentrate, most likely due to differences in concentrate viscosity. Decreasing the protein content also brought about an increase in bulk, tapped, and particle density of the MPC powders. The wetting and dispersion of the powders were improved by decreasing the protein and increasing the lactose content of the blends. The rehydration and physical properties of the MPC powders were significantly altered by changes in concentrate composition, but did not appear to be affected by the method of manufacture (i.e., concentrate standardisation with milk permeate compared with direct membrane concentration).

Author Contributions: Designed the experiments, analysed the data, and prepared the manuscript, D.J.M. and N.A.M.; Performed the experiments, analysed the samples, and collated the data, D.J.M.; Analysed samples, V.M.; Performed sample collection and analysis, S.M.; Reviewed the manuscript and provided academic guidance, J.A.O. All authors have read and agreed to the published version of the manuscript.

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Influence of nitrogen gas injection and agglomeration during spray drying on the physical and bulk handling properties of milk protein concentrate powders

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ABSTRACT

This study investigated the influence of injecting nitrogen (N_2) gas under high pressure into milk protein concentrate (80%, w/w, protein; MPC) prior to spray drying and examining the physical and bulk handling properties of regular (non-agglomerated) and agglomerated powders. MPC powders produced using the N_2 injection (NI) process had significantly lower bulk density and flowability, higher wall friction angles and increased levels of interstitial and occluded air. Agglomerated MPC powders had higher flow index values, lower wall friction angles, but were more friable, compared to regular powders. Surface composition analysis of MPC powders showed that NI caused fat to preferentially migrate to the surface in comparison to powders spray dried without NI. The results obtained in this study demonstrate that the injection of N_2 into liquid MPC directly prior to spray drying, as well as agglomeration by fines return, can produce ingredients with unique particle and bulk powder properties.

1. Introduction

The adoption of membrane filtration technology has enabled the dairy industry to produce high-protein, casein-based dairy powders such as milk protein concentrate (MPC). In the preparation of MPC using ultrafiltration and diafiltration of skim milk, caseins and whey proteins are retained, while lactose and minerals pass through the membrane as permeate. This high-protein retentate is then evaporated and spray dried to form a powder (Mistry and Hassan, 1991). MPC ingredients are incorporated into a range of products due to their functional, sensory and nutritional properties, e.g., yogurt, cheese, low lactose beverages and medical nutrition products (Agarwal et al., 2015). However, commercially available high-protein, casein-dominant powders (e.g., MPC and micellar casein concentrate) generally have poor powder rehydration properties. This has mainly been attributed to hydrophobic interactions occurring between micellar casein proteins in close proximity and the low concentration of lactose facilitating close packing (Havea, 2006; Anema et al., 2006). Several approaches have previously been developed to improve the rehydration properties of MPC powders. These include chemical modifications such as the use of calcium chelating agents (McCarthy et al., 2017) and ion-exchange (Bhaskar et al., 2001), and physical high shear treatments such as microfluidisation or homogenisation of concentrates before spray drying (Augustin et al., 2012). However, limited research has been performed regarding the use of gas injection to alter powder particle structure and improve the subsequent rehydration of high-protein dairy ingredients.

Gas injection has been utilised in dairy products to modify the functionality of milk powders, butter and cheese (Bisperink et al., 2004; Adhikari et al., 2018). Hanrahan et al. (1962) investigated the influence of nitrogen (N_2) gas injection into whole milk concentrate before atomisation on the characteristics of the spray dried powder and reported an improvement in dispersibility and an increase in powder particle size. Similarly, Bell et al. (1963) produced a skim milk powder with higher dispersibility via the injection of compressed air into the concentrate between the high-pressure pump and nozzle. More recently, Bouvier et al. (2013) used a novel technology known as extrusion-porosification to create MPC powders with improved dispersibility due to increased particle porosity. This involved mixing carbon dioxide gas with a high-solids concentrate using a twin-screw extrusion-aeration system. The influence of carbonation on the physical and functional properties of whole milk powder has been reported by Kosasih et al. (2016), whereby the addition of CO_2 prior to spray

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drying increased powder porosity, occluded air content and dispersibility. Aside from dairy, confectionary products (e.g., marshmallows, nougat and taffy) are often aerated during manufacture to modify relative density, texture and appearance (Hartel et al., 2018). Modifying powder particle structure may influence the physical attributes of the bulk powder (e.g., density, porosity, friability, particle size and morphology), and these factors can play an important role in bulk handling of powders industrially. After spray drying, powder is usually transferred to storage containers (e.g., bins, silos) and can undergo numerous handling (e.g., pneumatic conveying) and processing (e.g., packaging) steps (Ilari, 2002). To alter the physical and bulk handling properties of powders, the process of agglomeration may be used whereby small powder particles, collected from the cyclone (i.e., fines), can be pneumatically conveyed and returned to the top of the spray dryer main chamber and introduced near the nozzles to combine with atomised milk droplets (Gianfrancesco et al., 2008; Murrieta-Pazos et al., 2012). Agglomeration is used extensively in the dairy industry for whole milk, fat-filled and infant formula powders in which the physical (e.g., flow behaviour, density and porosity) and rehydration (e.g., wetting) characteristics are significantly improved (Palzer, 2007). However, agglomeration is seldom used in high-protein dairy ingredients as it generally increases rehydration times (Crowley et al., 2016; Gaiani et al., 2007). The combined effect of gas injection and agglomeration on the physical properties of high-protein powders has not been previously investigated but may facilitate the manufacture of MPC powders with improved flow behaviour and rehydration performance. Therefore, the objective of this paper was to characterise the physical and bulk handling properties of MPC powders produced using N_2 gas injection prior to spray drying and agglomeration.

2. Materials and methods

2.1. Rehydration of milk protein concentrate powder

Milk protein concentrate (MPC) powder (80%, w/w, protein) was supplied by a local dairy ingredient manufacturer. All subsequent processing was carried out using the pilot-plant facilities at Moorepark Technology Limited (Teagasc, Moorepark, Fermoy, Co Cork, Ireland) To obtain a rehydrated MPC dispersion, reverse osmosis water (1800 kg) was weighed into a 5000 L capacity, jacketed, stainless steel tank, attached to a continuous in-line Crepaco high shear mixer (APV Pulvermixer, SPX Flow Technology, Pasteursvej, Silkeborg, Denmark), configured in a "squirrel cage" design. MPC powder (~500 kg) was inducted directly into the recirculating water stream (50 °C) as it passed through a high shear mixing head. Once dispersed, it was recirculated for 30 min and stored at 5 °C overnight under gentle agitation.

The MPC dispersion (21.2%, w/w, total solids) was passed once through an SPX hydrodynamic cavitator (Model P286184-12 R4; SPX Flow Technology, Pasteursvej, Silkeborg, Denmark) equipped with a proprietary dispersion head (300 mm diameter), consisting of 160 discrete fluid channels, at a rotational speed of 2914 rpm to ensure complete rehydration of the powder. The rotor speed, which determines the extent of cavitation, was driven by a 30 kW motor at a frequency of ~40 Hz. The MPC dispersions were transferred through the cavitator using a centrifugal pump at a feed flow rate of 1287 L/h. The flow rate, and thereby residence time in the cavitation zone, was controlled by a manual back-pressure valve on the system outlet (1.18 bar) with a product change in temperature of 15 °C.

2.2. Nitrogen gas injection, spray drying and agglomeration

Immediately after hydrodynamic cavitation, the MPC dispersion was heated from 18 to 70 °C using a scraped surface heat exchanger and passed through two filters (pore size of 800 μm) before being pumped to the atomisation nozzles using a high-pressure pump (HPP). Nitrogen (N_2) gas was injected (3.5 kg/h) at a pressure of ~190 bar into the feed line, after the HPP and prior to atomisation, using a pressurised injection unit (Carlisle Process Systems, Farum, Denmark). Concentrates were dried using a NIRO Tall Form spray dryer (TFD-0025-N, Soeborg, Denmark), with air inlet and outlet temperatures set at 185 and 75 °C, respectively, for manufacture of regular and agglomerated powders. Air inlet and outlet temperatures for concentrates with N_2 injection were set at 180 and 75 °C, respectively, for both regular and agglomerated variants. First- and second-external fluid bed temperatures were set at 50 and 25 °C, respectively. Agglomeration was performed by returning all fines collected in the cyclone to the top of the spray dryer. For regular powders, all fines were returned to the second external fluid bed. A process flow diagram for the production of powders is provided in Fig. 1. Four MPC powders were produced in total: regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN).

2.3. Compositional analysis

The free moisture and ash content of the MPC powders were determined using a TGA701 thermogravimetric analyser (LECO Corporation, St Joseph, Michigan, USA) at 102 and 550 °C, respectively. The protein nitrogen values were obtained using a LECO FP628 nitrogen analyser (LECO Corporation, St Joseph, Michigan, USA); the protein content was determined using a nitrogen-to-protein conversion factor of 6.38. The fat content was determined using the Rose Gottlieb method (ISO, 2008). The lactose content was calculated by difference. All analyses were carried out in triplicate, except for fat, which was conducted in duplicate. The mean protein, lactose, fat, and ash content of the MPC powder was 80.5, 5.10, 1.54, and 7.49% (w/w), respectively.

2.4. Scanning electron microscopy

Samples of each MPC powder were attached to double-sided adhesive carbon tabs mounted on scanning electron microscope (SEM) stubs, and then coated with chromium (K550X, Emitech, Ashford, UK). Images were collected using a Zeiss Supra 40P field emission SEM (Carl Zeiss SMT Ltd., Cambridge, UK) at 2.00 kV. Representative micrographs were taken at 1000 × magnification.

2.5. Surface composition

Surface composition analysis of the powders was determined using a Kratos Axis Ultra X-ray photoelectron spectrophotometer (XPS; Kratos Analytical, Manchester, UK), equipped with a monochromatic Al Ka X-ray source (1486.58 eV) at 150 W (15 kV, 10 mA). Using elemental composition, i.e., carbon (C), oxygen (O) and nitrogen (N), data derived from experimental analysis of milk protein isolate (C = 68.4, O = 17.6 and N = 12.85%), lactose (C = 55.75, O = 44.25 and N = 0%), and anhydrous milk fat (C = 90.3, O = 9.7 and N = 0%) reference samples, a matrix formula was used to determine relative amounts of protein, lactose and fat on the MPC powder particle surface, as described by Faldt et al. (1993).

2.6. Colour

The colour of each MPC powder was measured using a Chroma Meter CR-400 (Konica Minolta Sensing Europe B.V., Nieuwegein, the Netherlands). The colour measurement was determined according to the three colour coordinates: L*, a*, and b*. The value L* represents the sample luminosity or brightness, varying from black (0) to white (100); a* represents the colour varying from green (−) to red (+); b* represents the colour varying from blue (−) to yellow (+). Each reported colour value was the mean of three different measurements. Total colour difference (ΔE) was calculated for RN, A and AN-MPC powders using equation (1), as reported by Kelleher et al. (2020):

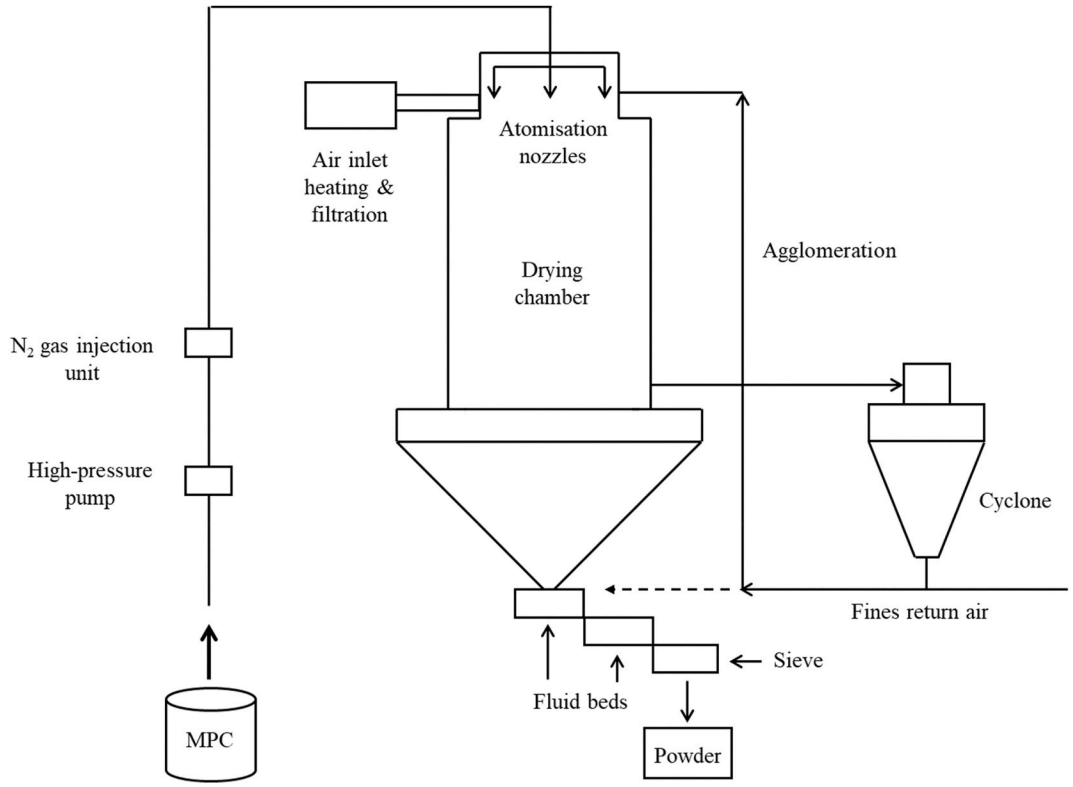

Fig. 1. Process flow diagram for the production of milk protein concentrate (MPC) powders.

$$\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \quad (1)$$

2.7. Particle density, bulk density, porosity, occluded and interstitial air

Particle density of the MPC powders was measured using an AccuPyc II 1340 gas pycnometer (Micromeritics Instrument Corporation, Norcross, Georgia, USA) according to the air pycnometry method of GEA Niro (2006a). The volume of interstitial and occluded air was calculated as described in the GEA Niro method (2006a). The loose and tapped (100 taps) bulk density of the MPC powders was measured as per the GEA Niro method (2006b), using a jolting volumeter STAV II (Funke Gerber, Berlin, Germany). The porosity (ε) of each MPC was calculated using equation (2), as described by Li et al. (2016):

$$\varepsilon = 1 - (\text{tapped density}/\text{particle density}) \quad (2)$$

2.8. Powder particle size and friability

The particle size and friability of the MPC powders were determined using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with an Aero S dry powder dispersion unit. The refractive index and absorption index were set at 1.45 and 0.1, respectively. The air pressure used was 2 bar and the feed rate was adjusted (from 25 to 100%) to compensate for innate differences in flowability of the powder samples. Size measurements were recorded as the median particle diameter (D_{50}) and cumulative diameters (D_{10}) and (D_{90}), whereby 10, 50 and 90% of the sample volume is represented by particles smaller than the size indicated. The volume-weighted mean particle diameter ($D_{[4,3]}$) was also calculated.

Friability, the ability of powder particles to fragment during processing, was measured according to the method of Schuck et al. (2012b), using a Malvern Mastersizer equipped with an Aero S dry powder dispersion unit. The compressed air pressure was set at either 0.5 or 4 bar, the feed rate was adjusted (from 20 to 100%) to compensate for innate differences in flowability of the powder samples, and the D_{50} was subsequently recorded. Each powder was analysed in triplicate and friability was calculated using equation (3) as follows:

$$F = \left(\frac{[d(0.5@50\ kPa) - d(0.5@400\ kPa)]}{d(0.5@50\ kPa)}\right) \text{ x } 100 \quad (3)$$

2.9. Specific surface area

Specific surface area (SSA) values for MPC powders were determined using a Gemini VI Surface Area Analyzer (Micromeritics, Norcross, GA, USA). Powder particles (0.1–0.5 g) were first loaded into a glass tube and degassed at 25 °C, overnight, before analysis using a FlowPrep™ 060 degassing unit (Micromeritics, Norcross, GA, USA); nitrogen was used as the adsorbate and the operating pressure set at 1 bar. The SSA was calculated from a nine-point sorption isotherm (liquid nitrogen at −196 °C was used to maintain isothermal conditions) using the Brunauer-Emmett-Teller equation (Brunauer et al., 1938). The technique determined SSA of powder particles by correlating it to the flow of nitrogen through the column of packed particles (Buma, 1971a). All measurements were carried out in triplicate. SSA can be inferred from particle size distribution data but are representative only of the SSA of equivalent spheres, while analysis of SSA by nitrogen adsorption does not include an assumption of sphericity (Crowley et al., 2014).

2.10. Bulk powder properties

The powder bulk handling and flowability properties (i.e., flow index, the effective angle of internal friction, bulk density and compressibility) were measured using a Brookfield Powder Flow Tester (PFT; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). Powder samples were prepared for analysis as described by Crowley et al. (2014). Briefly, a standard flow function (FF) test was carried out to determine the flowability of MPC powders by applying five normal stresses (1.0–4.8 kPa) and three over-consolidation stresses at each

normal stress. Values for the effective angle of internal friction were obtained from FF analysis, and the value at 4.8 kPa was reported. Loose bulk density (p_b) and tapped bulk density (p_t) were recorded at the minimum and maximum major principal consolidating stress, respectively. A standard wall friction test was performed whereby ten normal stresses (0.5–4.8 kPa) were applied to determine wall friction angle (ϕ_w) values. The ϕ_w was reported at a normal stress of 4.8 kPa. Compressibility index (CI) was calculated using equation (4) as described by Schuck et al. (2012a):

$$CI = \frac{p_t - p_b}{p_t} X\ 100 \quad (4)$$

Powder flowability was also measured by determining the time required for a defined volume of powder to leave a rotating drum (GEA Niro, 2019). The flowability of powder was expressed using equation (5) as follows:

$$F_d = (g_{p1} - g_{p2})/\text{time} \quad (5)$$

where F_d is the drum flowability (g/s) and g_{p1} and g_{p2} correspond to the amount (g) of powder in the container at the beginning and end of the test, respectively (Murphy et al., 2020).

2.11. Statistical analysis

Measurements of powder characteristics were performed in triplicate, with results presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was carried out using IBM SPSS (version 24; Armonk, New York, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. Microstructure

Scanning electron microscopy images showed significant differences between the morphology of regular and agglomerated milk protein concentrate (MPC) powders (Fig. 2). Regular (R) MPC powder displayed shrivelled or collapsed particles (Fig. 2A), which resembled the wrinkled native phosphocaseinate (NPC) particles reported by Sadek et al. (2014), while agglomeration resulted in the formation of clearly defined powder clusters composed of several closely linked particles (Fig. 2C). The injection of nitrogen (N_2) gas resulted in significantly different morphology for regular and agglomerated powders, as evidenced by the spherical, fractured and porous appearance of the particles (Fig. 2B and D). The higher porosity was likely a result of the foam structure formed in the liquid MPC following the injection of N_2, and the subsequent rapid removal of N_2 from the atomised droplets in the drying chamber, as suggested by Bouvier et al. (2013) for an MPC into which carbon dioxide gas was incorporated. Furthermore, the change in particle shape following N_2 injection (NI), whereby the particles were puffed and inflated, could be explained by increases in occluded air content. Bouvier et al. (2013) showed that extrusion-porosification produced particles with a more spherical appearance compared to a conventionally spray dried MPC powder. Spray drying temperature can also play a role in powder morphology as Fang et al. (2012) reported that smooth MPC powder particles were produced at low drying temperature (i.e., 77 °C) whereas higher drying temperatures (i.e., 178 °C) generated wrinkled powder particles, likely due to differences in the rate of water removal and particle shrinkage.

3.2. Surface composition

In this study, x-ray photoelectron spectroscopy (XPS) was used to investigate whether NI altered powder particle surface composition as it has been reported that higher levels of surface fat result in greater inter-particle cohesiveness and impaired powder flowability (Fitzpatrick et al., 2007; Kim et al., 2005; Silva and O'Mahony, 2017). Surface composition analysis showed that all MPC powders had a greater coverage of fat at the particle surface compared to the bulk fat content of the powders (Table 1). It is worth noting that as the melting point of milk fat is ~36 °C (O'Callaghan et al., 2016), significantly lower than the

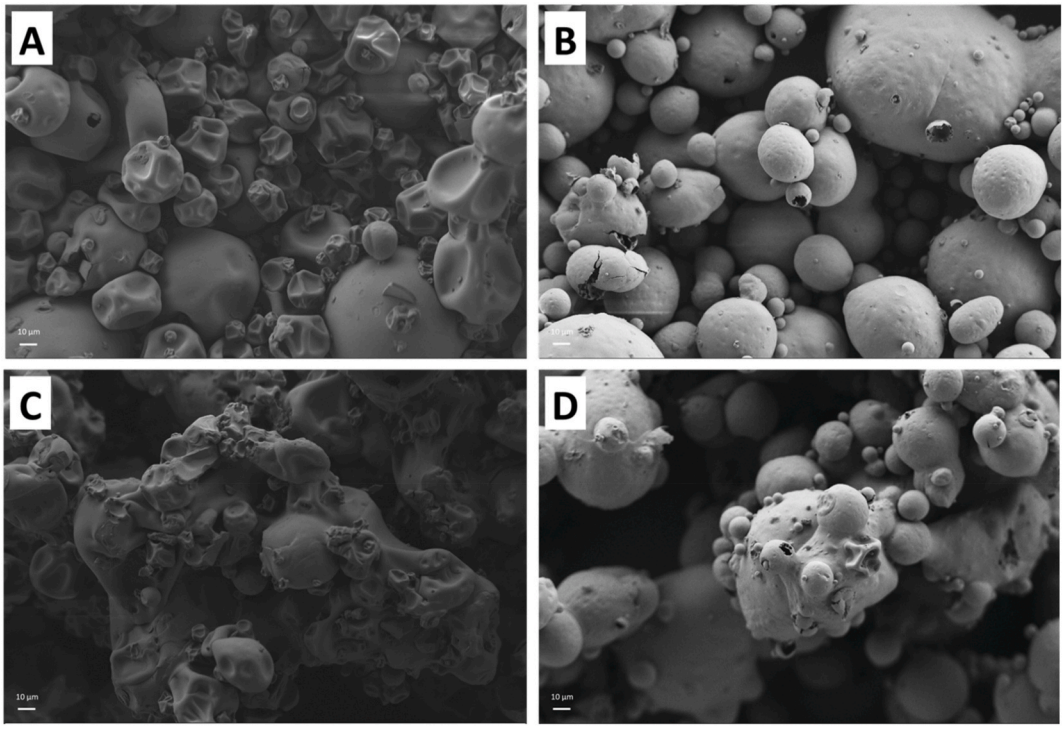

Fig. 2. Scanning electron micrographs showing the microstructure of [A] regular (R), [B] regular with N_2 injection (RN), [C] agglomerated (A) and [D] agglomerated with N_2 injection (AN) milk protein concentrates powders at a magnification of 1000x. Scale bar represents 10 µm.

Table 1
Surface composition (%) of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders, as determined by X-ray photoelectron spectroscopy (XPS) analysis.

MPC	Protein	Lactose	Fat
R	$96.9^{a} \pm 0.55$	$0.91^{a} \pm 0.70$	$2.02^{a} \pm 0.02$
RN	$87.9^{bc} \pm 1.10$	$0.90^{a} \pm 0.32$	$11.1^{b} \pm 1.27$
A	$89.5^{b} \pm 2.20$	$2.61^{b} \pm 0.29$	$7.68^{b} \pm 1.96$
AN	$83.3^{c} \pm 1.10$	$4.72^{c} \pm 0.03$	$11.4^{b} \pm 1.13$

[a-c] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

temperature of the concentrate to the dryer (i.e., 70 °C), it was in liquid form throughout the spray drying process (Liu et al., 2020). R-MPC powder particles had the highest proportion of surface protein (96.9%) and the lowest amount of surface fat (2.0%). However, for regular with N_2 injection (RN) MPC, surface protein and fat coverage were 87.9% and 11.1%, respectively. Therefore, the NI process had a significant impact on the migration of fat to the surface of the powder particles. Lactose was present in significantly higher proportions on the surface of agglomerated MPC powders, compared to regular powders; 0.9% for RN-MPC and 4.7% for agglomerated with N_2 injection (AN) MPC. Previous studies have shown that for dairy powders such as MPC, the level of fat at the surface of particles is over-represented when compared to the level of fat in the bulk powders. For example, Kelly et al. (2015) reported that fat was present in a higher quantity at the surface of an MPC powder (8.2%) compared to the bulk (1.2%), while for a native phosphocaseinate (NPC) powder, Gaiani et al. (2006) reported surface and bulk fat values of 5.3 and 0.4%, respectively. Kim et al. (2009) demonstrated that spray drying temperature plays an important role in surface fat content of skim milk powder, with a decrease in the air inlet temperature causing an increase in the migration of fat to the surface of the powder particle, possibly due to slower particle skin formation. Furthermore, Gaiani et al. (2009) attributed the migration of lipids to the surface of NPC powder during storage, measured using XPS, to the development of pores throughout the powder matrix. In the current study, the increased porosity of the powder particles that were produced using the NI process may have facilitated the movement of fat to the powder surface. Another contributing factor may be that as N_2 gas bubbles within the liquid droplets escaped, creating pores and voids in the powder particles, they promoted the transfer of hydrophobic lipids towards the surface. Although surface free fat differs from surface fat measurements by the use of organic solvents to extract fat from the powder, it should be mentioned that Buma (1971b) reported a strong correlation between surface free fat and porosity of whole milk powder. Additionally, Hansen (1980) suggested that fat-filled milk powders had higher surface free fat contents when the powders were more aerated, with numerous capillaries and vacuoles, as the fat was less protected.

3.3. Moisture, colour, density, porosity, occluded and interstitial air

The mean moisture contents of MPC powders were 5.37, 5.59, 4.96 and 5.16%, for R-, RN-, A- and AN-MPC, respectively. The significantly lower moisture content of A-MPC compared to R-MPC powder, could be due to the recirculation of fines through the dryer main chamber, resulting in the removal of additional moisture. Similarly, Gaiani et al. (2007) reported moisture contents of 5.4 and 4.5% for non-agglomerated NPC and whey protein isolate (WPI) compared to 4.8 and 3.9% for agglomerated NPC and WPI, respectively. The injection of N_2 did not significantly increase the moisture content of MPC powders.

Significant ($P < 0.05$) differences were observed in the colour of the MPC powders (Table 2). The L* values were higher for RN- and AN-MPC (95.5 and 93.6, respectively) compared to R- and A-MPC powders (93.3 and 91.5, respectively), indicating higher overall whiteness of the NI

Table 2
Colour space values (L*, a*, b*) and total colour difference (ΔE) for regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

MPC	L*	a*	b*	ΔE
R	$93.3^{a} \pm 0.52$	$-2.28^{a} \pm 0.01$	$11.0^{a} \pm 0.02$	–
RN	$95.5^{b} \pm 0.06$	$-1.07^{b} \pm 0.01$	$5.48^{b} \pm 0.02$	6.08 ± 0.19
A	$91.5^{c} \pm 0.01$	$-2.68^{c} \pm 0.02$	$12.0^{c} \pm 0.02$	2.11 ± 0.46
AN	$93.6^{a} \pm 0.01$	$-1.42^{d} \pm 0.02$	$6.47^{d} \pm 0.01$	4.64 ± 0.04

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$).

powders. The b* values were higher for both R- and A-MPC powders (i.e., 11.0 and 12.0, respectively) compared to RN- and AN-MPC (5.48 and 6.47, respectively; Table 2), indicating a significant reduction in the yellowness of NI powders. Overall, the ΔE was highest for RN-MPC (6.08), followed by AN- and A-MPC (4.64 and 2.11, respectively), suggesting that N_2 injection influenced powder colour more than agglomeration. The differences in colour may be explained by the density data presented in Table 3. The process of agglomeration produced powders with lower loose bulk density values, e.g., 0.29 and 0.18 g/cm^3 for R- and A-MPC powders, respectively (Table 3). Similarly, Chever et al. (2017) reported lower loose (0.37 g/cm^3) and tapped (0.50 g/cm^3) bulk density values for agglomerated whole milk powders compared to non-agglomerated powders (0.41 and 0.72 g/cm^3, respectively). The loose bulk density was significantly lower for RN-MPC (0.09 g/cm^3) than for R-MPC (0.29 g/cm^3) due to the injection of N_2 into the concentrate. The tapped bulk density was significantly lower for RN- and AN-MPC (0.11 and 0.08 g/cm^3, respectively) compared to the R- and A-MPC powders (0.34 and 0.21 g/cm^3, respectively). The particle density of MPC powders produced using NI was also significantly lower. For example, the particle density was 1.09 g/cm^3 for R-MPC but 0.96 g/cm^3 for RN-MPC, while it was 0.99 and 0.87 g/cm^3 for A- and AN-MPC, respectively. With the decrease in powder density, there was a corresponding increase in the interstitial (between particles) and occluded (within particles) air content values (Table 3). The interstitial air was higher for RN-MPC (771 mL/100 g) and AN-MPC (1078 mL/100 g) compared to 202 and 372 mL/100 g for R- and A-MPC, respectively. Finally, the porosity was higher for RN- and AN-MPC (0.88 and 0.90, respectively) compared to R- and A-MPC powders (0.68 and 0.79, respectively). The higher porosity occurred as a result of the lower tapped and particle densities for NI powders. Previously, Bouvier et al. (2013) showed that non-agglomerated MPC powders produced using extrusion-porosification had loose bulk density, tapped bulk density and occluded air values of 0.22 g/cm^3, 0.43 g/cm^3 and 146 mL/100 g, respectively, compared to 0.36 g/cm^3, 0.52 g/cm^3, and 107 mL/100 g, respectively, for a conventionally spray dried MPC powder. A challenge

Table 3
Physical properties of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

MPC	p_p	p_b	p_t	V_{ia}	V_{oa}	ε
	g/cm^3			mL/100 g		
R	$1.09^{a} \pm 0.02$	$0.29^{a} \pm 0.01$	$0.34^{a} \pm 0.01$	$202^{a} \pm 8.00$	$24.2^{a} \pm 1.27$	$0.69^{a} \pm 0.01$
RN	$0.96^{b} \pm 0.00$	$0.09^{b} \pm 0.00$	$0.11^{b} \pm 0.00$	$771^{b} \pm 16.3$	$36.3^{b} \pm 0.33$	$0.88^{b} \pm 0.00$
A	$0.99^{b} \pm 0.01$	$0.18^{c} \pm 0.00$	$0.21^{c} \pm 0.00$	$372^{c} \pm 9.38$	$33.7^{b} \pm 1.47$	$0.79^{c} \pm 0.00$
AN	$0.87^{c} \pm 0.01$	$0.07^{d} \pm 0.00$	$0.08^{d} \pm 0.01$	$1078^{d} \pm 70.8$	$47.5^{c} \pm 1.91$	$0.90^{d} \pm 0.01$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). p_p = particle density; p_b = loose bulk density; p_t = tapped bulk density; V_{ia} = volume of interstitial air; V_{oa} = volume of occluded air; ε = porosity.

of producing dairy powders with a low loose bulk or particle density is their suitability for export due to volume constraints during handling and packaging (Barbosa-Canovas et al., 2005).

3.4. Powder particle size and friability

The effect of NI and agglomeration on powder particle size is displayed in Fig. 3, whereby the D_{90} values were 134 and 148 μm for R- and RN-MPC, compared to 244 and 256 μm for A- and AN-MPC powders, respectively (Table 4). The incorporation of N_2 into the concentrate significantly ($P < 0.05$) increased the size of the regular MPC powder particles across all size measurements (Table 4). This slight increase in particle size for NI powders may be due to the expansion of gas bubbles within the liquid droplets directly after exiting the spray nozzles. As expected, agglomerated samples had a significantly higher particle size than regular MPC powders due to the return of fines through the drying chamber. The R-MPC powder had a higher friability value (20.6%) compared to RN-MPC (19.1%; Table 4). However, the injection of N_2 did not influence the breakdown of the agglomerated samples as they both had a friability of approximately 33% (Table 4). Therefore, the friability results suggest that regular MPC powders would retain their shape and structure to a greater extent and be less likely to break during handling and processing, as they possessed the higher particle strength, in comparison to the agglomerated powders. Attrition of agglomerated products can negatively impact powder functionality, with Hazlett et al. (2020) reporting that pneumatic conveying of an agglomerated whey protein concentrate powder (80% protein, w/w) caused agglomerate breakdown, resulting in lower powder bulk density, flowability, wettability and dispersibility. The significant difference in friability between regular MPC powders is most likely due to the greater cohesiveness, or poorer flow, of the RN-MPC powder, thus making it less friable than R-MPC. In addition, the significantly lower particle density and higher occluded air content of RN-MPC may also play a role in its lower friability.

3.5. Flowability, specific surface area, wall friction angle and compressibility

The injection of N_2, as well as agglomeration by fines return, altered the flow properties of the MPC powders (Fig. 4). The flow index values were 5.14, 2.71, 7.73 and 3.68 for R-, RN-, A- and AN-MPC, respectively (Table 5). According to the Jenike classification, powders with a flow index value between 4 and 10 are easy flowing, while cohesive powders present flow index values of less than 4 (Jenike, 1964). Therefore, both powders which underwent NI were categorised as cohesive over the range of consolidating stresses applied and as mentioned in Section 3.2.,

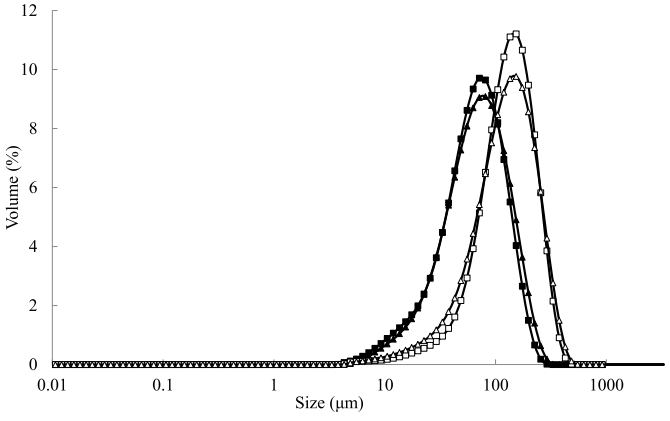

Fig. 3. Particle size distribution of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders.

Table 4
Particle size distribution parameters, friability (F) and specific surface area (SSA) of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

MPC	D_{10}	D_{50}	D_{90}	$D_{[4,3]}$	F	SSA
	μm				%	m^2/g
R	$22.8^{d} \pm 0.0$	$65.3^{d} \pm 0.1$	$134^{a} \pm 0.0$	$73.0^{a} \pm 0.2$	$20.6^{a} \pm 0.1$	$0.65^{b} \pm 0.03$
RN	$24.8^{c} \pm 0.2$	$68.5^{c} \pm 0.3$	$148^{b} \pm 1.5$	$78.9^{b} \pm 0.6$	$19.1^{b} \pm 0.3$	$2.82^{a} \pm 0.05$
A	$55.5^{a} \pm 0.2$	$132^{a} \pm 0.0$	$244^{c} \pm 1.5$	$142^{c} \pm 1.0$	$32.6^{c} \pm 0.3$	$0.50^{b} \pm 0.02$
AN	$45.5^{b} \pm 0.1$	$126^{b} \pm 0.0$	$256^{d} \pm 3.0$	$140^{c} \pm 1.5$	$33.1^{c} \pm 0.4$	$2.62^{a} \pm 0.16$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). D_{10} = particle size below which 10% of sample volume exists; D_{50} = particle size below which 50% of sample volume exists; D_{90} = particle size below which 90% of sample volume exists; $D_{[4,3]}$ = volume-weighted mean particle diameter.

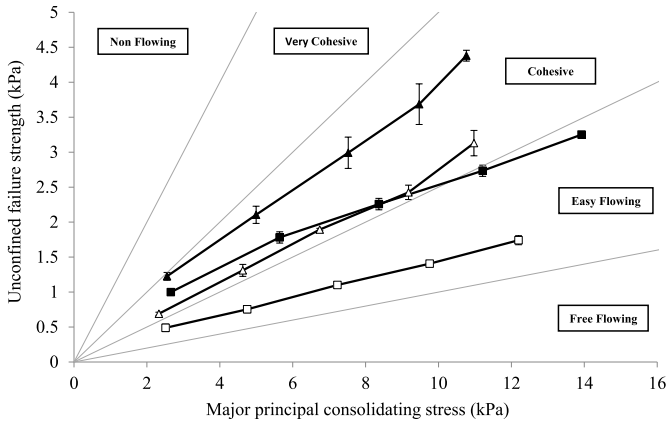

Fig. 4. Flow function profiles showing unconfined failure strength as a function of major principal consolidating stress (kPa) for regular (■), regular with N_2 injection (▲) agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders.

surface fat can play a detrimental role in powder flowability. Conversely, the regular and agglomerated MPC powders were easy flowing. Assessment of powder flowability by the drum method supported the results obtained by the Brookfield powder flow tester; agglomeration improved flowability while the injection of N_2 into the concentrate produced a powder with poorer flowability, e.g., 2.24 g/min for R-MPC compared to 0.31 g/min for RN-MPC (Table 5). The

Table 5
Bulk-handling and flowability properties of regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with nitrogen injection (AN) milk protein concentrate (MPC) powders.

MPC	i	JC	F_d (g/min)	δ_e (°)	ϕ_w (°)	CI (%)
R	$5.14^{a} \pm 0.16$	Easy flowing	$2.24^{a} \pm 0.14$	$46.4^{a} \pm 0.6$	$16.2^{a} \pm 0.6$	$41.5^{a} \pm 2.4$
RN	$2.71^{b} \pm 0.18$	Cohesive	$0.31^{b} \pm 0.02$	$46.8^{a} \pm 2.4$	$18.3^{b} \pm 0.6$	$50.4^{b} \pm 3.2$
A	$7.73^{c} \pm 0.36$	Easy flowing	$8.30^{c} \pm 0.11$	$42.0^{b} \pm 0.1$	$13.6^{c} \pm 1.3$	$24.9^{c} \pm 0.8$
AN	$3.68^{d} \pm 0.38$	Cohesive	$1.29^{d} \pm 0.04$	$42.7^{b} \pm 0.2$	$14.1^{c} \pm 0.4$	$48.2^{ab} \pm 3.4$

[a-d] Values within a column not sharing common superscripts differ significantly ($P < 0.05$). i = flow index; JC = Jenike classification; F_d = drum flowability; δ_e = effective angle of internal friction; ϕ_w = wall friction angle; CI = compressibility index.

size of powder particles affects the bulk properties (e.g., flowability) and it has been suggested by Rennie et al. (1999) that powder cohesiveness decreases as particle size increases. However, AN-MPC was classified as cohesive, despite having a D_{90} of 256 μm compared to A-MPC which was easy-flowing with a D_{90} of 244 μm. This suggests that the NI process counteracts the improved flowability that agglomeration typically provides. The differences in flowability between NI and non-NI powders could be due to a difference in powder particle shape, as shown in Fig. 2, but was most likely caused by the significant difference in specific surface area (SSA). The SSA was almost four times higher for the NI samples in comparison to non-NI MPC powders (Table 4) which may be due to the greater porosity of the NI MPC powders. A-MPC had a lower SSA than R-MPC, likely due to its larger powder particle size. The greater SSA of RN- and AN-MPC would facilitate a greater number of attractive surface interactions between powder particles and restrict movement. It was previously found by Fu et al. (2012) that lactose powder with the highest sphericity had better flowability. However, in this study, RN-MPC had the most spherical powder particles (Fig. 2B) but had the lowest flow index value (Table 5). These differences in particle structure and shape appear to have a large impact on its physical characteristics and will likely influence the rehydration properties also.

The NI process did not alter the effective angle of internal friction, however; it was significantly different for the agglomerated powders (~42°) in comparison to the regular MPC powders (~46°; Table 5). This suggests that less resistance to flow occurs between the agglomerated powder particles in comparison to regular MPC powders. In this study, the wall friction angle was increased by the NI process as it was highest for the RN-MPC and lowest for the A-MPC powder (Table 5 and Fig. 5). An increase in wall friction can cause greater stress on the perimeter or wall of silos and significantly hinder the removal and emptying of powders (Fitzpatrick et al., 2004). The wall friction angle obtained for A-MPC (13.6°) is similar to that reported by Teunou et al. (1999) for an agglomerated skim milk powder (13.0°). Furthermore, Crowley et al. (2014) reported a wall friction angle of 21.7° for a regular MPC80 powder, which differs from the value of 16.2° for R-MPC in the current study, possibly due to differences in powder particle size; D_{90} of 134 μm in the current study (Table 4) compared to 58 μm reported by Crowley et al. (2014). Of all powders analysed, RN-MPC had the highest wall friction angle and the highest effective angle of internal friction. The strong attractive forces between both the powder and a surface, and between the powder particles themselves, were probably attributed to the larger SSA of RN-MPC. Furthermore, NI-MPC powders were found to be more compressible; RN-MPC had a compressibility index of 50.4% compared to 41.5% for R-MPC (Table 5). This can be explained by the large volume of interstitial air present between these powder particles. The voids would be largely removed during compaction, with a corresponding decrease in the distance between powder particles. It was evident that the more compressible a powder was, the lower its flow index. A relationship between poor flowability and high compressibility was also previously reported by Crowley et al. (2014) for high-protein MPC powders. Compression of such MPC powders during handling and storage is known to have important implications for the functionality of these powders for end-users, e.g., changes in powder density and loss of dispersibility due to removal of air voids.

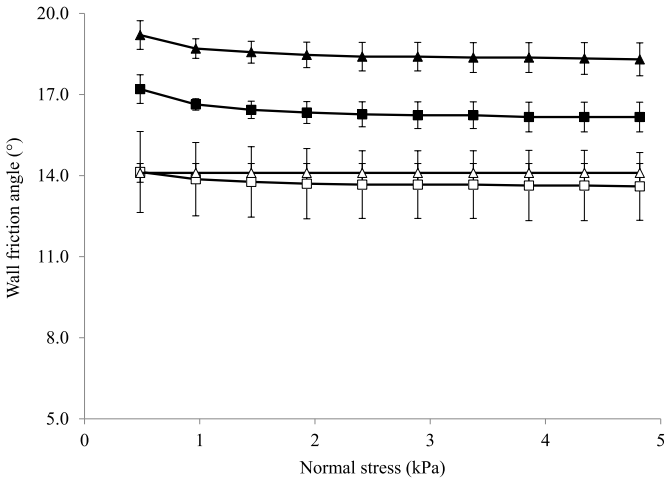

Fig. 5. Wall friction angle as a function of normal stress (kPa) for regular (■), regular with N_2 injection (▲) agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders.

4. Conclusion

The injection of nitrogen (N_2) gas into the concentrate prior to spray drying can significantly alter the physical and bulk handling characteristics of milk protein concentrate (MPC) powders. In particular, the bulk density and flowability of MPC powders were significantly changed by this process. Flow index values were lower and wall friction angles were higher with the use of N_2 injection (NI). This was attributed to the alterations in surface composition and powder particle structure, as well as the higher specific surface area and porosity. The NI process significantly increased the compressibility of MPC powders which may cause changes in powder properties during handling and storage and subsequently alter their reconstitution properties. Agglomeration by fines return during spray drying generated powders with improved flowability but increased friability, which suggests their functional properties could be impaired during bulk powder handling and conveying. This study has provided essential information regarding the influence of NI and agglomeration on powder functionality and will support both the processing operations (i.e., storage, handling) and technological development of milk protein ingredients.

Credit author statement

David J. McSweeney: Conceptualization, Methodology, Formal Analysis, Investigation, Visualization, Writing – Original Draft. Valentyn Maidannyk: Methodology, Investigation. James A. O'Mahony: Funding acquisition, Supervision, Writing – Review and Editing. Noel A. McCarthy: Funding acquisition, Project Administration, Conceptualization, Supervision, Writing – Review and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Rehydration properties of regular and agglomerated milk protein concentrate powders produced using nitrogen gas injection prior to spray drying

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ABSTRACT

This study evaluated the effect of high-pressure nitrogen (N_2) gas injection prior to spray drying on the subsequent rehydration properties of regular and agglomerated milk protein concentrate (MPC) powders. Conductivity measurements demonstrated a slower release of ions for powders produced using N_2 injection (NI) as they took longer to wet and sink due to their lower density. However, analysis of particle size distribution on reconstitution at both 23 and 50 °C showed an improvement in powder dispersion with NI. Powder solubility, when measured at 23 °C, was higher for the NI powders, while agglomeration negatively impacted solubility. Confocal laser scanning microscopy analysis showed a faster diffusion of dye into MPC powder particles produced using NI. The improvement in powder dissolution with NI was attributed to higher porosity and the presence of air voids which facilitated increased water transfer and accelerated the breakdown of primary powder particles.

1. Introduction

The rehydration of high-protein, casein-dominant dairy powders, including milk protein concentrate (MPC), is currently a significant challenge encountered by the food and beverage industry. These value-added ingredients provide unique nutritional (e.g., high-protein and calcium, low lactose content) and functional (i.e., heat stability, gelation) properties. To exploit the functionality of dry dairy ingredients, rapid and complete powder rehydration is generally required. However, this is impaired by reduced water transfer due to non-covalent protein-protein interactions (Havea, 2006) and high micellar casein content (Schuck et al., 1998, 2002), with dispersion of primary powder particles regarded as the rate-limiting stage of rehydration due to the presence of a network of casein micelles at the powder particle surface (Mimouni et al., 2009).

A water temperature of approximately 50 °C, in combination with high-shear treatment and extended mixing times, are normally required to accelerate the rehydration of casein-dominant powders (Gaiani et al., 2006b; McCarthy et al., 2014), but this is not desirable for ingredient manufacturers and end-users. Ideally, rehydration should take place within a short time period at ambient temperature (~20 °C) and low shear to minimise manufacturing time and production costs (Saggin and Coupland, 2002). Previous research has proposed several processing and formulation strategies to promote the rehydration of casein-dominant powders, including cold microfiltration during micellar casein concentrate manufacture (Crowley et al., 2018), high-pressure treatment (Udabage et al., 2012), acidification of skim milk before membrane filtration (Liu et al., 2019), and the incorporation of monovalent salts (e.g., sodium chloride) into the concentrate before spray drying (Schuck et al., 2002; Sikand et al., 2013).

Gases have been used in dairy processing to alter the functional properties of a range of products. For example, carbon dioxide (CO_2) has been used to improve the shelf life and quality of milk, cheese and fermented beverages (Hotchkiss et al., 2006). However, only a limited number of studies have reported their impact on the rehydration properties of dairy powders. Marella et al. (2015) investigated the effect of CO_2 injection into skim milk before and during membrane filtration and reported the subsequent characteristics of the MPC powder. An improvement in cold water (10 °C) solubility was observed, which was attributed to the solubilisation of colloidal calcium phosphate during membrane fractionation due to the decrease in pH and a reduction in micellar casein interactions. However, the incorporation of CO_2 into dairy streams during processing may change product composition, presenting challenges for some existing applications. Bouvier et al.

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(2013) used CO_2 during extrusion-porosification to manufacture MPC powders with enhanced dispersibility compared to MPC produced using conventional spray drying. The achievement of a sub-micron particle size distribution after only 2 h of rehydration was attributed to the partial dissociation of casein micelles as well as increased porosity of powder particles. Kosasih et al. (2016a, 2016b) investigated the addition of dry ice (i.e., solid CO_2) to whole milk concentrate prior to spray drying and showed an improvement in the dispersibility of powder particles. Nitrogen (N_2) gas has also been used in dairy processing to modify ingredient functionality (Adhikari et al., 2018). One apparent benefit of using N_2 gas is that, unlike CO_2 and compressed air, it is inert, so is unlikely to alter the pH of the dairy concentrate or promote oxidation in the final product. Hanrahan et al. (1962) reported an improvement in whole milk powder dispersion when N_2 gas was incorporated into the concentrate before spray drying. Similarly, Bell et al. (1963) enhanced the dispersibility of skim milk powder by injecting compressed air into the concentrate between the high-pressure pump and atomisation nozzle.

Aside from the incorporation of gas into dairy streams, powder particle structure and physical properties can be modified *via* a process known as agglomeration. It can be performed by returning fine powder particles from the cyclone to the top of the drying chamber during droplet dehydration or by combining the spray dried powder with water or a binder in the fluidised bed (Gianfrancesco et al., 2008). The process of intentionally mixing the atomised spray with small, dry powder particles is known as forced secondary agglomeration (Pisecky, 2012). The effects of fluid bed agglomeration on the physicochemical properties of milk protein isolate powders have been reported by Ji et al. (2015, 2016, 2017), whereby improvements in powder wettability were achieved, with no improvement in solubility. Gaiani et al. (2007) reported that agglomeration using fines return was effective in accelerating rehydration of whey protein powder, while it resulted in impaired rehydration performance for casein-dominant powder. Furthermore, the rehydration characteristics of MPC powders produced using both agglomeration and N_2 gas injection have not been established. A previous study (McSweeney et al., 2021) by the current authors investigated the influence of N_2 gas injection directly prior to spray drying, agglomeration by fines return, and a combination of these approaches, on the physical and bulk handling properties of MPC powders. The MPC powder produced using N_2 gas injection had lower density and flow index values, with higher air content, specific surface area, porosity and surface fat, compared to the powders produced without N_2 gas injection, while agglomeration also decreased powder density but improved flowability. Given the significant changes to the structure of the powder particles, the current study was designed to investigate the rehydration properties of these MPC ingredients. Several techniques were employed to elucidate the impact of these processing modifications on the performance of the powders throughout the main stages of rehydration (i.e., wetting, dispersion and dissolution).

2. Materials and methods

2.1. Powder manufacture

The manufacture, composition and basic physical properties (e.g., density, morphology, porosity, powder particle size) of the regular (R), regular with nitrogen gas injection (RN), agglomerated (A) and agglomerated with nitrogen gas injection (AN) milk protein concentrate (MPC) powders used in this study were described by McSweeney et al. (2021). Briefly, concentrate (21.2% total solids, w/w) was first prepared from MPC powder using high-shear treatment and hydrodynamic cavitation. Prior to spray drying, the concentrate was pre-heated to 70 °C and pumped to the atomisation nozzle using a high-pressure pump (HPP). Regular (R) MPC powder was produced using a conventional spray drying process. Agglomerated (A) powders were manufactured by returning all fines collected in the cyclone to the atomisation zone of the spray dryer main chamber. For MPC powders produced with nitrogen (N_2) gas injection (i.e., RN- and AN-MPC), N_2 was injected (3.5 kg/h) at a pressure of ~190 bar into the feed line, after the HPP and prior to atomisation, using a pressurised injection unit (Carlisle Process Systems, Farum, Denmark).

2.2. Immersional and capillary rise wetting behaviour

Immersional wetting was measured using the GEA Niro method (GEA Niro, 2009) with one modification; 4 g of each powder sample was added to the beaker of water (250 mL; 25 °C). Capillary rise wetting was measured using a modified Washburn method with 2 g of each powder sample added to a cylindrical stainless-steel tube (diameter = 2.4 cm) with an open base covered by filter paper and parafilm (Ji et al., 2015). The analysis was first carried out with no powder to determine the quantity of water absorbed by the filter paper and parafilm (i.e., control), and subsequently this value was subtracted from the test values. The weight of the tube was recorded before and after the addition of powder. The top of the tube was submerged in 25 °C ultrapure water and the wettability was quantified by measuring the additional mass of the wetted powder after 20 min, with results presented as the mean of three independent measurements.

2.3. Confocal laser scanning microscopy and liquid phase water diffusion

A Leica TCS SP5 confocal laser scanning microscope (CLSM; Leica Microsystems CMS GmbH, Wetzlar, Germany) was used for the real-time visualisation of dye penetration into powder particles, as described by Power et al. (2020). Liquid phase water diffusion in MPC powders was measured using the novel method presented by Maidannyk et al. (2019). Rhodamine B was added to anhydrous powders which allowed diffusion of the dye molecules into the particles without changing particle morphology and preventing solubilisation, thereby providing an indicator of powder hydration. The CLSM images were obtained at fixed time intervals and represent real-time water diffusion. Diameters of particles were detected using Leica TCS SP5 software in the size range 6–142 μm. The areas of individual powder particles were measured using spherical approximation and this information, combined with the time of dye penetration, enabled the local effective diffusivity of the liquid phase in individual powder particles to be calculated. Initially, powder particles appear as dark particles with a dark green background. However, during the water diffusion process, the fluorescent dye penetrates the particles and changes their colour to bright green.

2.4. Water sorption isotherms

Water sorption analysis was carried out as described by Maidannyk et al. (2020), with one modification: powders were weighed at intervals of 0, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 h. The water content in each system was plotted as a function of time, and the Guggenheim-Anderson-de Boer (GAB) relationship was fitted to data to relate water activity and water content of anhydrous powders, as shown in equation (1):

$$\frac{m}{m_0} = \frac{Cka_w}{(1 - ka_w)(1 - ka_w + Cka_w)} \quad (1)$$

Where m is the water content (g of water/100 g of dry solids), m_0 – the monolayer value of water content, C, k – constants, which can be calculated from m_0.

2.5. Measurement of mineral release using conductivity

Conductivity of MPC dispersions (1.5% protein, w/w; 300 mL of ultrapure water in a 400 mL beaker) was measured using a Titrando autotitrator equipped with a five-ring conductivity measuring cell and

accompanying Tiamo v2.3 software (Metrohm Ireland Ltd, Athy Road, Co. Carlow, Ireland). The probe was calibrated at 25 °C with a KCl solution of known conductivity (12.9 mS/cm) and a temperature coefficient of 2.07 was used (Crowley et al., 2015). Before the addition of powder to the beaker of water, 1 min was allowed to elapse to establish a baseline reading of conductivity and powder was then added over a period of 1 min. It is expected that cations and anions found in the serum phase (e.g., H^+ and Cl^-) would contribute most to conductivity measurements rather than minerals found in the colloidal phase, e.g., calcium (Zhuang et al., 1997; Schuck et al., 2007).

2.6. Particle size distribution of milk protein concentrate dispersions

The particle size distribution of MPC dispersions was measured using a laser-light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens, as described by McSweeney et al. (2020). Additionally, powders were reconstituted using low-speed mixing for 1 h at 23 °C. Size measurements were recorded as the volume-weighted mean particle diameter ($D_{[4,3]}$), median diameter (D_{50}) and cumulative diameters (D_{90} and D_{10}), whereby 10, 50 and 90% of the powder volume is represented by powder particles smaller than the size indicated. Particle size measurements were recorded when the laser obscuration reached 3–4%.

2.7. Powder solubility

The solubility of MPC powders was measured as described by McSweeney et al. (2020). Powder solubility was given by the total solids content of the supernatant (obtained following centrifugation at 3000*g* for 10 min) expressed as a percentage of the total solids content of the initial dispersion.

2.8. Statistical analysis

Measurements of the powder rehydration characteristics were performed in triplicate and results presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was performed using the IBM SPSS (Version 24; Armonk, NY, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. Wetting behaviour of milk protein concentrate powders

Wettability analysis by the GEA method showed that all milk protein concentrate (MPC) powders did not completely wet or submerge below the surface of the water within 10 min. However, the water became increasingly turbid for the regular (R) and agglomerated (A) nitrogen (N_2) injection powders (i.e., RN- and AN-MPC) compared to R- and A-MPC, in which the water remained relatively clear (data not shown). Bouvier et al. (2013) reported that extrusion-porosification, which created MPC powder particles with high porosity, did not improve wetting time as it had the same wettability index (>120 s) as a conventionally spray dried MPC powder. The capillary rise wetting behaviour, observed using the modified Washburn method, is shown in Fig. 1. A-MPC absorbed the most water (1.0 g) and R-MPC absorbed the least (0.43 g). The N_2 injection (NI) process appeared to improve capillary rise wetting for the regular powders as RN-MPC absorbed 0.74 g of water. However, for the agglomerated powders, AN-MPC absorbed a lower quantity of water (0.61 g) than A-MPC despite having a higher porosity. The difference in capillary rise wetting between R- and A-MPC may be explained by the differences in powder particle size; A-MPC had a $D_{[4,3]}$ of 142 μm while R-MPC had a $D_{[4,3]}$ of 79 μm. Similarly, Ji et al. (2016) reported water absorption levels of 0.24 and 1.0 g for non-agglomerated and agglomerated milk protein isolate (MPI) powders, respectively. It has been previously reported that agglomeration

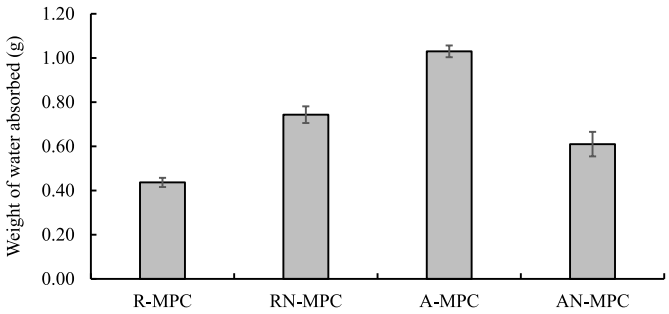

Fig. 1. Mean weight of water absorbed for regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders after submerging in ultrapure water (25 °C) for 20 min using a modified Washburn method.

improved the wetting behaviour of a native phosphocaseinate (NPC) powder due to the large powder particle size (mean = 285 μm) and high porosity (Gaiani et al., 2005, 2007). One of the main factors influencing powder wettability is the surface composition (Gaiani et al., 2006a), and the presence of fat specifically on the surface of spray dried powders would be expected to influence the wetting behaviour by increasing surface hydrophobicity. The surface composition of the powders in the current study were established previously by McSweeney et al. (2021), where it was reported that the NI powders had significantly higher amounts of surface fat (e.g., 2.02% for R-MPC and 11.1% for RN-MPC), while these samples still performed relatively well in powder wetting experiments. Kim et al. (2002) reported that surface fat had a strong, negative impact on the wettability of several dairy powders (e.g., cream, skim and whole milk powder), while Gaiani et al. (2006a) did not find a clear relationship between the surface fat of NPC powders and wetting times.

3.2. Visualisation of liquid phase water diffusion and effective diffusivity

Confocal laser scanning microscopy images showing the movement of the rhodamine B dye into R- and RN-MPC powder particles are displayed in Fig. 2. Complete diffusion of rhodamine dye into R-MPC powder particles took 1563 s (Fig. 2A) compared to 196 s for RN-MPC (Fig. 2B). This was likely caused by the significantly higher occluded air (R-MPC = 24.2 mL/100 g, RN-MPC = 36.3 mL/100 g) and porosity (R-MPC = 0.69, RN-MPC = 0.88) values reported by McSweeney et al. (2021) for this powder.

Large differences were observed in effective diffusivity between agglomerated and regular MPC powders (Fig. 3). AN-MPC had the highest effective diffusivity value of 8.09^{-12} m^2/s compared to 4.09^{-12} m^2/s for A-MPC. Conversely, the movement of rhodamine dye into R-MPC occurred at the slowest rate among all powders at 3.29^{-13} m^2/s, with RN-MPC slightly higher at 4.18^{-13} m^2/s. It is apparent that NI prior to spray drying assisted the transfer of the aqueous dye into the powder particles. The rate of diffusion was most likely higher for agglomerated MPC powders due to the larger powder particle size compared to regular MPC powders. The link between higher effective diffusivity and increasing powder particle size has been reported previously by Power et al. (2020) for enzymatically crosslinked MPC powders.

3.3. Water sorption isotherms

Water sorption profiles for MPC powders are displayed in Fig. 4. It is evident that water content and the time to reach equilibrium increased with increasing relative humidity (RH). Lactose crystallisation did not occur, as this is generally indicated by a sudden decrease in water content, while Kelly et al. (2015) also reported the absence of lactose crystallisation in MPC powders containing ~80% protein (w/w). NI promoted a faster uptake of moisture during the early stages of RN-MPC

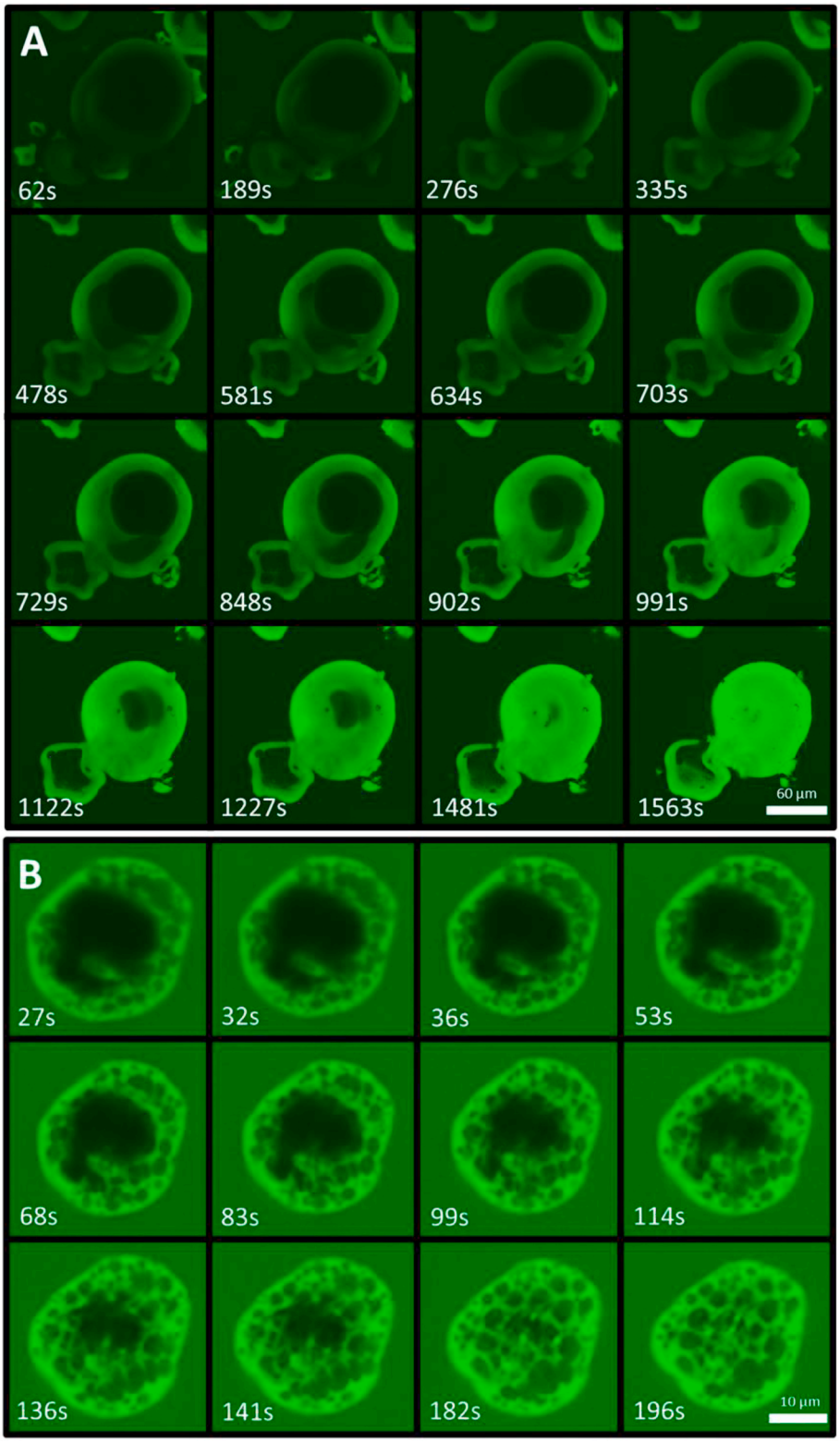

Fig. 2. Confocal laser scanning microscopy images showing the movement of rhodamine B dye into (A) regular and (B) regular with N_2 injection milk protein concentrate powders over time (s).

powder storage compared to R-MPC powders (Fig. 4A and B). For example, after 8 h at 85% RH, R-MPC had a water content of 7.2 g/100 g compared to 9.5 g/100 g for RN-MPC. However, after 144 h, R-MPC (18.2 g/100 g) and RN-MPC (17.8 g/100 g) powders had similar water contents. Agglomerated powders (Fig. 4C and D) absorbed more water overall than their non-agglomerated counterparts. After 8 h at 85% RH, AN-MPC had a water content of 14.9 g/100 g compared to 11.8 g/100 g for A-MPC. This trend was also evident for the effective diffusivity analysis presented in Section 3.2. whereby larger powder agglomerates favoured the movement of water into the particles. Particle size distribution has been previously identified as an important determinant of a materials water sorption behaviour. Mathlouthi and Roge (2003)

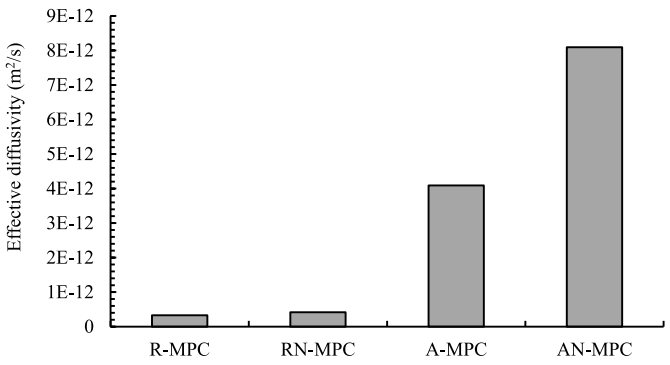

Fig. 3. Effective diffusivity (m^2/s) for regular (R), regular with N_2 injection (RN), agglomerated (A) and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) powders.

reported that smaller particles of sugar were capable of absorbing more water than larger particles, while Murrieta Pazos et al. (2014) observed a similar trend for durum wheat semolina. However, Ji et al. (2017) reported that MPI powders agglomerated using fluidised bed granulation showed similar water sorption, despite differences in particle size. In the current study, the surface composition of powders may have been a contributing factor as the surface of agglomerated powder particles was significantly higher in lactose than that of regular powders (McSweeney et al., 2021).

3.4. Measurement of mineral release using conductivity

The release of minerals from powder particles was complete by approximately 3000 s (Fig. 5). It is evident that R- and A-MPC released ions at a faster rate than both RN- and AN-MPC powders. The R- and A-MPC powders underwent wetting and sinking after approximately 600 s (time to reach steady state), which can be inferred from the beginning of the plateau on the graph. However, a surface barrier was evident during stirring for powders produced using NI and it took ~1400 s for this plateau to be reached. This result is likely related to the physical properties of the NI powders as they had lower bulk and particle density values and higher air contents. Masters (1985) reported that sinking of powder particles is supported by high particle density and low occluded air, while a low particle density will cause the powder to float on the surface of the water. Fitzpatrick et al. (2016) attributed the poor wettability (>1 h) of an MPI powder to its low apparent density (0.81 g/cm^3), which was similar to the particle density values for RN-MPC (0.96 g/cm^3) and AN-MPC (0.88 g/cm^3). It has been previously reported by Mimouni et al. (2010) that minerals (non-micellar material) are freely released during rehydration of MPC but that protein dispersion is the rate-limiting stage.

3.5. Particle size distribution and solubility of milk protein concentrate dispersions

NI significantly enhanced the dispersion of MPC powder particles following reconstitution (Table 1). When powders were mixed for 30 s at 23 °C, sub-micron particles were not present and all samples had monomodal volume-based distributions, suggesting that casein micelles were not released from primary powder particles (Fig. 6A); however, a significantly smaller particle size was observed for RN-MPC ($D_{[4.3]}$ = 32.6 μm) compared to R-MPC ($D_{[4.3]}$ = 79.6 μm). For agglomerated powders, the $D_{[4.3]}$ was significantly lower for AN-MPC (41.8 μm) in comparison to A-MPC (119 μm) under these conditions. The improvement in dispersion of RN and AN-MPC is likely accounted for by the powders lower loose bulk density and the higher porosity and interstitial space. The more porous structure of NI powder particles and the presence of large air voids between these particles would facilitate increased

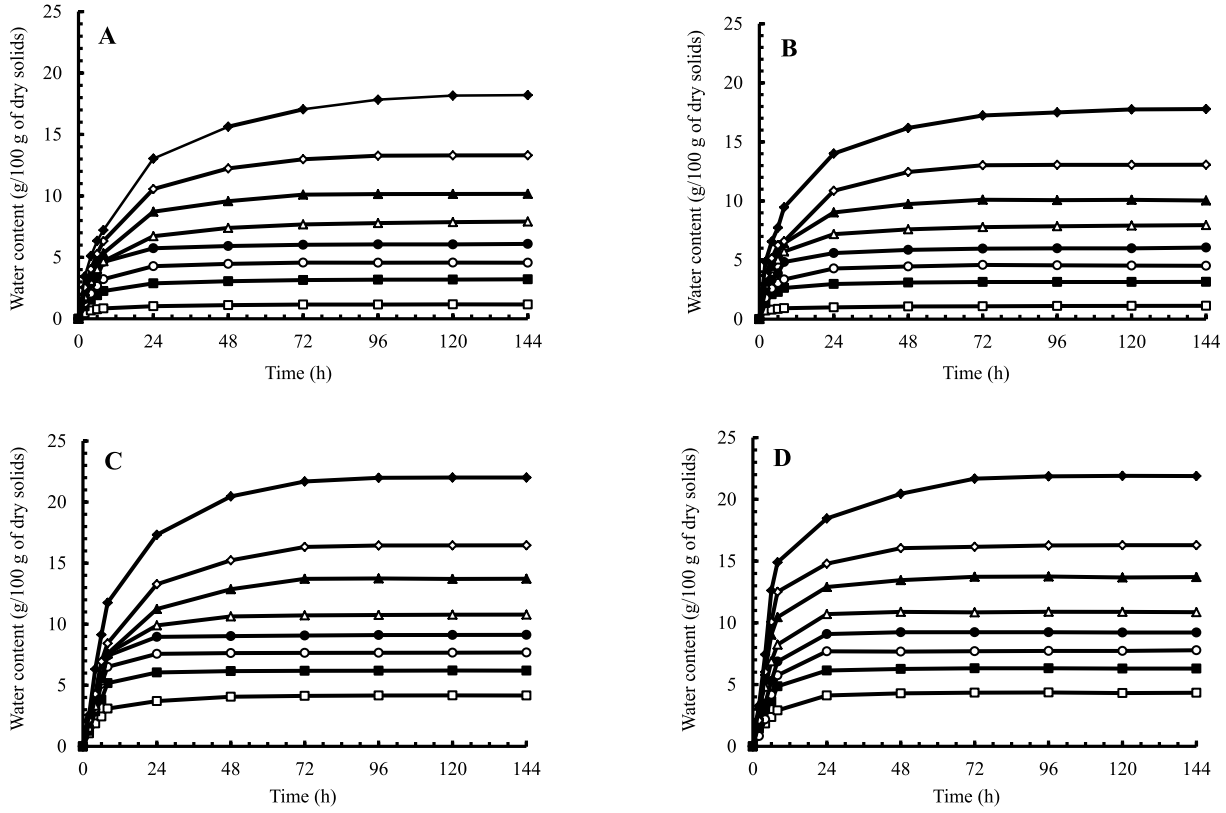

Fig. 4. Water sorption isotherms for (A) regular, (B) regular with N_2 injection, (C) agglomerated and (D) agglomerated with N_2 injection milk protein concentrate powders at relative humidity values of 11 (□), 23 (■), 33 (○), 44 (●), 55 (Δ), 65 (▲), 76 (◊) and 85% (◆) over 144 h.

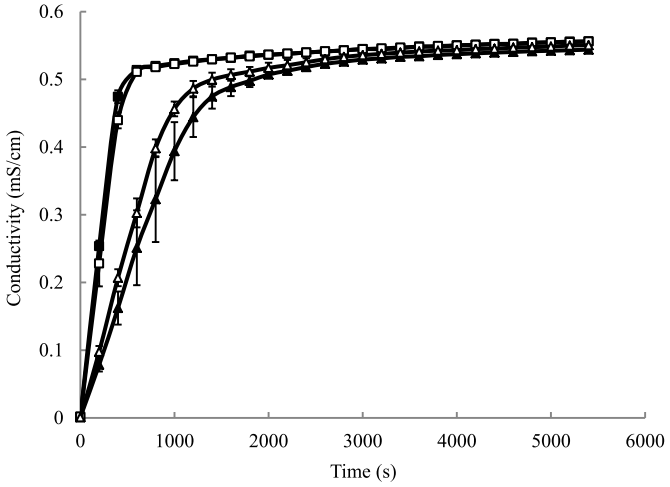

Fig. 5. Conductivity profiles of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders measured while stirring in ultrapure water (25 °C) for 90 min.

Table 1
Particle size distribution parameters for regular (R), regular with N_2 injection (RN), agglomerated (A), and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) dispersions after high-speed (HS) mixing at 23 °C and 50 °C for 30 s using a solubility index meter and after low-speed (LS) mixing at 23 °C for 1 h using a magnetic stirrer.

Rehydration conditions	MPC	D_{50}	D_{90}	$D_{[4,3]}$
		μm		
HS mixing 30 s at 23 °C	R	$68.4^{a} \pm 3.16$	$155^{a} \pm 6.83$	$79.6^{a} \pm 3.38$
	RN	$25.6^{b} \pm 0.85$	$66.0^{b} \pm 2.19$	$32.6^{b} \pm 0.81$
	A	$108^{c} \pm 4.09$	$224^{c} \pm 5.87$	$119^{c} \pm 3.67$
	AN	$35.5^{d} \pm 1.27$	$79.8^{d} \pm 1.75$	$41.8^{d} \pm 1.07$
HS mixing 30 s at 50 °C	R	$0.20^{a} \pm 0.07$	$66.0^{a} \pm 10.9$	$18.4^{a} \pm 4.69$
	RN	$0.09^{b} \pm 0.00$	$0.42^{b} \pm 0.02$	$1.59^{b} \pm 0.13$
	A	$0.33^{c} \pm 0.15$	$56.7^{c} \pm 5.29$	$19.6^{a} \pm 3.10$
	AN	$0.10^{b} \pm 0.00$	$0.52^{b} \pm 0.06$	$2.21^{b} \pm 0.36$
LS mixing 1 h at 23 °C	R	$76.6^{a} \pm 4.67$	$156^{a} \pm 6.46$	$83.7^{a} \pm 4.01$
	RN	$0.16^{b} \pm 0.05$	$51.4^{b} \pm 11.3$	$13.6^{b} \pm 3.97$
	A	$55.2^{c} \pm 7.67$	$129^{c} \pm 17.3$	$66.3^{c} \pm 8.44$
	AN	$0.19^{b} \pm 0.04$	$51.7^{b} \pm 5.78$	$14.4^{b} \pm 2.45$

[a-d] Values within columns not sharing common superscripts differ significantly ($P < 0.05$).

water transfer, while also increasing the physical space between casein micelles and reducing protein-protein interactions. This would appear to promote the structural collapse of powder particles when added to water, as interactions between poorly dispersible micellar casein, particularly at the particle surface, are considered to be responsible for the slow rehydration of MPC (Anema et al., 2006; Mimouni et al., 2009, 2010).

The water temperature used during reconstitution significantly affected the particle size distribution, with a higher temperature enhancing the fragmentation of MPC powder particles (Fig. 6B). When the temperature of the reconstitution water was 50 °C, the $D_{[4.3]}$ values were 18.4 μm and 1.59 μm for the R- and RN-MPC powders, respectively (Table 1). All powders had a bimodal volume-based distribution, with a peak <1 μm and a second peak in the size range of 8–300 μm. However, the volume of sub-micron particles was higher for RN- and AN-MPC compared to R- and A-MPC. This implies that a large quantity of particles present in the NI powder dispersions were casein micelles, suggesting higher levels of dissolution were achieved.

A-MPC powder had poorer dissolution properties as indicated by the larger particle size ($D_{[4.3]}$ = 119 μm) after reconstitution at 23 °C compared to R-MPC ($D_{[4.3]}$ = 79.6 μm). Therefore, the agglomeration of high-protein powders during spray drying appears to be counter-productive for improving rehydration, unlike its use in the production of skim and whole milk powders (Pisecky, 2012). Gaiani et al. (2005, 2007) reported similar results, whereby agglomeration increased the overall rehydration time of NPC powders as it delayed the dispersion process. However, in the current study, reconstitution at 50 °C resulted in no significant differences between agglomerated and regular (non-agglomerated) powders, with $D_{[4.3]}$ values of 20 and 18.4 μm for A-MPC and R-MPC, respectively (Table 1). This suggests that increasing the water temperature may moderately alleviate this issue with A-MPC dispersion.

MPC powders were also analysed after magnetic stirring for 1 h in ultrapure water at 23 °C, with bimodal particle size distributions obtained for NI powders compared to monomodal size profiles for non-NI powders (Fig. 6C). This corresponded to $D_{[4.3]}$ values of 13.6 μm for RN-MPC and 14.4 μm for AN-MPC compared to 83.7 and 66.3 μm for R- and A-MPC, respectively (Table 1). This result further highlights the improved dispersibility of the NI powders, at a relatively low reconstitution temperature and agitation rate, compared to non-NI powders. Mimouni et al. (2009) reported that 480 min of stirring at 24 °C was required to fully solubilise a MPC powder (85%, w/w, protein). Similarly, Gaiani et al. (2007) reported that 807 and 572 min of stirring at 24 °C were required to dissolve agglomerated and non-agglomerated micellar casein powders, respectively.

Aside from the particle size data of reconstituted powders, solubility results confirmed the superior dissolution of NI powders, particularly at 23 °C (Table 2), with R- and RN-MPC having 83.6 and 96.2% solubility, respectively. The lower loose bulk density and higher porosity for RN-MPC are also likely to be responsible for the higher levels of solubility (McSweeney et al., 2021). Similarly, A-MPC had a solubility value of 62.6% compared to 92.1% for the AN-MPC powder (Table 2). However, when powders were reconstituted at 50 °C, no significant differences in solubility were observed. It has been previously reported by Mimouni et al. (2009) that an increase in solvent temperature accelerates the release of constituent materials from MPC powder particles into the aqueous phase. Overall, these results indicate that physical and structural properties (e.g., density, air content and porosity) play a significant role in the rehydration of high-protein MPC powders.

4. Conclusion

This study demonstrated that the dissolution of MPC powder is enhanced by the injection of N_2 gas into the concentrate prior to spray drying. It is proposed that higher powder particle porosity and lower density are responsible for the improvement in rehydration performance. Water transfer during reconstitution was promoted by the presence of large air voids and pores throughout the powder particles, resulting in a large volume of small, dispersed particles (i.e., <1 μm). Agglomeration alone favoured powder wetting, water uptake and particle hydration; however, it had a negative impact on powder particle dissolution. Combining N_2 gas injection with agglomeration resulted in further improvements in diffusion and wetting behaviour but did not confer any additional improvement in dispersion and solubilisation of powder particles compared to N_2 gas injection alone. The injection of N_2 gas into high-protein concentrate prior to spray drying is a relatively simple and effective processing technology to enhance powder particle dispersibility and solubility, while avoiding the use of chemical additives which may disrupt casein micelle integrity. The impact of N_2 gas injection on bulk handling and other functional properties of MPC powders (e.g., density and dissolution) after storage and transport should be considered in future research.

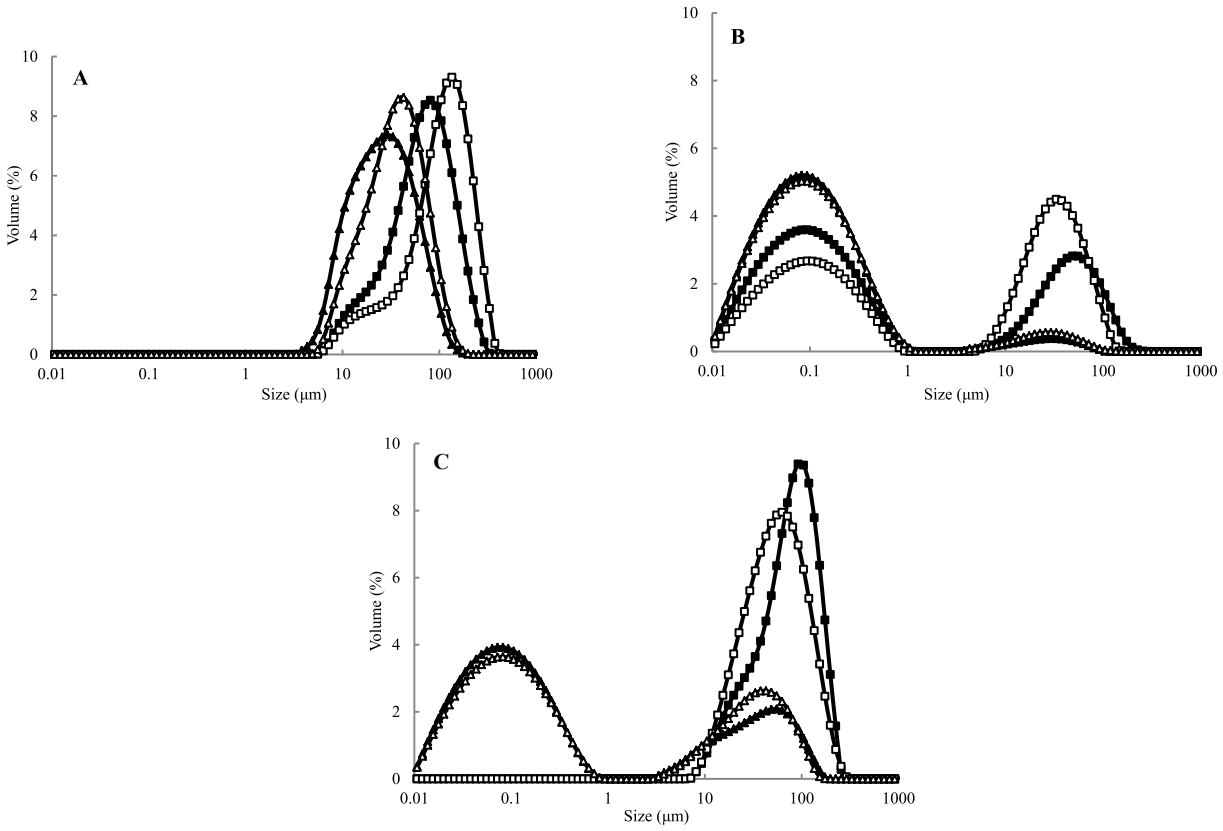

Fig. 6. Particle size distribution profiles of regular (■), regular with N_2 injection (▲), agglomerated (□) and agglomerated with N_2 injection (Δ) milk protein concentrate powders measured after reconstitution in ultrapure water for (A) 30 s at 23 °C, (B) 30 s at 50 °C and (C) 1 h at 23 °C.

Table 2
Powder solubility (%) of regular (R), regular with N_2 injection (RN), agglomerated (A), and agglomerated with N_2 injection (AN) milk protein concentrate (MPC) samples taken after mixing at 23 °C and 50 °C for 30 s using a solubility index meter and centrifuged at 3000*g* for 10 min.

MPC	Solubility (23 °C)	Solubility (50 °C)
R	$83.6^{a} \pm 1.9$	$96.7^{a} \pm 0.9$
RN	$96.2^{b} \pm 1.3$	$98.2^{a} \pm 1.1$
A	$62.6^{c} \pm 6.9$	$96.6^{a} \pm 0.7$
AN	$92.1^{a} \pm 3.1$	$98.1^{a} \pm 0.3$

[a-c] Values within columns not sharing common superscripts differ significantly ($P < 0.05$).

Credit author statement

David J. McSweeney: Conceptualization, Methodology, Formal analysis, Investigation, Visualisation, Writing – original draft, Valentyn Maidannyk: Methodology, Investigation, James A. O'Mahony: Funding acquisition, Supervision, Writing – review & editing, Noel A. McCarthy: Funding acquisition, Project administration, Conceptualization, Supervision, Writing – review & editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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