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Investigation of the impact of pre- and post- drying treatments on the physicochemical and functional properties of whey powders

Thesis presented by

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B.Sc. Food Science, University College Cork

for the degree of

Master of Science

in

Food Science and Technology

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Declaration

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

Signature:

Emma Gordon

Emma Jane Gordon Date: 07/07/2025

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Dedication

This thesis is dedicated to my parents for their continuous support and encouragement.

Abstract

Whey powder is a widely utilised ingredient produced in large volumes by the dairy industry. It is a versatile ingredient used in many products including animal feed, soups, bakery, confectionary, powder beverages, snack seasonings and dairy products.

Physicochemical and functional properties of whey powder are key in determining the processability, handling, storage, use and overall quality of the ingredient. Various unit operations and process technology interventions can be implemented to modify these properties. Thus, creating innovative powders that are tailored and optimised for particular end use application. These processes can be applied not only on the finished powder, but also to the wet feed prior to drying. The objectives of the work reported in this thesis were to investigate the impact of selected pre- and post-drying treatments on the physicochemical and functional properties of whey powder at three different points throughout the year, to take account for seasonality.

The first study (Chapter 2) explored the impact of in-line shearing of precrystallised whey concentrate on physicochemical and rehydration properties of the resultant powder. The results demonstrated that shearing whey concentrate prior to drying reduced the resultant powder particle size, along with significantly altering the powder colour, particle morphology, powder density, and viscosity of reconstituted whey powder. The shearing process had no effect on the flowability or rehydration properties of the powder.

Chapter 3 investigated the impact of altering the particle size post-drying on the physicochemical and functional properties of whey powder. The whey powder was agglomerated by coating the particles in a whey solution, while the powder was fluidised using a pilot-scale fluid bed dryer. The results showed that the agglomeration process, caused an

increase in particle size, with concomitant effects on particle morphology, colour, density, flowability and protein content.

In addition to agglomeration, the whey powder was also milled to two different extents, producing two whey powders with reduced particle size. The milled powder's colour, morphology, shape, density, flowability, and levels of protein denaturation were all altered as a result. In addition, the performance of the agglomerated and milled powders was also analysed in a simulated beverage and snack seasoning application systems.

The results from these two experimental studies demonstrate that, by shearing the crystallised concentrate pre-drying, the particle size of the resultant powder can be reduced, while the physicochemical and rehydration properties are not impacted as severely as they are for post-drying treatments (Chapter 3). The findings from this study will allow dairy researchers and processors to deepen their scientific understanding of the impact of pre- and post- drying treatments on the physical and functional properties of whey powder. This will enable the production of whey powders with physical and bulk handling properties optimised for selected applications.

Chapter 1

Introduction and review of relevant scientific literature

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Declaration

The relevant literature was critically reviewed by Emma J. Gordon (E.J.G.). This chapter was written by author E.J.G. and reviewed by all co-authors

1.1 Introduction

It is only in the last 3-4 decades that whey has been viewed as a nutritional and economic opportunity, having originally been disposed of in water ways, spread on the land and/or fed to animals (Macwan *et al*., 2016). Today, whey is generally regarded as a valuable co-product from the production of casein and cheese (Adamson, 2015). Globally, approximately 121 million tons of liquid whey is processed each year (Ozel *et al*., 2022) of which, approximately 60% is converted into value-added whey products through additional processing (Macwan *et al*., 2016). These products include whey powder, whey protein concentrate, whey protein isolate, lactose, whey permeate and demineralised whey powder (Ozel *et al*., 2022).

Regular whey powder is essentially the most basic product than can be produced from whey, and has a wide range of applications, including but not restricted to; animal feed, soups, bakery, confectionary, powder beverages, snack seasonings and dairy products (Chegini and Taheri, 2013; Adamson, 2015). Due to its versatile nature, 1.89 million tons of whey powder was produced in the European Union in 2019, with levels projected to reach 2.44 million annually by 2030 (Ozel *et al*., 2022). The global whey powder market size expanded to €5.15 billion in 2023 and is expected to grow further in the years to come (IMARC, 2024). Ireland has a significant role in the global whey processing sector, with the value of Irish exports of whey powder estimated at €250 million in 2024; however, this was €90 million lower than the previous year due to a reduction in the value of whey, as well as lower volumes of regular whey powder being produced (Bord Bia, 2024).

Whey powder production processes involve the integration of several relatively basic unit operations to separate the whey stream from casein, remove water to concentrate the solids,

crystallise the lactose and finally, dry whey concentrate to a dry finished powder. The liquid whey stream can be separated from casein enzymatically through rennet and/or starter culture addition, utilizing microfiltration technology, or through the addition of an acidogen to decrease the pH (Jelen, 2011). Such whey streams contain approximately 5-6% solids, which needs to be further concentrated to achieve suitable conditions for the crystallisation of lactose. This is completed by the removal of water, traditionally using evaporation. Nowadays, this is mostly done through a combination of evaporation and membrane concentration technologies such as nanofiltration (NF) and/or reverse osmosis (RO) (Kumar *et al*., 2013; Reig, Vecino and Cortina, 2021).

To further concentrate the whey solids up to approximately 60%, water is generally removed through evaporation, most commonly, using a falling-film vacuum evaporator (Cunningham *et al.,* 2006) which can be done under vacuum to allow operation at lower temperatures, usually 30-70 °C (Zhang *et al*., 2018).

This low temperature operation provides functional benefits to the whey as it protects the naturally heat-sensitive whey proteins (Gandhi, Amamcharla and Boyle, 2017). Once the whey feed has been sufficiently concentrated, it is rapidly cooled to 30°C and transferred to whey crystallisation tanks with continuous agitation to initiate the crystallisation of lactose (Kilara, 2016). These tanks further cool the whey down to 15-20°C over a period of 12-16 h, to promote the growth of small uniformly sized, tomahawk-shaped lactose crystals. This crystallisation process is extremely important in the production of whey powder. This is due to the associated high lactose of the product causing several challenges during processing and in the finished powder product. If the lactose is not sufficiently crystallised (i.e., a target of 75-80%

crystallised) (O'Donoghue *et al*., 2019), and remains in the glassy state prior to drying, this can result in detrimental effects such as product sticking to equipment during drying and caking of the powder during storage (Huppertz and Gazi, 2016). The pre-crystallised whey concentrate is then subsequently spray-dried, with rotary wheel atomisers generally used for whey products due to its ability to handle products with crystals as well as high viscosities (Refstrup and Bonke, 2011).

Whey powders, along with other dairy powders, are principally characterised by their composition, such as concentrations of protein and ash; however, physical characteristics and rehydration properties of the powders can be equally important (Chegini and Taheri, 2013). Physicochemical and functional properties such as particle size, particle shape, morphology, colour, flowability, density, rehydration ability and denatured protein content are all essential characteristics of whey powder. These properties can be included in quality specifications; if these specifications are not met, they can be rejected by the customer (Chegini and Taheri, 2013). In order to continually meet the adapting needs of industry and end-use consumers, these properties can be targeted and altered pre- and post-drying in a variety of different ways to produce powders with optimal physicochemical characteristics depending on industry processing needs and end-use application.

A number of studies have investigated pre-drying modifications to dairy powders in order to alter the functional and/or physicochemical properties of the resultant feed and/or powder. This includes using an inline high-shear mixing method to produce fat-filled milk emulsions (O'Sullivan *et al*., 2018), injection of carbon dioxide before and during ultrafiltration to reduce mineral content and viscosity for more optimal functionality (Marella *et al*., 2015), and

utilizing twin screw extrusion porosifcation technology (EPT^{TM}) to reduce viscosity of the feed and improve rehydration properties of the powder (Patil *et al*., 2021). All of these treatments have successfully affected the resultant feed and/or powder in a variety of ways; however, in-line shearing aimed at reducing the particle size of powder post-drying has not been investigated. The effect of inline shearing on crystallised whey concentrate and the subsequent impact on the physicochemical and functional properties of the resultant whey powder is investigated in Chapter 2.

Many post-drying alterations of dairy powders have also been investigated, in particular, utilising techniques to alter particle size post-drying such as through agglomeration and milling. These techniques have been examined to understand their effects on physicochemical and functional properties. Agglomeration methods to improve the flowability and initial rehydration of high protein dairy powders have also been studied (Hazlett, Schmidmeier and O'Mahony., 2021b; Wu *et al*., 2022). As well as increasing the particle size through agglomeration, superfine grinding is an emerging technique that can be implemented post-drying during food powder processing (Gao *et al*., 2020). Superfine ground food powders can have improved physicochemical properties such as flowability (due to altered particle surface composition) and density, as well as optimised functional properties of the powder including higher bioavailability and improved flavour release and mouthfeel (Gao *et al*., 2020). However, the studies detailed in these review papers did not investigate the post-drying alterations on a lactose rich powder such as whey powder as investigated in Chapter 3.

1.2 Components of milk

All dairy products are derived from liquid milk, and the composition of this milk varies due to a variety of factors such as breed of the cow, seasonality and health status (Chandan, 2016). As reported by Lindmark-Månsson (2008), the average composition of raw milk throughout an entire year was 87% water and 13% milk solids, with the milk solids consisting of 4.0% fat, 3.5% protein, 4.5% lactose and 1% minerals.

1.2.1 Protein

Milk proteins provide young animals with essential amino acids, vital for their growth and development. Protein in milk can be classified into two major fractions; casein and whey proteins; these fractions are present in bovine milk at an ~80:20 ratio (Fox *et al*., 2015a). The characteristics of many dairy products depend on the properties of milk proteins, and these characteristics can be altered during processing, which can impact on the resulting products in desirable and undesirable ways (Fox *et al*., 2015a).

1.2.1.1 Casein

Caseins are small, random coil, unstructured proteins. There are four primary classes of casein; α_{s1}-, α_{s2}-, β-, and κ-casein (Deeth and Hartanto, 2009). Caseins are present in milk as colloidal particles known as micelles, with the hydrophobic caseins preferentially at the core of the micelle while the more hydrophilic caseins, i.e., κ-casein, are present on the surface of the micelle (Kailasapathy, 2016). In the presence of calcium ions (Ca^{2+}), at the isoelectric point (pH 4.6) and at approximately 30°C, casein and whey protein can be separated (Fox *et al*., 2015), producing acid casein. Casein and whey protein can also be separated with the addition of enzymes such as rennet. Under these conditions, caseins precipitate out of solution, which,

with further processing, produces rennet casein, whereas whey protein remains in a soluble form in the serum phase (Fox *et al*., 2015a). Casein can also be separated from whey protein through filtration, creating micellar casein concentrate (MCC) with the permeate from such production being known as ideal whey (Hammam, Martínez-Monteagudo and Metzger, 2021).

1.2.1.2 Whey protein

Whey protein consists of a variety of different proteins including, β-lactoglobulin (β-Lg), α-lactalbumin (α-La), glycomacropeptide (GMP), blood serum albumin (BSA), immunoglobulins and lactoferrin (LF) (Deeth and Hartanto, 2009). β-Lg, α-la and GMP are present in whey at higher proportions compared to the other minor proteins such as BSA, immunoglobulins and LF. The whey protein fraction also contains a small amount of proteose-peptone, which is a complex mixture of peptides (Fox *et al*., 2015a). Compared to the heat-resistant caseins, the heat stability of whey protein is low, as denaturation and aggregation of whey proteins can occur at temperatures of 75°C and above (Kailasapathy, 2016).

1.2.1.2.1 Major whey proteins

β-Lg is the principal whey protein in bovine milk and represents 50% of total whey protein and approximately 10% of total milk protein. There are 162 amino acids present in β-Lg and with a molecular weight of 18 kDa (Kilara, 2016). It is a compact globular protein with two principal genetic variants, and existing in milk as a dimer (Sawyer, 2013). β-Lg unfolds and denatures at temperatures greater than 75°C, when a free sulfhydryl group in cysteine residues is exposed, which can then participate in protein-protein interactions by creating disulphide linkages (Deeth and Hartanto, 2009). In β-lg, the disulphide bond only occurs between Cys_{106} and Cys_{119} (Creamer, Loveday and Sawyer, 2011). Such disulphide bonding can also occur between β-lg

and κ-casein proteins with the potential to significantly affect the rennet coagulation and heat stability characteristics of milk (Fox *et al*., 2015a).

α-La is the second most prevalent protein found in whey (Kilara, 2016), making up approximately 13% of the total whey protein, which equates to 2% of the total milk protein (Kilara, 2016). It consists of 123 amino acids (Kilara, 2016) and has a molecular mass of 14 kDa, which is a lot smaller than other whey proteins (Brew, 2013). The α-la molecule consists of four disulfide linkages with no phosphate groups (Kilara, 2016). In milk, the function of α-la is to bind one calcium ion (Ca^{2+}) in a pocket in its structure with four aspartate residues. At pH 5 and below, it loses its ability to bind Ca^{2+}; once it has lost this calcium ion, α-la becomes particularly heat sensitive and can be denatured at low temperatures such at 78°C (Fox *et al*., 2015a).

GMP is a casein-derived peptide obtained from the glycosylated N-terminal region of κ-casein, which occurs due to the action of rennet on the κ-casein molecule (O'Mahony and Fox, 2013). GMP is the glycosolated version of caseinomacropeptide (CMP), meaning there are carbohydrates attached to the protein portion (Huppertz, 2013). When enzymes, such as rennet, are added to milk during cheese making, the Phe_{105}–Met_{106} bond of κ-casein is cleaved resulting in κ-casein being split into *para*-κ-casein and GMP. The GMP that was once attached to casein is then released into the whey stream (Brody, 2000; Huppertz, 2013), hence, GMP is only present in whey streams that are produced from enzymatic action.

1.2.1.2.2 Minor whey proteins

BSA is found in low amounts in bovine milk and is thought to be present because of leakage from the blood in the animal. It has a molecular mass of approximately 65 kDa (Fox *et al*, 2015a) and contains 583 amino acids (O'Mahony and Fox, 2013). In blood, it is known to serve several functions; however, in milk, it performs little to no function, due to its relatively low concentration (O'Mahony and Fox, 2013; Fox *et al*., 2015a).

Immunoglobulins are a set of minor proteins present in low concentrations in whey. Depending on the stage of lactation, breed, age and health status of the cow, concentrations of immunoglobulins in milk can vary significantly. Out of five immunoglobulin classes, three of these exist in milk, (IgA, IgG and IgM), which occur as subclasses made up of heavy and/or light chains that are linked together, which is specific to each Ig type (O'Mahony and Fox, 2013; Fox *et al*., 2015a). These types of proteins are particularly rich in colostrum when compared to mature milk and can provide passive immunity to the neonate (Bell, 2000; O'Mahony and Fox, 2013). Immunoglobulins in general are made up of two light chains and two heavy chains, linked together by disulfide bonds and bind antigens. The purpose of immunoglobulins in bovine milk is to provide immunity to the calf against infections, as calves are born with little to no protective antibodies (Fox *et al*., 2015a).

LF is a red-coloured, iron-binding, glycoprotein present in low concentrations in cow's milk. Colostrum contains 5 g l^{-1} of LF and this reduces to around 0.1 g l^{-1} in mature milk (Korhonen and Marnila, 2011). In recent years, LF has gained commercial interest as a health promoting product due to the suggestion that LF is associated with the immune system in fighting diseases and enhancing iron absorption (Lönnerdal and Suzuki, 2013; Korhonen and Marnila, 2011). It

is a single-chain, glycosylated protein with 689 amino acids and has a molecular mass of approximately 80 kDa (Korhonen and Marnila, 2011; Lönnerdal and Suzuki, 2013). Not only can LF bind iron in biological systems, but it can also bind other metal ions such as copper and aluminium (Lönnerdal and Suzuki, 2013).

1.2.1.2.3 Whey protein denaturation and aggregation

Whey proteins are heat-sensitive proteins (Dissanayake *et al*., 2013). Whey protein denaturation is defined as a process by which globular proteins unfold and lose their native state, which is followed by aggregation, involving the denatured proteins linking together by bonds from binding sites which were made available due to the unfolding proteins. This aggregation can then result in gel formation and/or precipitation (Mulvihill and Donovan, 1987; Adel *et al*., 2008). The first step of denaturation is where weak bonds in the protein external structure break, which brings about unfolding of the protein. This unfolding causes exposure of hydrophobic groups, disulfide bonds and free sulfhydryl groups. Because of these available connections, new linkages form either intra- or inter- molecularly, which results in aggregation of protein molecules (Mulvihill and Donovan, 1987).

The denaturation temperature of β-lg is 78°C and, of all the whey proteins, heat affects it the most (Hollar *et al*., 1995). As it is the most abundant whey protein, it has the greatest impact of the effect of heating (Hollar *et al*., 1995). In whey products, reactions can occur between many different proteins, in particular β-lg and α-la. During denaturation, β-lg can self-assemble into thin and long fibrils and these structured aggregates of fibrils can cause a considerable increase in viscosity (Creamer, Loveday and Sawyer, 2011). Whey protein denaturation can be facilitated by a variety of different external factors including, changes in pH, addition of

destabilizing substances or enzymes, application of high pressure and the most common, the addition of heat (Mulvihill and Donovan, 1987). As heating is commonly used within industry as a method of preservation and protection, heat sensitive materials such as whey proteins can be irreversibly affected. If whey protein denaturation occurs during processing, this can be evident through an increase in viscosity of the product due to intra/intermolecular interactions occurring (Creamer, Loveday and Sawyer, 2011). This viscosity increase can then result in protein fouling on surfaces of equipment, which can build up to cause back pressure and blockages of pipes during processing such as in the evaporator (Deeth and Hartanto, 2009; Creamer, Loveday and Sawyer, 2011; Gandhi, Amamcharla and Boyle, 2017; Ozel *et al*, 2022). These issues are not only seen during processing, which lead to increased down time in the plant with additional use of chemicals and energy to remove this build up, but also affect finished product quality as this denaturation tends to impair the functional properties of whey proteins such as solubility and foaming (Mulvihill and Donovan, 1987).

1.2.2 Lactose

Lactose is the principal carbohydrate in milk and is produced in the mammary gland of lactating animals (Jelen, 2022). There are two main functions of lactose in milk, providing energy to the calf and is primarily responsible for the osmotic pressure during the synthesis of milk. The concentration of lactose in milk can dictate how much water is drawn through the Golgi vesicles, having an impact on milk volume produced (Fox et al., 2015b).

1.2.2.1 Structure and crystallisation

Lactose is a disaccharide made up of glucose and galactose, linked together by a β1-4 glycosidic bond (Fox, 2011a). It is a reducing sugar that can undergo Maillard browning in the

presence of amino acids and heat, creating a brown colour which can be desirable or undesirable depending on the desired colour in the finished product (Kaliaspathy, 2016). As lactose is a reducing sugar, it exists partly as an open-chain structure with an aldehyde group which can form a hemi-acetal and therefore a ring structure. The presence of a hemi-acetal creates an asymmetric carbon (Jelen, 2022). While the asymmetric carbon in the lactose structure is not stable, it allows lactose to occur interchangeably as two optical isomers, α-lactose and β-lactose (Fox, 2011a). When in solution, lactose can alternatively open and form the ring structure and the molecule can mutarotate between the α- and β- forms of lactose to reach equilibrium (Fox *et al*., 2015b). α and β-Lactose have very different properties in terms of solubility and specific rotation (Jelen, 2022). At 20 °C, a lactose solution in equilibrium is composed of 62.7% β-lactose and 37.3% α-lactose; thus, in these conditions, the equilibrium constant is 1.68 (Fox *et al*., 2015b). As the temperature of the solution increases, the proportion of α-lactose in equilibrium increases, and hence the equilibrium constant decreases. The rate of mutarotation between α- and β- forms is dependent on temperature and pH (Fox *et al*., 2015b).

Lactose crystallisation is a critically important step in the production of whey powders. This crystallisation step has a major impact on subsequent processing steps, including drying and storage stability (Schuck, 2011a). The optimal crystalline version of amorphous α-lactose is α-lactose monohydrate and contains 5% water of crystallisation (Fox *et al*., 2015b). If the lactose is not crystallised prior to being spray-dried (i.e., the lactose remains in the amorphous form) this can affect the quality of the final powder (Kailasapathy, 2016). Amorphous lactose, under certain conditions of temperature (glass transition temperature) and relative humidity, will transition into a crystalline form, which involves transitioning from a glassy to a rubbery state. As the conditions get closer to the glass transition temperature, the lactose molecules gain more

molecular mobility, thus resulting in a sticky behaviour, which may happen during spray drying or upon storage (O'Donoghue *et al*., 2019). This phenomenon is mainly due to the differing levels of hygroscopicity of crystallised lactose compared to that of amorphous lactose, which results in caking and stickiness of the finished product where high levels of amorphous lactose are present (Schuck, 2011a). This is due to crystalline α-lactose monohydrate binding one water molecule, and thus there is less free water in a powder composed of this crystalline substance compared to that of amorphous α-lactose (Jelen, 2022). Crystallizing 75-80% of amorphous lactose prior to spray-drying can alleviate some of these caking/sticking quality challenges in whey powders (O'Donoghue *et al*., 2019).

Before crystallisation occurs, seed crystals of α-lactose monohydrate may be added to the crystallisation tank to promote crystallisation of lactose (Deeth and Haratanto, 2009). Lactose seeds are added to induce secondary nucleation, which prompts lactose crystals to form at lower levels of supersaturation, helps to ensure more consistent size populations of the crystals and requires a lower activation energy (Wong and Hartel, 2014). Otherwise, if lactose seeds are not added, primary nucleation, also known as spontaneous crystallisation, must occur to produce lactose crystals. As seen in Figure 1.1, this occurs when the lactose solution is in the supersaturated state, which is dependent on the concentration of lactose present in solution as well as the temperature of the solution (Fox *et al*., 2015b). Once an adequate number of crystal nuclei have been formed, the growth rate of the crystals is affected by a number of factors, such as concentration, agitation, temperature and viscosity of the solution (Fox *et al*., 2015b). In general, lower temperatures and higher concentrations of lactose favour spontaneous crystallisation (Fox, 2011a),

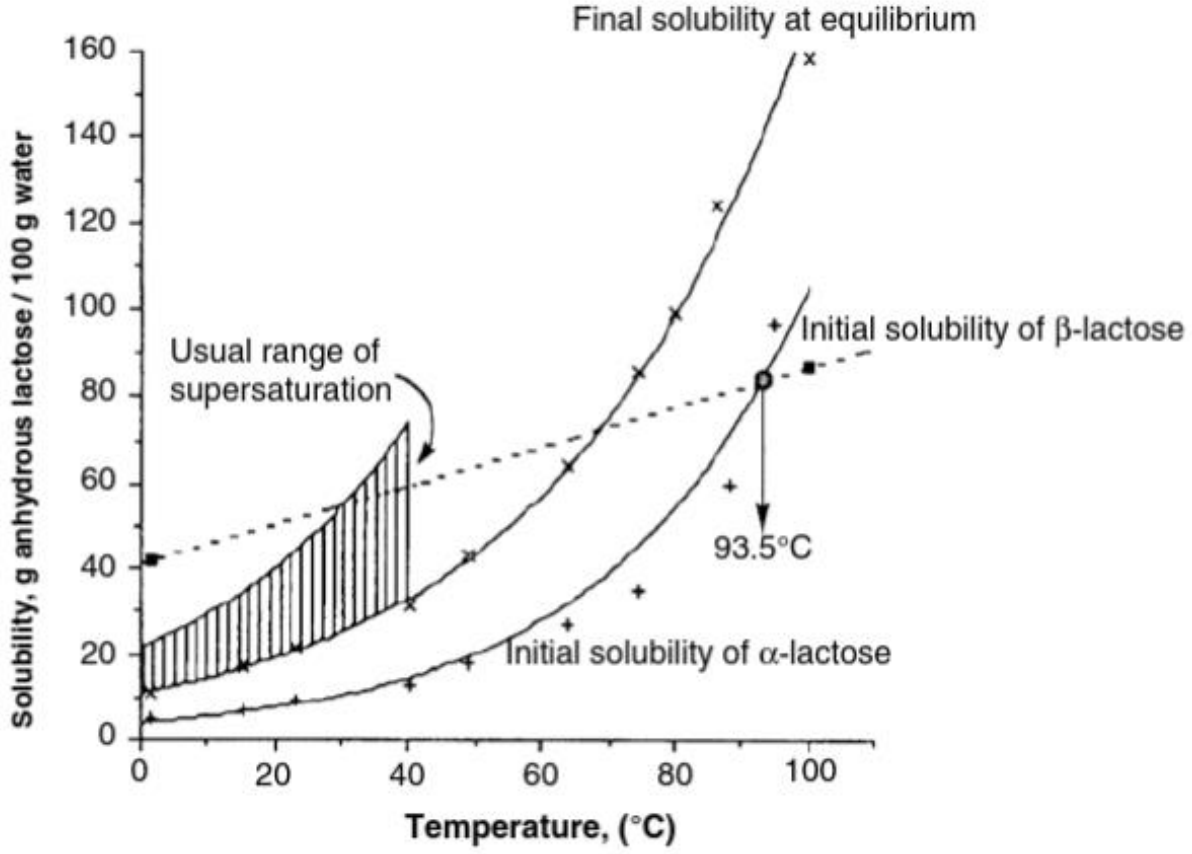

Figure 1.1: Solubility of lactose in water as a function of temperature, demonstrating how supersaturation can promote the crystallisation of lactose (Fox, 2011a)

During crystallisation, α-lactose is transformed into crystalline α-lactose monohydrate, which leads to the mutarotation of β-lactose to the α-form to keep the equilibrium constant (Kailasapathy, 2016). In isolation, crystalline α-lactose forms tomahawk shape crystals, as seen in Figure 1.2 (Fox *et al*., 2015b). However, processing conditions and the presence of other components, such as whey proteins, can interfere with the traditional shapes of the lactose crystals (Kailasapathy, 2016) and cause other crystal shapes such as flat triangles, needles, pyramids, prisms and half-moons to form (Jelen, 2022). α-Lactose monohydrate crystals can range in size; however, it is desirable for them not to exceed 50-100 μm, so as to not impede the subsequent spray-drying process or result in gritty dried powder (Jelen, 2009). During

production, to form small crystals, flash cooling followed by slow controlled cooling of 2°C/h over 12-16 h is best practice (Jelen, 2009).

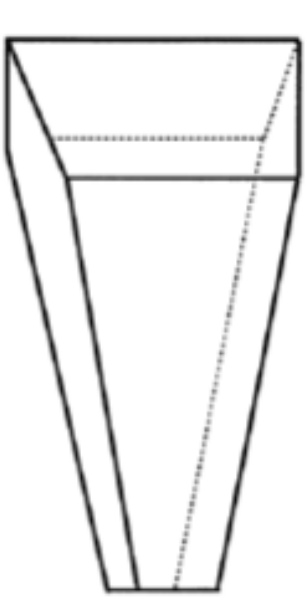

Figure 1.2: Schematic representation of α-lactose monohydrate crystal showing the typical tomahawk structure (Fox et al., 2015b)

1.2.2.2. Solubility

The solubility of a solute in a solvent is defined by the maximum amount of the solute that can be dissolved at a given temperature and pressure in the solvent. In terms of lactose, once the solution is saturated, the crystallisation process may begin (Schuck, 2011a). Compared to other sugars, lactose has a low solubility (Fox *et al*., 2015b); the solubility of α-and β-lactose are 74 g and 480 g/1000 g H_2O at 20°C, respectively. The equilibrium ratio of 62.7 β: 37.3 α between these two forms results in an overall solubility of approximately 18.2 g lactose per 100 g water at 20°C (Fox *et al*., 2015b).

Many factors are known to affect solubility of lactose. As temperature decreases, the solubility of lactose decreases, which can be exploited when crystallising lactose by cooling a lactose solution during processing to promote lactose crystallisation. The presence of other components can also have positive and negative effects on solubility. For example, whey proteins are said to reduce solubility of lactose (Bhargava and Jelen, 1996) and accelerate the rate of nucleation in the crystallisation process (Huppertz and Gazi, 2015). The impact of whey proteins on lactose crystallisation is not fully understood; however, it is possible that the water-binding ability of whey proteins may create areas of supersaturation of lactose, which leads to this reduction in solubility and increase in the rate of nucleation (Huppertz and Gazi, 2015).

1.2.3. Fat

Bovine milk fat can vary in concentration in raw liquid milk from 3.5-5% (Jensen, Ferris and Lammi-Keef*.,* 1991). There are approximately 400 different fatty acids in milk fat, which exist mostly (98%) in the form of triacylglycerols (Taylor and MacGibbon, 2011). Fat-soluble vitamins A, D, E and K, steroids, carotenoids and phospholipids make up the remaining 1-2% of milk fat (Kailasapathy, 2016). The concentration and type of fatty acids present in the milk fat is dependent on the feed source and microbial activity in the rumen (Lindmark-Månsson, 2008). Lipids in milk are present in the form of fat globules, which are protected and surrounded by a milk fat globule membrane (MFGM) (Taylor and MacGibbon, 2011). The MFGM acts as an emulsifier in the oil-in-water system of milk, protecting the fat globules from coalescence, as well as shielding the lipids from enzymatic degradation (Dewettinck *et al*., 2008). These globules are microscopic, with diameters ranging from ~0.1–20 mm; however, 75% of all globules have a diameter of <1 mm. Like many properties of bovine milk, the size

of these fat globules is dependent on many factors, including the breed and stage of lactation of the cow (Taylor and MacGibbon, 2011).

1.2.4 Minerals and other minor components of whey

Bovine milk minerals, also known as milk salts, are very important for the growth and development of young calves (Lucey and Horne, 2022). The most abundant minerals in milk are sodium, potassium, calcium, magnesium, phosphorous, chloride and sulphate. There are also other trace minerals present, which includes iron, zinc, copper, selenium, iodine, manganese and fluoride (Hill, 2022). These milk minerals constitute 8-9 g per litre of milk and are distributed between both the colloidal and serum phases of the milk; this equilibrium is referred to as the salts equilibria (Gaucheron, 2011). During the separation of casein and whey, minerals can move between the phases, which is largely dependent on the choice of manufacturing conditions. For example, when acid is used to disrupt the casein micelle, this results in calcium and phosphorus solubilizing into the serum phase. On the other hand, when rennet is used for destabilization of casein, the calcium and phosphorus present in the micellar phase remain in the micellar phase, which results in acid whey having higher contents of calcium and phosphorus compared to rennet whey (Deeth and Hartanto, 2009).

Different minerals are present in milk in various quantities, as depicted in Table 1.1 (Fox *et al*., 2015c). Factors such as the breed of cow and stage of lactation can alter the concentration of minerals present (Fox *et al*., 2015c). Milk salts can have a major influence on texture of final products, as well as protein stability during processing (Lucey and Horne, 2022). Some dairy products, such as demineralized whey, employ processing steps to remove minerals from the raw material, which is discussed in more detail in Section 1.3.3. This demineralisation process

is significant to meet customer demands, such as needs from the infant formula market or for flavour in some confectionary applications.

Table 1.1: Typical profile of minerals found in milk, adapted from Fox *et al*. (2015c)

Constituent	Average content (mg/l milk)
Sodium	500
Potassium	1,450
Calcium	1,200
Magnesium	130
Phosphorus (total)	950
Chloride	1,000
Sulphate	100
Carbonate (as CO_2)	200
Citrate (as citric acid)	1,750

In Western diets, the primary source of calcium is in milk and dairy products (Hill, 2022). Approximately two thirds of calcium in milk is present in the micellar phase, where it forms micellar calcium phosphate complexes (Gaucheron, 2011). The rest of the calcium in milk is soluble in the serum phase and present as non-micellar calcium and free ionic calcium (Deeth and Hartanto, 2009). Calcium in its ionic form, which represents 10% of the total calcium in milk, helps stabilize the casein micelle as it counteracts the negative charge associated with caseins at the pH of milk (Lewis, 2011). Phosphorus in bovine milk is present in organic and inorganic forms (Gaucheron, 2013). The organic form of phosphorus is mostly associated with

casein in the micellar phase, whereas the inorganic phosphorus is distributed evenly between the micellar and aqueous phases (Gaucheron, 2013). Potassium is an essential nutrient that can regulate cell membrane potential, which leads to the regulation of blood pressure, nerve and muscle activity. Bovine milk contains a significant amount of potassium when compared to human milk. Because of this, bovine milk can be a main contribution to potassium needs for a person consuming a western diet (Hill, 2022).

Vitamins are found in foods at very low concentrations, while they are essential for maintaining bodily function (Hill, 2022). Milk contains both fat-soluble vitamins (A, D, E and K), which are present in the lipid portion of milk, and water-soluble vitamins (B and C), which are found in the aqueous phase (Fox *et al*., 2015d; Hill, 2022). In particular, milk provides significant contribution of an adults daily required intake of vitamins A, D and B_{12} (Hill, 2022). Vitamin A has the same biological activity of retinol and aids the vision process. It is relatively stable during processing, unless temperatures exceed 100°C, such as through the production of ultra heat treated (UHT) milk, when there may be some loss (Fox *et al*., 2015d). Vitamin D maintains the level of plasma calcium in the body by stimulating the absorption from the gastrointestinal tract (Fox *et al*., 2015d). The majority of milk in the United States is fortified with vitamin D to aid consumers in reaching their daily intake. It is an essential vitamin which plays a critical role in the prevention of chronic diseases like cardiovascular diseases and diabetes (Fox *et al.*, 2015d; Zahedirad *et al*., 2019).

Non-protein nitrogen (NPN) is a fraction constituted of nitrogen containing compounds that are not protein (O'Mahony and Fox, 2013; Gowen *et al*., 2025). They are mostly produced from protein breakdown in the cow and are present in milk from the animal's blood (Ruska

and Jonkus, 2014). Generally, milk protein makes up 95% of the nitrogen content in milk, with NPN making up the other 5% (O'Mahony and Fox, 2013; Ruska and Jonkus, 2014). These NPN compounds include urea, creatine, α-amino nitrogen, uric acid, ammonia and creatinine (O'Mahony and Fox, 2013). Urea is the most abundant compound present in NPN, where it makes up approximately 30% of the total (O'Mahony and Fox, 2013). Urea concentration in milk varies throughout the season depending on the cow's diet, and this can be reflected in the changing heat stability of milk during the year (O'Mahony and Fox, 2013). Total protein content is generally calculated from the amount of nitrogen present, to calculate true protein content, the amount of NPN must be subtracted from the total nitrogen content. NPN is calculated from the amount of nitrogen soluble in 12% TCA (O'Mahony and Fox, 2013).

1.3. Whey and whey products

Whey is the yellow/green liquid obtained from milk after the separation of casein. It makes up 90% of the original milk volume and contains 50% of the original milk components (Jelen, 2009). As previously mentioned, whey protein makes up 20% of the protein found in milk. In order to obtain this fraction, milk must be collected from the farm, taken in by a dairy processing facility and separated into the respective casein and whey protein fractions. The starting material can either be whole milk or skim milk depending on the desired casein product. When making full-fat cheese, the starting material is whole milk, as the presence of fat in cheese is desirable. However, for acid/rennet whey, milk fat is not needed in the casein-derived product, so the fat is separated from the skim milk prior to acid/rennet addition (Meunir-Goddik and Sandra, 2011). Ideal whey is also made using skim milk as the starting material; however, in this process, casein and whey are separated using membrane filtration based technologies, such as microfiltration, in order to produce micellar casein concentrate

(MCC) and ideal whey, which is the membrane filtration permeate (Hammam, Martínez-Monteagudo and Metzger, 2021). As ideal whey is made in this way, it is seen as an unadulterated, pure whey stream. This is because it is free from starter cultures, CMP, GMP, rennet enzymes and acids that would have been used to alter the pH during processing (Fox *et al*., 2015b).

1.3.1 Sources of whey

There are two main classes of whey, which can be identified as acid whey and sweet whey (Jelen, 2011). Acid whey originates from acid addition to skim milk to precipitate casein, whereas sweet whey can come from a variety of sources (Božanić *et al*, 2014). These include rennet addition and membrane filtration to produce whey as the permeate of micellar casein concentrate production; in all these processes, whey is the by-product. The main compositional difference between the two major types of whey, acid and sweet whey, is the pH of the whey stream, mineral content, presence of starter cultures and the make-up of the whey protein fraction, in particular the presence or absence of GMP (Jelen, 2011).

Acid whey is the by-product of a variety of products such as acid casein, greek yoghurt and acid coagulated cheeses (Rocha-Mendoza *et al*., 2021). Approximately 25% of total cheese production is represented by acid-coagulated cheeses, which include cream cheese, quark and cottage cheese (Fox, 2011b). Acid whey can be made through a variety of processes, including the fermentation of milk by using lactic acid bacteria (LAB), the addition of acid such as hydrochloric acid (HCL), or the use of an acidogen such as glucono-delta-lactone (GDL) (Fox, 2011b) to decrease the pH of the system to approximately pH 4.6, which is the isoelectric point of casein (Ryan and Walsh, 2016). This causes casein to precipitate and separate from the whey

stream. Acid whey contains more minerals compared to other types of whey, due to calcium and phosphate present in the casein micelle being solubilised, and entering the whey stream, at this low pH (Jelen, 2011). Acid whey is used in food applications (Macwan *et al*., 2016) and is also used as a fertilizer on farmland. The use of acid whey as a fertilizer has a poor impact on the environment which is due to the nitrogen present being transformed into ammonia which is volatised (Ghaly *et al*., 2007). Because of this, new approaches to the utilisation of acid whey are constantly being investigated. There is promising research around its potential future use in health promoting products, such as being a starter material for fermented beverages (Rocha-Mendoza *et al*., 2021).

Sweet whey is a broad name for the by-product produced from the production of most cheeses, rennet casein and micellar casein concentrate (MCC). Sweet whey has a higher pH, of between pH 6-7, compared to that of acid whey (Rocha-Mendoza *et al*., 2021). Rennet whey is the by-product of the production of rennet casein and certain types of cheese where the casein is coagulated in the milk. This involves the use of a specific protease called chymosin which can be found in rennet or in fermentation produced chymosin. Chymosin cleaves a bond in κ-casein and causes the casein micelle to become destabilized, resulting in coagulation of casein (Ryan and Walsh, 2016); enzymatic cleavage of the Phe_{105}-Met_{106} bond of κ-casein by chymosin causes this coagulation of casein (Brody, 2000). Due to this bond breakage, the κ-casein protein is altered, forming *para*-κ-casein, which remains with the casein molecule and caseinomacropeptide (CMP) portion which is lost in the rennet whey (Huppertz, 2013). Once CMP is glycosylated it is referred to as glycomacropeptide (GMP), which is a peptide that makes up 20 % of the total protein in this type of whey (Jelen, 2011). Because the presence of GMP is based on enzyme action, it is not found in acid whey. The presence of GMP in products is seen as advantageous due to reports of it being beneficial for human health (Córdova-

Dávalos, Jiménez and Salinas, 2019). It is desirable to have GMP present in infant formula, for example, due to its possible positive effect on gut microflora (Brück *et al*., 2006). The presence of GMP also helps increase heat stability of whey proteins, due to GMP interacting with β-lg which enhanced the stability of the whey proteins by increasing the gelation temperature (Gaspard *et al*., 2021).

Cheese whey is the liquid by-product obtained due to the coagulation of casein during cheese production (Zotta *et al*., 2020). This type of cheese whey is made using a combined action of lactic acid bacteria (LAB) and proteinases to coagulate the curds which can create harder, lower moisture cheeses, due to better syneristic properties of these curds compared to acid coagulated cheeses (Fox, 2011b). Cheese whey contains mostly lactose; however, more importantly, it also includes whey proteins, minerals, lipids, lactic acids, citric acids and non-protein compounds (Zotta *et al*., 2020).

Ideal whey, also known as native whey, is the permeate by-product from the production of MCC. This is produced through microfiltration of milk using membranes with pore sizes of 0.05-0.2 μm to separate casein and whey proteins (Xia *et al*., 2022). Ideal whey is seen as being more biologically active compared to other types of sweet whey because of heat treatment, that usually causes denaturation of whey proteins, does not occur in the filtration-based technologies used to produce ideal whey (Muuronen *et al*., 2021). However, pasteurisation is important to ensure microbial safety for consumers of ideal whey. Brick *et al*. (2017) reported that approximately 20 % of whey proteins were denatured using typical pasteurisation parameters of 72 °C for 20 s. Not only is the production process of ideal whey very different

to other types of sweet whey. Indeed, ideal whey also contains no GMP due to no enzymatic actions occurring, which results in the GMP remaining with the casein micelles.

1.3.2 Whey powder

Whey powder is the most basic powder product than can be produced from whey. Regular whey powder has a wide range of applications, which include animal feed, soups, bakery, confectionary, powder beverages, snack seasonings and dairy products (Chegini and Taheri, 2013; Adamson, 2015). Liquid whey is produced using whole/skim milk as a starting material and separating casein from whey which can be done using a variety of different methods as mentioned in Section 1.3.1. After this process is complete, liquid whey with approximately 5% solids is produced (Kilara, 2016).

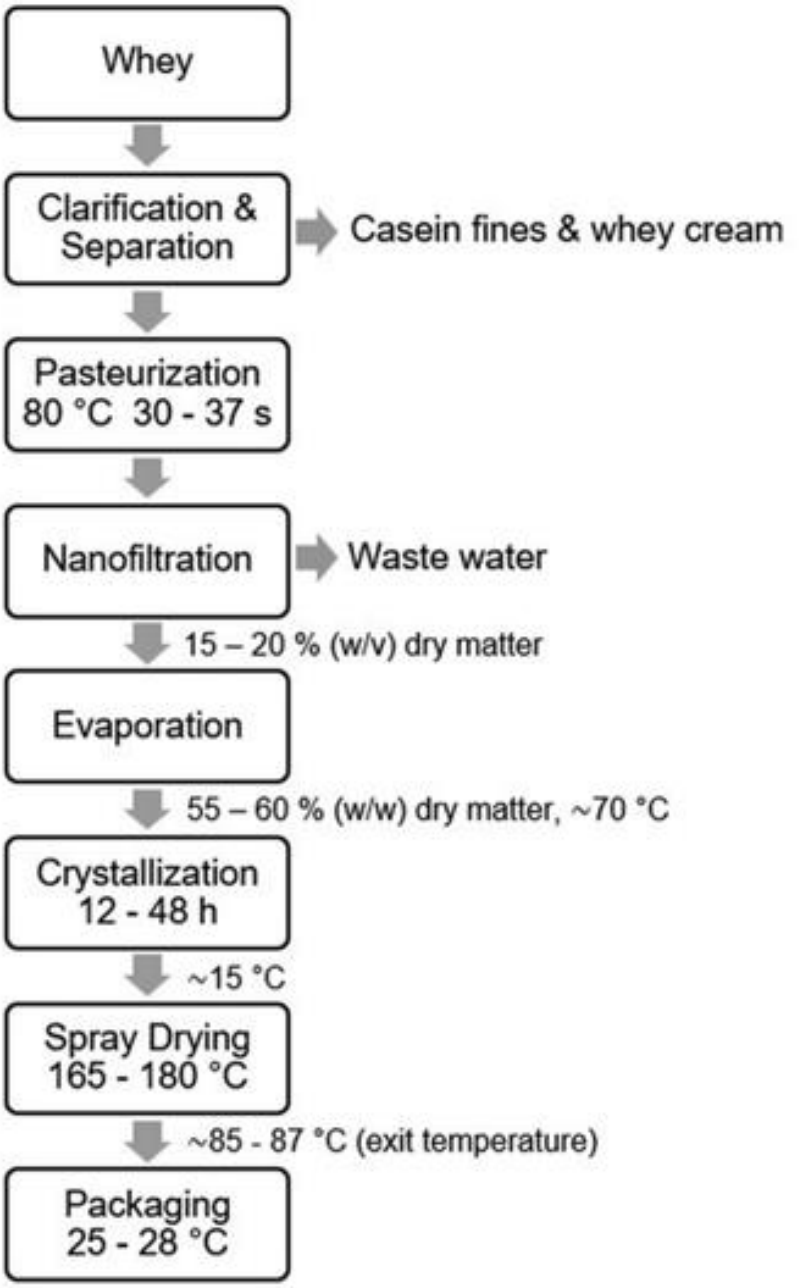

Figure 1.3: Flow diagram outlining the main steps for the industrial production of whey powder (Ozel et al., 2022)

Liquid whey is generally dried and sold as a powder with less than 4% moisture. There are many reasons why moisture is removed from products by drying, including the fact that it prolongs shelf-life and provides functional benefits to the powder, as well as convenience for the customer. Producers can also use less packaging and have reduced transportation costs due to the reduced volume/space required of the material (Park and Drake, 2014). Drying gives food producers the opportunities to create high quality food/food ingredients that are stable over extended periods of time (Courtois, 2013). Pre-drying concentration steps are used for a variety of products in the dairy industry to increase the total solids in a material, so that less energy is used in the generally energy intensive drying operations, as it would be uneconomical to dry the liquid whey straight without pre-concentration steps (Kilara, 2016). This concentration can be completed using membranes or evaporation, or often a combination of both, as depicted in Figure 1.3 (Ozel *et al*., 2022). Membrane technology is not only used for the removal of water, but is also used for demineralisation, bacteria reduction and segregating individual compounds to create value-added products (Reig, Vecino and Cortina., 2021).

Two different membrane processes can be used in concentrating whey, nanofiltration (NF) and reverse osmosis (RO), which can be performed separately or in combination. Membrane treatment is often a pre-requisite for other whey concentration steps such as evaporation and drying. It is advantageous compared to other concentrating technologies (e.g., vacuum evaporation) as it uses less energy (e.g., steam) and occurs at lower temperatures (and thereby preserves product functionality). NF is also used in demineralisation of whey, where it simultaneously removes some water which concentrates the level of whey solids (Kumar *et al.,* 2013). NF not only removes water but is also used to remove organic compounds with molecular weight smaller than 300 Da, due to the pore size of the membrane being 1 nm, through pressure-driven (5-40 bar) membrane filtration (Pan *et al*., 2011, Mohammad *et al.,*

2015). While removing certain organic compounds, such as minerals, it also performs simultaneous concentration of the whey solution. The demineralisation of whey will be discussed in further detail in Section 1.3.3. RO is a type of membrane process used for water-recovery and concentration of dairy products mainly (Reig, Vecino and Cortina., 2021). RO is a pressure-driven mechanism that involves product being pushed through a dense, semi-permeable, non-porous membrane (Deshwal *et al*., 2021). The greater amount of RO membranes/surface area present in the system, and the more often the product is being recycled through them, the more water can be extracted from the solution and thus higher levels of concentration can occur. RO configuration involves several membrane modules (membranes, housing for the membrane, feed inlet, retentate outlet and permeate outlet) along with high pressure pumps, heat exchanger and storage tanks (Carić *et al.,* 2009, Deshwal *et al*., 2021); Figure 1.4 depicts this configuration (Deshwal *et al*., 2021). This type of membrane technology involves high trans-membrane pressures with very low molecular weight cut-offs, so that only water is transmitted to the permeate and all solids are retained. RO membranes generally have a molecular weight cut off of <100 Da and transmembrane pressures of 30-100 bar (Reig, Vecino and Cortina., 2021).

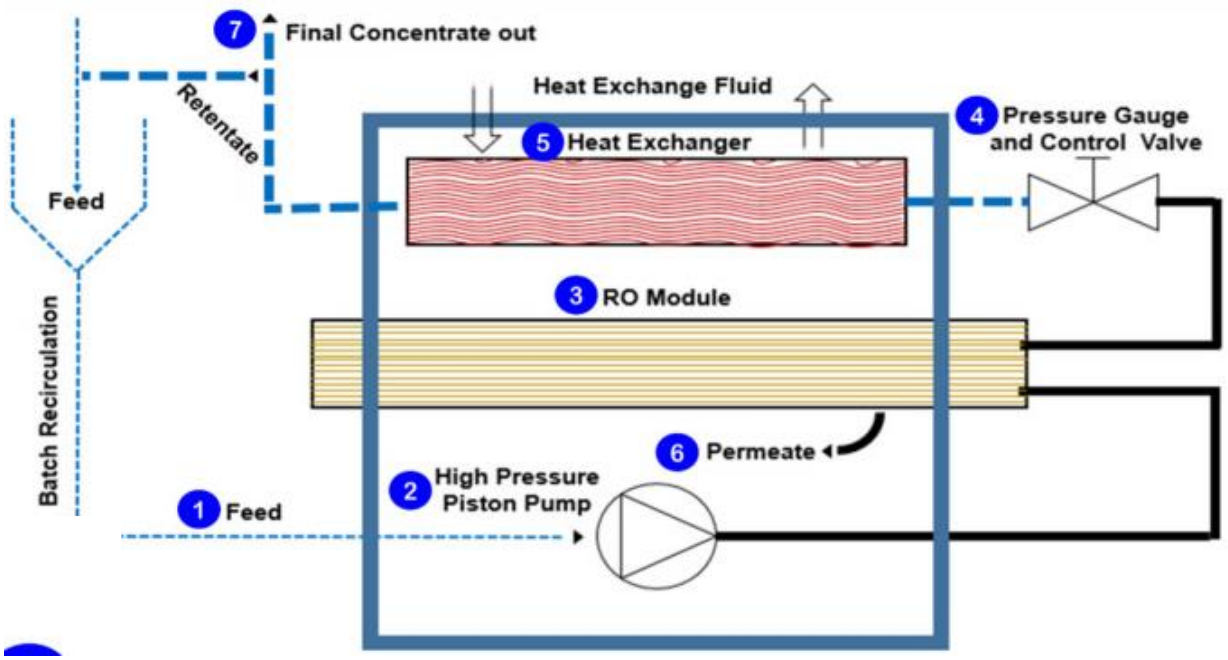

Figure 1.4: Schematic representation of reverse osmosis membrane plant used in the concentration of dairy products (Deshwal et al., 2021)

Along with membrane concentration techniques, evaporators are often used to concentrate liquid whey feed product even further and usually yield concentrated whey with 50-60% total solids prior to drying in traditional production processes (Sánchez *et al*., 2010). Evaporation is particularly important in the drying of whey to ensure that sufficiently high solids concentrations are achieved to facilitate the lactose crystallisation step (Zhang *et al*., 2018).

Membrane technology is limited by the extent of concentration that can be achieved due to the increase in viscosity of the feed that coincides with the removal of water. To further remove water from the feed, whey must be adjusted to high temperatures to remove water through evaporation. Whey proteins, in particular β-lg, are one of the principal components of whey and are naturally heat-sensitive (Gandhi, Amamcharla and Boyle, 2017), which leads to

denaturation occurring at high temperatures (Kailasapathy, 2016). This can cause fouling on the stainless steel surfaces in the evaporator (Gandhi, Amamcharla and Boyle, 2017), as well as having a detrimental impact on the quality of the finished product (Carić *et al*., 2009). Because of this, evaporators operate under vacuum, which allows lower temperatures, usually between 30-70 °C, to be used during this process. This enables the removal of water, increasing solids and protecting heat-sensitive materials such as vitamins and whey proteins (Zhang *et al*., 2018).

Prior to evaporation, it is important that the product is heated from a food safety perspective, to inactivate certain bacteria, but also to ensure that the product temperature is optimal for the first stage of evaporation. Depending on the end application of the product, this can be done at lower temperatures through pasteurisation or at higher temperatures using UHT sterilisation. (Carić *et al.,* 2009). There are a variety of different types of evaporators used in the dairy industry, including falling-film evaporators, plate evaporators and horizontal-tube evaporators (Hoffman, 2004; Zhang *et al.,* 2018). Of these, vertical falling film evaporators are the most widely used in the dairy industry (Cunningham *et al.,* 2006). In general, evaporators in a factory have two or more effects, with each consecutive effect operating at lower pressures and maintaining temperatures below approximately 70°C. Which thus induces lower boiling points. This reduces energy costs, as steam is only needed to be created once for the first effect and the vapour produced from the first effect is compressed, which then can be used as the heating medium for the second effect and so on (Cunningham *et al*., 2006). There are two different recompression methods used in the dairy industry for vapour recompression from evaporators, known as Thermal Vapour Recompression (TVR) and Mechanical Vapour Recompression (MVR). TVR involves using steam at high pressure to drive a steam jet ejector which is used to increase the temperature and pressure of the vapour produced from the previous effect,

whereas MVR uses a compression fan powered by electricity to increase the temperature and pressure of the vapour so that it can efficiently act as the heating mechanism on the next effect. Indeed, a combination of both can also be used (Gourdon and Mura, 2017).

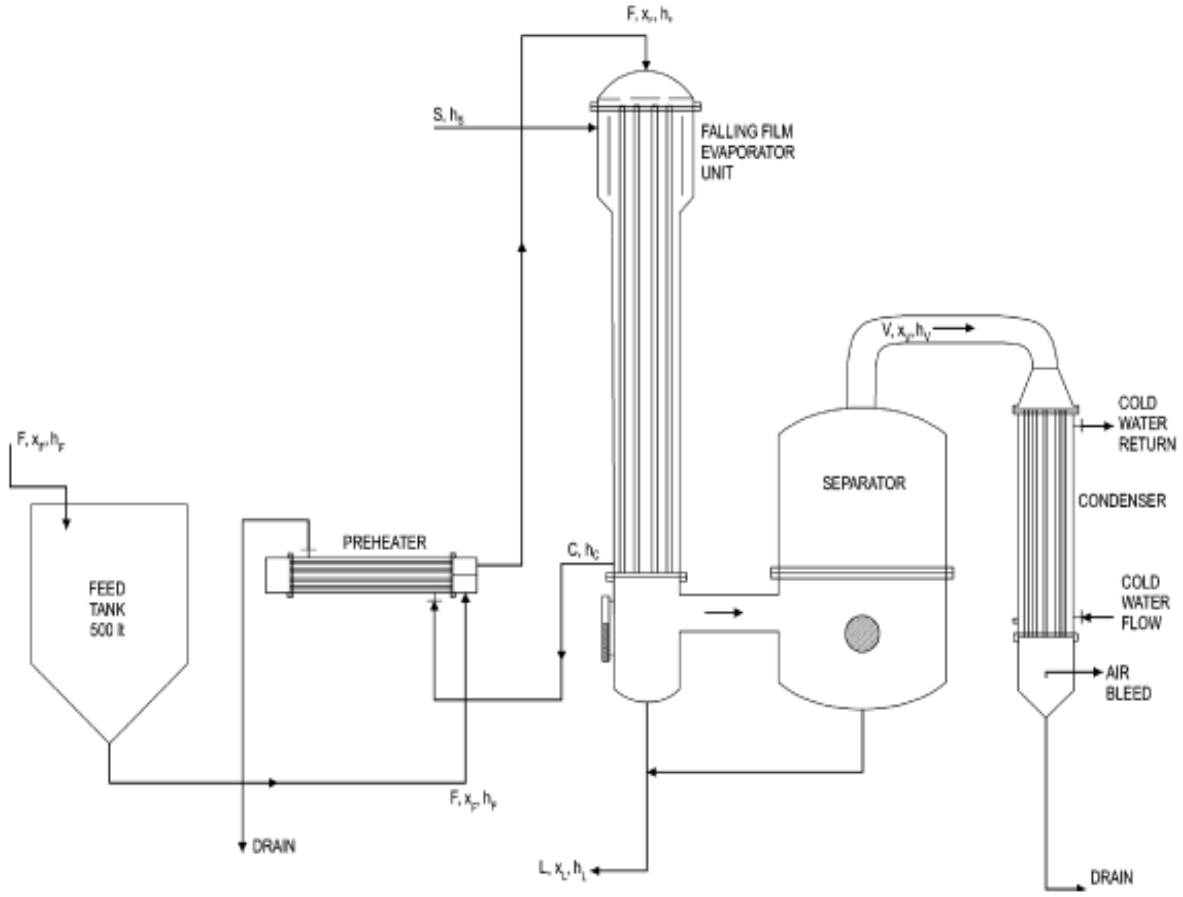

Figure 1.5: Schematic representation of a falling film evaporator used in the process of concentrating dairy products (Prost, Gonzalez and Urbicain, 2006)

A falling film evaporator is made up of multiple narrow tubes, up to 18 meters long, a bundle of these tubes are known as a calandria. As depicted in Figure 1.5, product is fed in through the top of the evaporator where it meets a distribution plate, where the product is evenly dispersed throughout the calandria tubes, such that a thin layer of liquid product flows down the inside of each individual tube due to gravity. The tubes are heated externally using steam, that condenses on the outside of the tubes and flows downwards when it indirectly meets the cooler whey, which is where the evaporation takes place. At the bottom of the effect, the concentrated product and vapour are separated, and the collected vapour is then used as the heating medium for the next effect (Gourdon and Mura, 2017). It is important that the flow of the product over the calandria tubes is even, and that the fluid has the correct viscosity and has the appropriate residence time to avoid fouling on the evaporator (Gourdon *et al*., 2015). If the product flowing down the narrow, heated tubes is too viscous, it may be unable to flow and therefore adhere to the calandria. If the feed sticks to the calandria, this will result in browning and crusting of the product onto the evaporator. This would cause major disruption and losses in production, as the evaporator would have to be thoroughly cleaned as well as the product requiring disposal (Gourdon *et al*., 2015).

Once the whey concentrate has been evaporated, it is rapidly cooled to approximately 30°C, using a plate heat exchanger or a flash cooler, and transferred to whey crystallisation tanks to initiate the lactose crystallisation process (Kilara, 2016). These crystallisation tanks are specifically designed with refrigerated jackets to further cool the concentrate down to 15-20°C over a period of 12-16 h to produce uniformly sized small crystals (Jelen, 2009). Also, during this time, the whey concentrate is being constantly agitated to allow the lactose crystallisation process to occur evenly throughout the tank. Lactose is encouraged to crystallise due to the high solids and low temperature environment. This crystallisation step has a major impact on

subsequent processing steps, including drying and storage stability (Schuck, 2011a; Ozel *et al*., 2022). If this crystallisation step does not occur, the lactose will remain in the glassy state which is much more unstable compared to lactose in a crystalline state and can result in product sticking to equipment during drying and caking during storage (Huppertz and Gazi, 2016).

Once the whey concentrate has a sufficient level of lactose crystallisation achieved, the concentrate can be dried. Spray-drying is the most common drying technology used in the dairy industry (Cama-Moncunill *et al*., 2015; Kelly and Fox, 2016). However, there are a variety of different ways a product can be dried, including roller drying, ring drying, attrition drying, freeze drying, fluid bed drying and spray drying (Refstrup and Bonke, 2011). The spray drying process is a mostly automated, continuous system and can be used to produce a variety of dried dairy products, including milk powders and whey powders. A variety of parameters in the dryer can be adjusted (i.e., pressure, air temperature, relative humidity and velocity) to produce powders with different characteristics such as particle size, bulk density and final powder moisture (Schuck, 2002; O'Sullivan *et al*., 2019).

Spray drying has strengths and shortcomings over the other drying technologies. It is a very rapid process, as the maximum time for which the powder particles are in the chamber is generally 30 s. Also, due to the fast process and relatively low temperatures used, the powder properties are particularly good, i.e., limited protein denaturation and/or vitamin degradation occur during this process (Anandharamakrishnan and Padma Ishwarya, 2017) when compared to other drying technologies. However, spray-drying is an energy-intensive operation due to the amount of water needed to be removed which results in high utility costs (Patel *et al*., 2010), as well as the expensive, complicated equipment.

Spray-dryers can have different configurations, they can be one-, two- or three- stage. When a spray dryer is of multi-stage configuration, this involves the spray dryer being the first drying step and a fluidised bed performing some secondary and/or tertiary drying (Anandharamakrishnan and Padma Ishwarya, 2017).

Spray drying begins with concentrated liquid being sprayed through an atomiser into the top of the drying chamber. This is where the bulk fluid stream is converted into droplets in order to substantially increase the surface area and allow quick evaporation of the concentrate during drying (Kelly and Fox, 2016; O'Sullivan *et al*., 2019). There are two main types of atomisers used across the dairy industries: rotary atomisers and nozzle atomisers. A rotary atomiser utilises centrifugal force for atomisation, while droplets from a nozzle atomiser form due to pressure applied as the liquid is forced through a narrow opening (Anandharamakrishnan and Padma Ishwarya, 2017; O'Sullivan *et al*., 2019). Other novel atomisers include ultrasonic nozzles and electrospray technology which may be utilised to tackle more difficult to handle concentrates such as a more cohesive fluids or those demonstrating highly non-Newtonian rheological behaviour (O'Sullivan *et al*., 2019). Depending on the properties of the feed (O'Sullivan *et al*., 2019) and the desired finished powder characteristics, the most suitable atomiser can be chosen, a rotary atomiser is used for whey products due to its ability to handle products with crystals as well as high viscosities (Refstrup and Bonke, 2011).

The drying chamber into which the droplets are dispersed, contains filtered, dry air, usually heated to temperatures between 150-300°C (Refstrup and Bonke, 2011). The inlet and outlet temperatures, which are the temperatures of the air going in and out of the drying chamber, has a strong influence on the drying process, and will have an impact on the final powder's

characteristics such as moisture content and functionality (Anandharamakrishnan and Padma Ishwarya, 2017). The free water on the surface of the powder particles is evaporated instantly on contact from the hot air and the remaining water migrates through the powder particle until no more can evaporate (Keshani *et al.,* 2015; Anandharamakrishnan and Padma Ishwarya, 2017). Due to the large surface area of these small droplets, the majority of the evaporation occurs instantly and has a cooling effect on the particles. This cooling effect is caused by the migration of water through the particle, which means that the particles never reach the same temperature as the inlet air. This is important for product functionality as, if the particles reach very high temperatures, this can have detrimental effects on the powder such as protein denaturation and/or visible browning (Anandharamakrishnan and Padma Ishwarya, 2017). The outlet air exiting the drying chamber has entrained fine powder particles that are too light to fall to the bottom of the chamber. This air is also significantly more humid and cooler than the inlet air due to the water removal from the droplets that take place in the chamber (Singh and Singh, 2016).

A cyclone or bag house is utilised to recover the fine powder particles that do not fall to the bottom of the chamber, but ordinarily leave with exiting air. This air leaving the chamber can contain between 10-30% of the overall powder (Skanderby, 2011). Cyclones are based on the theory of centrifugal force, whereby the powder entrained air enters the cyclone tangentially at speed. This results in the powder particles being thrown to the walls of the cyclone and exiting the cyclone through the bottom while the clean air exits the cyclone at the top (Skanderby, 2011). On the other hand, a washable baghouse contains many long fabric filter bags or "socks", which act as mechanical filters for the exhaust air. This is done using a vacuum sucking the air through the socks and the powder particles becoming caught on and coating the outside of the socks. The socks of the baghouse are purged periodically, releasing the powder

particles to allow them to be reintroduced into the main bulk of the powder. The bag house does not disrupt particles and is a more gentle powder recovery system when compared with cyclones (Gabites, Abrahamson and Winchester *et al.,* 2008).

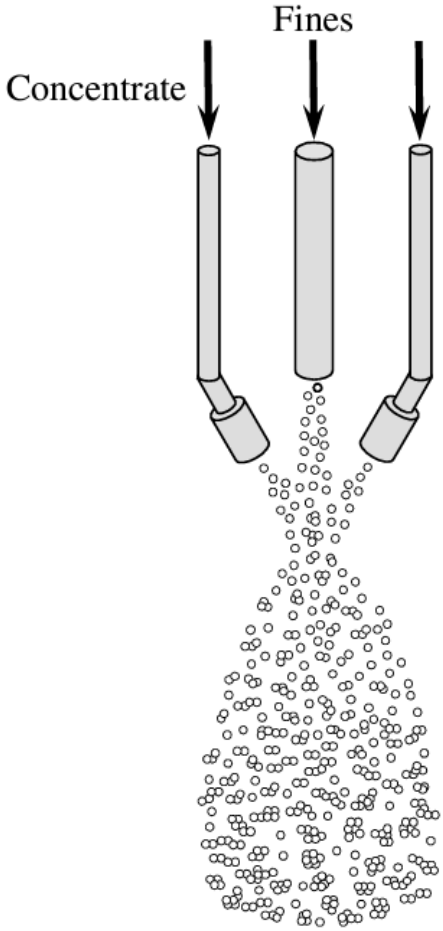

Figure 1.6: Schematic representation of an example of agglomeration where powder fines are being reintroduced near the concentrate feed to the dryer (Skanderby et al., 2009).

The small powder particles in the exhaust air, known as fines, can be reintroduced at two different stages, at the top of the drier, near the atomiser, or with the particles as they exit the drier (Carić *et al.,* 2009). As seen in Figure 1.6, by reintroducing these particles at the top of the dryer the fine powder particles can stick to the liquid being dispersed from the atomiser, which causes larger particles to form as they stick together and create agglomerates. Agglomerates can be advantageous by creating powders with enhanced rehydration properties (Fang, Selomulya and Chen 2007; Schuck, 2011b). Fine particles can also be reintroduced at the bottom of the dryer in the fluidised bed.

With either of these technological approaches, it is important to ensure the air leaving the cyclone/ baghouse is free from particles both due to efficiency of powder recovery as well to comply with directives on air quality (Cummins, McDonnell and Ward, 2006).

Fluid bed dryers are usually used in a secondary dryer application in a multi-stage dryer to control the final moisture and density of the powder. In a three-stage dryer, the spray dryer is usually the first stage with two fluid bed dryers making up the other two stages; a static fluid bed integrated into the base of the drying chamber and finally an external vibrating fluidised bed (VFB) as the final stage (Carić *et al*., 2009).

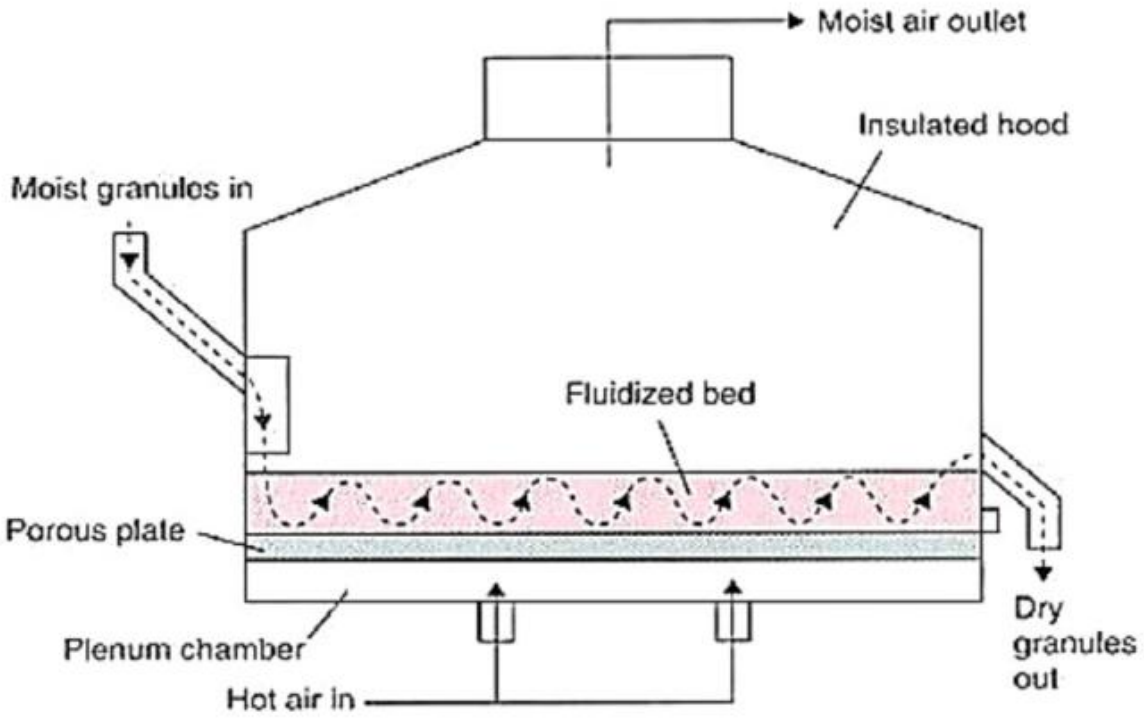

Figure 1.7: Schematic representation of a fluid bed dryer generally used as a secondary drying application in a multi-stage drying system in order to control the final moisture of dairy powders (Sivakumar et al., 2016)

As seen in Figure 1.7, fluid bed dryers consist of a rectangular-shaped chamber that is divided by a perforated air distributor plate to perform some secondary drying and cool the powder (Refstrup and Bonke, 2011). As fluidised beds can perform some secondary drying, this allows less water to be removed in the main drying chamber, i.e., lower temperatures may be used, which can cause less thermal damage to the powder, such as protein denaturation or vitamin degradation, and thus allow more control over final functional properties (Anandharamakrishnan and Padma Ishwarya, 2017).

Vibrating fluidised beds (VFB) work in stages and usually use temperatures 15 to 25°C lower than the spray dryer outlet temperature (Anandharamakrishnan and Padma Ishwarya, 2017). Generally, the first stage involves warm air blown up through perforated screens to induce further drying. The second and third stages involve cooler air used to both evaporate more water and cool powder down enough that it can be sieved and packaged (Anandharamakrishnan and Padma Ishwarya, 2017). Fine powder from the cyclone/baghouse can be recombined with the bulk of the powder at the beginning of the VFB, which can cause some powder particles to stick together, which is known as secondary agglomeration. This can result in changes to the particle size of the powder as well as the rehydration (i.e., sinkability and wettability characteristics). Lecithin can be coated onto particularly hydrophobic particles such as whole milk powder, which improves wettability and sinkability of a powder by acting as an emulsifier that interacts with the aqueous phase (Szulc and Lenart, 2010). An additional fluidised bed can be added to this sequence to make a multi-stage dryer; this fluidised bed would be added between the spray dryer and the fluidised bed previously mentioned. If it is integrated onto the main chamber, it is not vibrating.

Spray-drying is the most popular drying technique used in the dairy industry. However, there are other possible options to transform a concentrate to a powder. Roller drying, also known as drum drying, involves two co-/counter- rotating steam-heated cylindrical drums. A thin film of product is distributed across the outside of the drums, where water is evaporated and the dried product is scraped off the drum and ground up, which produces flaky irregularly-shaped powder particles (Courtois, 2013). Compared to other concentration steps, in roller drying, the vapour coming off the product is not recycled. Milk powders are the most popular dairy product produced on roller dryers. The high heat involved in roller drying can cause severe thermal damage, which has an impact on the finished powder properties, including taste and colour,

which can give milk powders a cooked flavour and cause high levels of free fat (Courtois, 2013). Depending on the powders application, this may be advantageous, i.e., high levels of free fat in powder particles are sought after for chocolate applications. In a study conducted by Mustafa, Alsaed and Al-Domi, (2014), sweet whey was dried using roller dryers as a substitute for sugar in a French-type bread and butter cookie application; corn starch (2%) was added to the whey before it was dried. It was concluded that sweet whey can be dried using this drying technique; however, without corn starch, there would have been negative effects on the final products quality such as browning. In the past, roller dryers were often used; however, the majority of roller dryers have now since been replaced with spray dryers (Kelly and Fox, 2016). Roller dryers have lower energy costs compared to spray-drying but are still used to produce dairy products in some industrial applications (Schuck *et al.,* 2015).

Post drying, the powder can be transported around the plant using a fluidised bed or a pneumatic powder transport system. As pneumatic conveying can break down agglomerates due to the use of pressurised air, this type of system would be used for more dense powders (Kelly and Fox, 2016). Depending on the powder, particularly common in caseins, it may be sifted to further classify the powder particles by size. The powder may then be directly packaged or held in silos until packaging (Kelly and Fox, 2016).

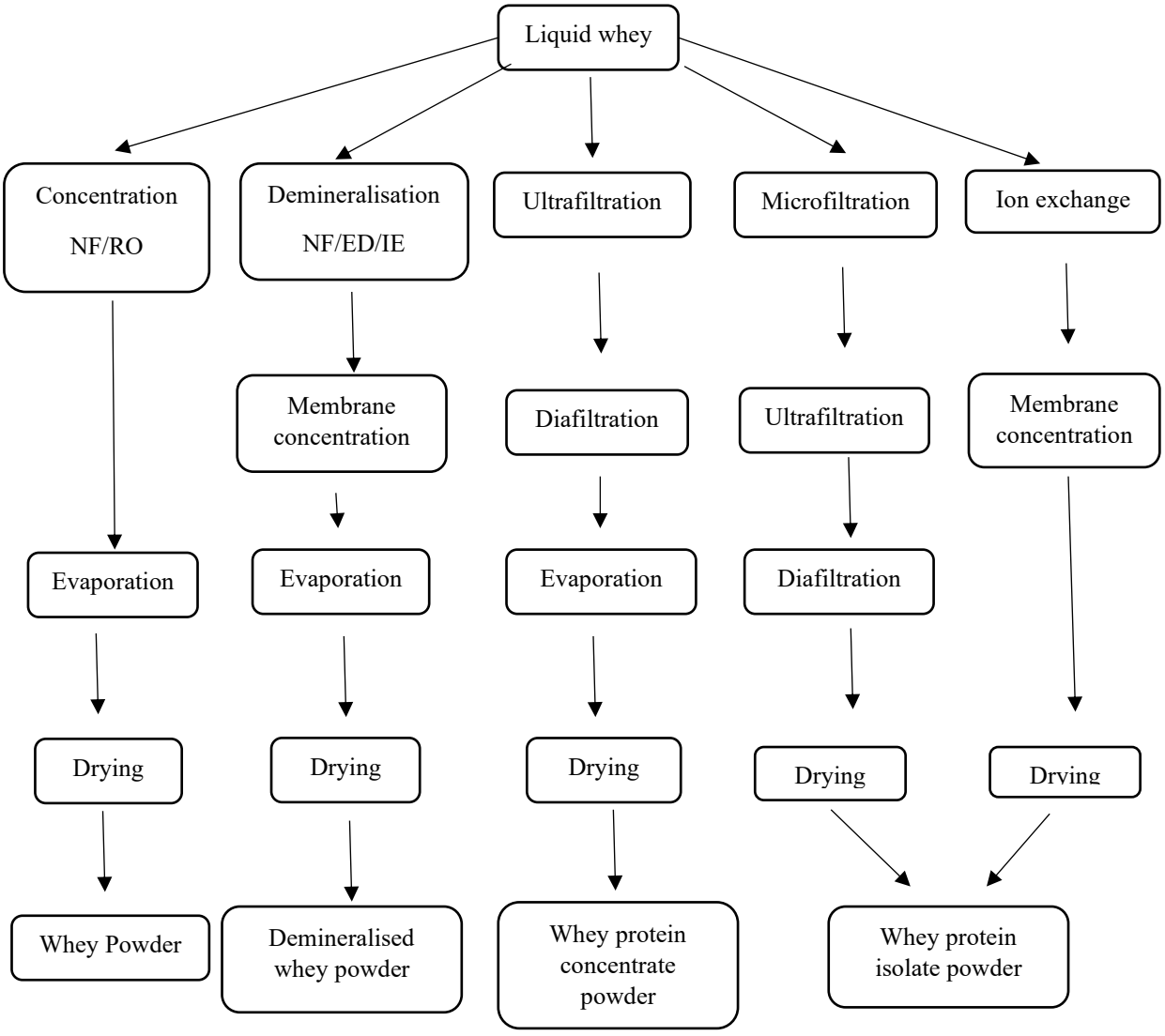

Figure 1.8: Process flow diagram outlining the major processing steps involved in the production of whey powder, demineralised whey powder, whey protein concentrate powder and whey protein isolate powder, *NF- nanofiltration, RO- reverse osmosis*, *ED- electrodialysis*, *IE- ion exchange*

1.3.3 Demineralised whey powder

Regular whey powder usually contains 6-10% minerals, with the variation depending on seasonality and source of the whey. This high mineral content can restrict the scope of whey being utilised in certain products and applications, particularly due to the nutritional impact of high levels of minerals, thus, regular whey powder cannot be used in infant formula products (Gernigon *et al*., 2011), due to such powder having a much higher concentration of minerals compared to the mineral content of human milk. To match the mineral profile present in human milk, it is necessary to remove minerals from cows milk/whey. This allows demineralised whey powder to be a suitable source of protein and lactose for infants when trying to replicate breast milk (Mclouchlin, Bernhart and Tomarelli, 1963). Whey that has been dried without any demineralisation step also has a bitter salty taste due to these high levels of minerals, representing another reason for demineralisation in altering the taste of whey powders to have a sweet taste so that it can be used in confectionary such as the manufacturing of chocolate, bakery or ice-cream (Prosekov *et al.,* 2013). There are many different manufacturing processes that can be used, individually or in combination, to remove minerals in whey (Figure 1.8), including nanofiltration (NF), electrodialysis (ED) and ion exchange (IE). Depending on the equipment available and the required levels of demineralisation for the application, different processes can be chosen. It has been reported that combining these three technologies can produce whey that is highly demineralised (>90%) (Kumar *et al.,* 2013).

NF, which has been previously mentioned, is a membrane technology that not only is used in the demineralisation process, but is also used as a method of concentrating liquid whey. The molecular weight cut off of 300 Da allows the NF membrane to permit most monovalent salts to pass through; however, it restricts the majority of larger components such as divalent/

polyvalent salts and other organic compounds (Kumar *et al.,* 2013). This results in the retentate being higher in total solids and lower in minerals than the original whey before demineralisation (Alkhatim *et al*, 1998). Through NF alone, the mineral content of whey can be reduced by 35-50%; if diafiltration is implemented into the NF process, the level of demineralisation can increase substantially. Diafiltration involves diluting the retentate with water and passing it through the ultrafiltration unit to reduce the concentration of soluble permeate components in the retentate, and simultaneously increase the concentration of retained components (Kumar *et al*., 2013). The combination of NF and diafiltration can remove as much as 60-70% of the original mineral content (Gernigon *et al*., 2011). Depending on the type of membrane used, and the characteristics of the feed solution, such as pH, the extent of demineralisation will differ, due to factors such as membrane charge and mean pore size, as well as membrane fouling (Cuartas-Uribe *et al.,* 2007).

ED reduces mineral content of whey through non-selective semi-permeable membranes, while being under an electric field supplied by an applied direct current source (Gernigon *et al.,* 2011). A large number of compartments are packed very close together within the ED unit and are separated by alternating cation- and anion-selective membranes, with electrodes at each end of the unit (Kilara, 2016). A direct current is then applied to the system, which causes positive ions to be attracted to the cathode, resulting in them passing through the negatively-charged cation-exchange membrane, but being retained by the positively-charged anion-exchange membrane; the opposite is seen for negatively-charged ions, which are attracted to the anode (Demircioglu *et al*, 2003). This results in ionised minerals being separated based on charge, allowing the demineralised whey to pass through the membranes. Demineralisation through ED can be a batch system, where the whey is processed through the same stack multiple times until sufficient demineralisation has occurred, or can be done continuously, with

multiple stacks through which fresh whey constantly runs. By controlling the density and viscosity of the whey, as well as the residence time of whey within the unit, the extent of demineralisation can be manipulated. It is optimal to run the ED unit at temperatures between 30–40°C, which can confer microbial risks to the product, and it is important to re-cool the whey once it has exited the system (Kilara, 2016). As with any membrane system, it is important that the membranes are cleaned and have proper flow design to prevent fouling from mineral and/ or protein build up which would result in reduced membrane separation performance.

Ion exchange (IE) is another technology used in the demineralisation of whey. The types previously mentioned, NF and ED involve using membranes to remove minerals from the product; however, IE utilises columns full of polymeric beads that are loaded with ions (Gernigon *et al*., 2011). The beads are spherical in shape and have diameters ranging from 0.4-0.8 mm (Greiter *et al*., 2002). The ions in the beads are exchanged for ions in the whey, which results in the minerals being removed from the whey. This occurs as positively-charged ions in whey are replaced with H^+ ions through the cation-exchange resin and negatively charged ions in whey are replaced with OH^- ions through the anion-exchange resin (Greiter *et al*., 2002). Once all the ions in the beads have been exchanged, the polymeric beads must be regenerated. This is completed by washing the resin, followed by elution to elute any bound analytes and then by passing a regenerative liquid through the bead-filled column (Gernigon *et al*., 2011). It is necessary to use extensive amounts of water as well as chemicals for regeneration of columns, which is a limitation of IE technology for industrial applications.

Once the required extent of demineralisation has taken place, the demineralised whey follows the same steps as the standard whey powder in terms of processing, i.e., evaporation, crystallisation of the lactose in the concentrate, spray drying and packing.

1.3.4 Whey protein concentrates and isolates

Whey protein concentrate (WPC) is another popular product made from the starting material of liquid whey. It involves similar processing steps as used for the production of regular whey powder but has the additional step of ultrafiltration, which increases the content of protein in the finished product, as seen in Figure 1.8 (Bronsveld, 2015). In addition to ultrafiltration, microfiltration is also sometimes used to remove fat and spores, depending on customer specifications and protein content of the finished powder to be achieved (Bansal and Bhandari, 2015). WPC is defined as having a minimum protein content of 25% in the finished dried powder (Bansal and Bhandari, 2015). However, it can come in different whey protein concentrations; WPC35, WPC50, WPC65 and WPC80 (with 35, 50, 65 and 80% protein content, respectively) being the most common (Bansal and Bhandari, 2015; Bronsveld, 2015). Details of the typical composition of WPC powders can be seen in Table 1.2, which shows the other major component of WPC to be lactose (Bansal and Bhandari, 2015; Bronsveld, 2015). WPC's have a wide range of applications, including soups, bakery, ice-cream, confectionary, beverages, clinical and infant and sports nutrition (Adamson, 2015).

Table 1.2: Typical composition of various whey protein concentrate powders (%) (Bansal and Bhandari, 2015)

Constituent	WPC35	WPC50	WPC65	WPC80
Lactose	46.50	30.90	21.10	3.50
Protein	36.20	52.10	63.00	81.00
Moisture	4.60	4.30	4.20	4.00
Ash	7.80	6.40	3.90	3.10
Lactic Acid	2.80	2.60	2.20	1.20
Fat	2.10	3.70	5.60	7.20

UF is a pressure-driven membrane separation process where whey proteins and fat are separated from smaller compounds such as lactose and minerals due to their differences in molecular weight (Baldasso, Barros and Tessaro, 2011; Rebouillat and Ortega-Requena, 2015). This is an excellent approach to concentrating and fractionating heat-sensitive materials as the process is not influenced by extreme temperatures (Baldasso, Barros and Tessaro, 2011). The ideal operating temperature for ultrafiltration is >50°C to avoid membrane fouling and maximise flux (Bansal and Bhandari, 2015; Kelly, 2019). It can also be completed at temperatures <15°C to protect heat sensitive material and control microbial growth. The retentate is fed to the dryer and made into WPC powder, while the co-product of this process is known as whey permeate. Whey permeate is mostly made up of lactose, along with low contents of ash and non-protein nitrogen (NPN). Whey permeate can be dried and used as is, or further processed into lactose powder, either edible or pharmaceutical-grade, depending on the levels of purity in the final product (Adamson, 2015). To produce powders with higher contents of protein (>50%), it is necessary to increase the volume concentration factor to

achieve higher protein content. This can be achieved by adding water through a process known as diafiltration, which further removes lactose and ash from the whey feed allowing the protein content of the retentate to be increased (Baldasso, Barros and Tessar, 2011; Bansal and Bhandari, 2015; Adamson, 2015). The higher protein content can be achieved by using a higher volume concentration factor and water for diafiltration. Evaporators are generally not used in the production of WPC's with >60% whey protein due to the heat-sensitive nature of the whey proteins as well as high viscosity levels (Adamson, 2015).

Whey protein isolate (WPI) is a whey protein ingredient that is broadly similar compositionally to that of high protein content WPC; however, it contains >90% protein (Bansal and Bhandari, 2015; Kelly, 2019). WPI has a strong market demand due to claims regarding nutrition and health (Kelly, 2019). It has a range of applications, including body building supplements, due to its high protein content, and egg white replacement, because of its foaming capacity and stability (Adamson, 2015). WPI can be produced using ion exchange methods or, it can also be produced using membrane filtration-based approaches, similar to the production process of WPC, as seen in Figure 1.8 (Bansal and Bhandari, 2015). Production of WPI through ion exchange involves altering the pH of the solution to give the whey protein a net positive/negative charge, allowing fractionation of the whey to occur by passing the pH-adjusted whey through an ion exchanger which adsorbs the charged proteins in the whey, while the deproteinised whey exits the ion exchange reactor (Bansal and Bhandari, 2015). The pH is then readjusted, and the proteins are desorbed from the resin, concentrated using UF and dried to produce a WPI powder (Bansal and Bhandari, 2015). This method can also be used to separate different whey protein fractions based on their differences in charge and isoelectric point (Doultani, Turhan and Etzel, 2004). As described in Section 1.3.4, WPI can also be produced using membrane filtration techniques. Along with ultrafiltration, it is common

practice when producing WPI to microfilter the liquid whey to remove fat and bacteria (Adamson, 2015). The fat composition of WPI must be ≤1% to be classified as an isolate (Kelly, 2019). It is necessary to reduce the fat to low concentrations so that the protein content can reach >90% in the finished powder and also so that the fat doesn't adversely affect the functionality of the WPI (Bansal and Bhandari, 2015; Adamson, 2015).

1.3.5 Hydrolysates and other whey protein fractions

Hydrolysing whey proteins involves cleaving the peptide bonds of proteins to different extents, which results in shorter chained peptides and free amino acids being produced (Bansal and Bhandari, 2015). This method alters the molecular, nutritional, functional and sensory properties of standard whey proteins (Bansal and Bhandari, 2015; Jeewanthi, Lee and Paik, 2015). This hydrolysis of bonds can occur through addition of enzymes or altering the pH (Bansal and Bhandari, 2015). Enzymatic hydrolysis is the most common in the food industry, as this is a very selective method based on the proteases utilised in the process (Jeewanthi, Lee and Paik, 2015).

Whey protein hydrolysates have advantages over intact whey proteins for applications such as nutritional products and infant formula due to more rapid digestion and lower allergenicity due to the smaller peptides present (Bansal and Bhandari, 2015; Jeewanthi, Lee and Paik, 2015). Hydrolysis of whey proteins also improves their functionality, such as increasing solubility and decreasing viscosity of the whey (Jeewanthi, Lee and Paik, 2015). Fragments of whey hydrolysates are demonstrated to have attributes of functional food ingredients (Dullius, Goettert and De Souza, 2018), providing health benefits in various systems such as the immune and cardiovascular system (Chatterjee *et al*., 2015). However, if the degree of hydrolysis is

medium-high (>8%), it can cause a bitter taste due to the small peptides present (Jeewanthi, Lee and Paik, 2015).

Whey protein is a family of different proteins which are present in different ratios and have varying molecular masses and isoelectric points (Etzel, 2004). These differences can be exploited through fractionation and segregation to extract individual proteins using a variety of different methods such as selective precipitation, adsorption and elution, they can also be separated through membrane filtration (Etzel, 2004). Each of the individual whey proteins have varying attributes which can be exploited to provide tailored nutrition to individuals (Etzel, 2004).

1.3 Physical, functional and quality attributes of dairy powder

Whey powders, along with other dairy powders, are principally characterised by their composition, such as concentrations of protein and ash; however, physical characteristics and rehydration properties of the powders can be equally important (Chegini and Taheri, 2013). Appropriate physical and functional characteristics of the powder are essential, not only for the final application of the powder, but also for industrial applications when these powders are being processed through manufacturing facilities and held during storage (Ozel *et al*., 2022). These characteristics include particle size distribution, morphology, bulk density, colour, flowability and rehydration properties. These properties can be included in quality specifications and, if they do not meet these requirements, the powders can be rejected by the customer (Chegini and Taheri, 2013).

1.4.1 Particle size distribution

Particle size distribution is an important factor that determines many other properties of dairy powders such as density and rehydration performance (Kelly and Fox, 2016). The particle size of spray dried powders can be influenced by the viscosity of the concentrate as well as atomization conditions (Schuck, 2011b). Wider particle size distributions can be achieved by drying higher viscosity concentrates and using larger nozzle sizes/reducing the velocity of the wheel (Skanderby *et al*., 2009). In terms of transport, wider particle size distributions are advantageous. The less interstitial air between particles, the higher the bulk density of the finished powder, which is due to smaller particles occupying the space between larger particles and thus results in a higher bulk density (Skanderby *et al*., 2009). The particle size distribution can also be altered post drying through agglomeration.

1.4.2 Morphology

Powder particle morphology, which incorporates particle shape, can have an impact on other physical characteristics such as bulk density and flowability. There are many factors that affect the morphology of powder particles, such as method of drying and composition of powder. Powder particles tend to obtain a spherical shape when spray dried due to the surface tension when drying (Carić *et al.,* 2009), whereas powders produced using roller drying tend to have a more irregular, compact shape with uneven edges (Caric and Kalab, 1987). Fat content of the powder can have an impact on particle shape; for example, it was reported by Malafronte *et al.* (2015) that powders with lower fat contents shrivelled under the same drying conditions as powders with higher levels of fat present. This was attributed to the fact that, at a microscopic level, the protein membrane is very porous within the particle. The fat present in the particle embeds within the porous membrane and helps support the shape of the particle whereas, when this fat is not present, it causes the powder particle to shrivel. As previously discussed,

agglomeration can alter the shape of powder particles quite considerably. Depending on the binder being used, and the chosen agglomeration process, the morphology of the finished powder particle can be quite different in terms of elongation, porosity, fragility, and agglomerate size (Turchiuli *et al*., 2005).

1.4.3 Bulk density

Bulk density is described as the weight of a given volume of powder and is generally expressed in g mL^{-1} (Skanderby *et al*., 2009). It is an important characteristic of dairy powders as it determines packaging and transport costs, where high bulk density is advantageous as the powder occupies less space (Ding *et al*., 2020). It is measured by placing a fixed mass (typically 100 g) of powder into a graduated cylinder and measuring the volume that the powder occupies (poured bulk volume). The powder-filled graduated cylinder is then placed into a device which taps the cylinder for a defined number of taps, and the tapped bulk volume is recorded by reading the height of the powder in the graduated cylinder (Skanderby *et al*., 2009). This can be converted to bulk density by dividing the weight of the powder by the final volume (Wang *et al*., 2021). Factors that affect bulk density of powder includes interstitial air, density of the particles themselves and processing steps such as evaporation and heat treatments (Kelly and Fox, 2016). Agglomerated powders generally result in lower bulk densities due to increasing particle size and interstitial air (Ding *et al*., 2020). Particle density can be determined by the composition of the powder as well as occluded air within the powder particle (Kelly and Fox, 2016). Heat treatments, such as pasteurisation and evaporation, can cause denaturation of whey proteins which lead to higher bulk densities. This is because native whey proteins increase air occlusion, which in turn decreases bulk density, whereas denatured whey proteins cause higher densities of the bulk powder (Deeth and Hartanto, 2009). During drying, powder bulk density can be affected by the inlet/outlet air temperature of the spray dryer, the atomizer speed and

the feed flow rate (Chegini and Taheri, 2013). Nozzle atomisers produce higher bulk density powder compared to those produced using a centrifugal atomizer, due to less air being incorporated in the atomisation process (Kelly and Fox, 2016). Bulk density values can significantly impact the handling and rehydration properties of dairy powders. In a study conducted by Hazlett, Schmidmeier and O'Mahony (2021a), the impact of agglomerate breakdown (increase in bulk densities) on bulk handling and rehydration properties of dairy powders were investigated. The flowability of the powders decreased upon an increase in bulk density, due to increased particle-particle interactions. It was also shown that the initial stages of rehydration, wettability and dispersibility, were impeded due to a reduction in volumes of interstitial air, which limited the movement of water through the powder during rehydration (Hazlett, Schmidmeier and O'Mahony, 2021a).

1.4.4 Colour

Colour is an important attribute of powders and can influence consumers interpretation of the quality of the product (Milovanovic *et al*., 2020). In terms of dairy powders, generally cream-coloured powders are desirable. The Maillard reaction is a three-stage chemical reaction that occurs between amino acids and reducing sugars in dairy products (Zhou and Langrish, 2022). The result of this reaction is a change in colour due to the formation of high molecular weight nitrogenous brown pigments known as melanoidins (Zhou and Langrish, 2022). This colour is desirable in some applications (e.g., caramel); however, in dairy products, this is generally seen as a quality defect (Kelly and Fox, 2016). Scorched particles are powder particles that are black/brown in colour. These are produced as a result of Maillard reactions due to exceeding normal residence times in the evaporator and/or spray dryer (Schuck, 2011b). Along with the production of this brown colour during processing, it can also be produced during storage of dairy powders (Li *et al*., 2019).

1.4.5 Flowability

Flowability is very important characteristic of dairy powders, and is defined as the resistance of the powder to flow under its own weight (Kelly and Fox, 2016). The extent of a powder's flowability can be determined by pouring out a fixed quantity of powder and measuring the angle of repose of the pile formed (Kelly and Fox, 2016). It is important that the powder is free-flowing and not cohesive to allow the powder to move easily and avoid mechanical caking and flow problems occurring during processing operations (Fitzpatrick *et al*., 2007; Ozel *et al*., 2022). Flowability of a powder may be affected by interactions taking place between powder particles, which are determined by, the shape and size of powder particles, the composition of the powder particle and the environment the powder is subjected to (Kelly and Fox, 2016; Hazlett, Schmidmeier and O'Mahony, 2021b). Higher contents of fat in the powder, particularly at the surface of the particle are associated with a more cohesive powder (Fitzpatrick *et al*., 2007; Kelly and Fox, 2016). This is exaggerated at higher temperatures, due to some fat becoming liquid, and thus liquid bridges occurring between particles (Fitzpatrick *et al*., 2007). High temperatures also influence the flowability of powders with high levels of lactose, due to the formation of liquid bridges (Hazlett, Schmidmeier and O'Mahony, 2021b), with moisture contents >6% also demonstrated to contribute to cohesiveness in powders (Fitzpatrick *et al*., 2007). As protein contents increased, so too did the cohesiveness of a powder, which is due to smaller powder particles and increased occluded air, resulting in increased compactness and cohesiveness (Hazlett, Schmidmeier and O'Mahony, 2021b). As small powder particles are associated with a cohesive powder, one method to improve flowability of a dairy powder is to increase the particle size through agglomeration. This helps combat cohesive powder as there is less interactions occurring between the particles, thereby improving the flowability (Hazlett, Schmidmeier and O'Mahony, 2021b). Along with agglomeration, additional components can be sometimes added to the powder to help increase

flowability, such as silica containing compounds (Kelly and Fox, 2016, Hazlett, Schmidmeier and O'Mahony, 2021b).

1.4.6 Rehydration properties

Good powder rehydration properties are essential functional characteristics of most dairy powders. It is important that the powders interact in a desirable manner with the solvent, as these properties can determine factors of the finished product quality, including the structure, texture, appearance, mouthfeel and flavour (Morr and Ha, 1993). The composition of the powder, processing conditions, as well as the physical characteristics listed above, all play a part in the rehydration properties of a powder.

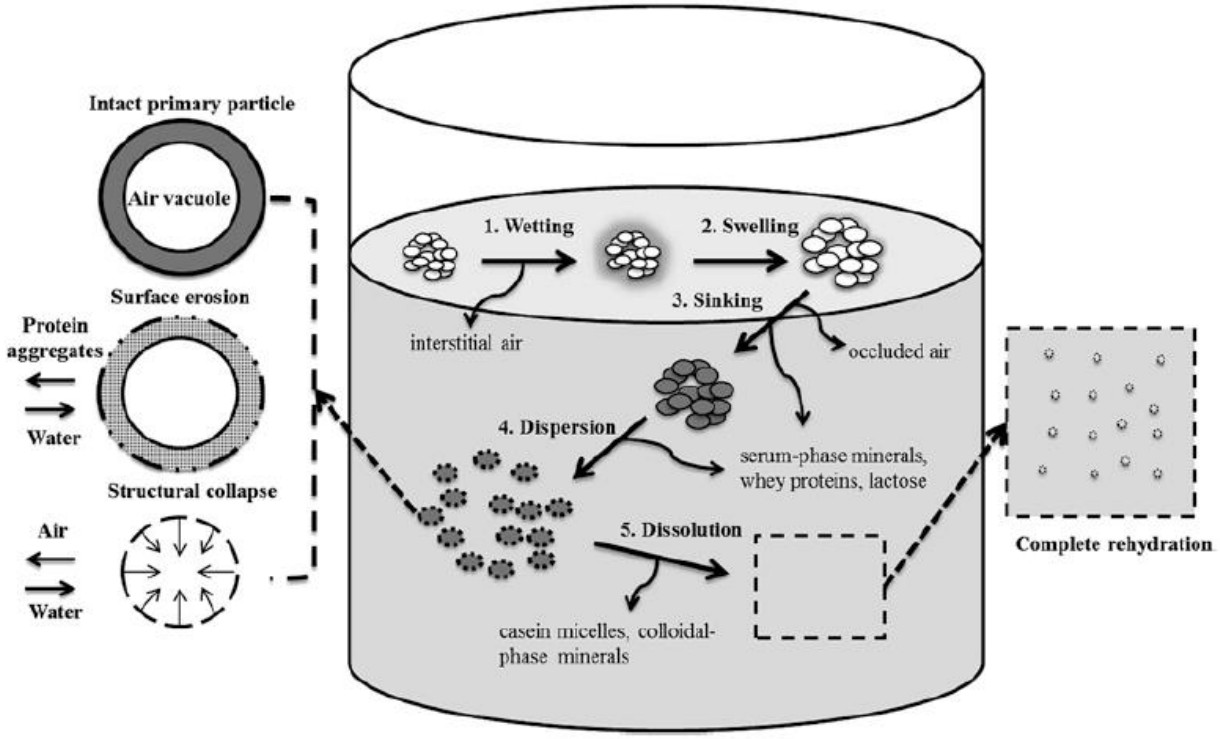

Figure 1.9: Schematic representation of the rehydration process of an agglomerated high protein dairy powder (Crowley *et al*., 2015)

Figure 1.9 depicts the rehydration process of a high protein, agglomerated, dairy powder, generally involving 5 stages; wetting, swelling, sinking, dispersion and dissolution (Crowley *et al*., 2015). The wettability of a powder is an important characteristic for dairy powders; it is defined as the time it takes for a given amount of powder to penetrate the still surface of water (Schuck, 2011b). It is described as the ability of the powder particles to overcome the surface tension between themselves and the water. When the powder begins to wet, a molecular interaction occurs between the solid (powder) and the liquid phase (Fang, Selomulya and Chen, 2007). If the powder fails to wet adequately, lumps will form and a layer may appear on the surface and around the walls of the container. A variety of factors affect wettability, which include, composition of the dairy powder, in particular at the surface of the powder particle, and powder particle size. Free fat on the particle surface, which can lead to hydrophobicity in powders, can impede the powders from wetting adequately (Fang, Selomulya and Chen, 2007). Agglomerated powder favours good wettability due to the inclusion of interstitial air, increasing particle size and inhibiting the formation of a surface film by minimising the specific surface area (Skanderby *et al*., 2009). Seo (2022) investigated the wetting times of regular skim milk powder (SMP) compared with SMP that had been agglomerated, increasing the average particle size from 87 to 169 μm. This increase in particle size resulted in a decrease in wetting time from >120 s to approximately 5 s.

The swelling step is most evident for casein-based dairy powders (Crowley *et al*., 2015), where an increase in viscosity of the solution occurs as the particles begin to swell with the uptake of particles in the aqueous solution. Whey protein-dominant powders follow the same rehydration steps, but without the swelling step as it can not be distinguished in their rehydration process (Gaiani *et al*., 2007). Sinking is the next step in the rehydration process, which occurs when the gaseous phase surrounding each powder particle begins to be replaced with the aqueous

phase, which can be seen when the powder particles begin to go below the aqueous solution's surface (Fang, Selomulya and Chen, 2007). The results of this test are expressed as milligrams of powder that submerge beneath the surface of the water per min and is also proportional to the surface area (Schuck, 2011b). The higher the density of a powder, combined with less occluded air within the powder particles, results in the fastest sinking time. Powders with high contents of fat can have issues with poor sinking due to their hydrophobic nature (Fang, Selomulya and Chen, 2007).

Once the powder particles have wet and begin to sink, the individual powder particles then tend to disperse into the solution and this includes the breakdown of agglomerates, which is known as the dispersion stage (Fang, Selomulya and Chen, 2007). It is known that the larger the particle size (i.e., the more agglomeration that has occurred), the better a powder's ability to disperse. Poor agglomeration or a high level of very fine powder particles can result in poor dispersibility and may even result in clumps of slurry-like powder visible at the bottom of the container (Fang, Selomulya and Chen, 2007; Schuck, 2011b). It has been reported that the higher the number of fine particles present, i.e., those below 90 μm, the poorer the dispersibility of a powder (Fang, Selomulya and Chen, 2007). Neff and Morris (1968) reported that, by agglomerating non-fat dry milk powders, the dispersibility could be increased significantly. This study also demonstrated that, when non-fat dry milk was size-fractioned, the smallest particles had the worst dispersibility characteristics (Neff and Morris, 1968). Agglomeration is not the only processing strategy that can improve the dispersibility of dairy powders, other approaches include the use of ultrasonication, injection of gas (to influence powder structure), ion exchange and pH adjustment (McSweeney *et al*., 2021).

Dissolution is the final step/end point in the powder rehydration process (Crowley *et al.*, 2015). In general, food powders must be able to provide effective dissolution to be able to interact with other food ingredients. This step allows the release of molecules present in the powder into solution (Crowley *et al*., 2015). The amount of insoluble material present after the powder has been fully dispersed indicates how soluble the powder is (Schuck, 2011b). This characteristic is measured by dispersing a powder in water, followed by centrifugation of the sample. The insoluble components of the powder form a pellet at the bottom of the graduated centrifuge container; the larger the size of the pellet at the bottom of the container, the poorer the solubility of the powder.; this is known as the solubility index, as described by the International Dairy Federation (IDF) (Skanderby *et al*., 2009; Schuck, 2011b).

Solubility is an important quality parameter, as insoluble components are undesirable for the consumer. Proteins in dairy powder can become denatured during processing, due to the instability at high temperatures or large changes in pH (Mulvihill and Donovan, 1987). These denatured proteins contribute to the level of insoluble components. Preheat treatment of milk prior to processing can improve the stability of the powders and thus result in less insoluble particles being present (Schuck, 2011b).

1.5 Whey powder as an ingredient in applications

Regular whey powder has a wide range of applications which include, animal feed, soups, bakery, confectionary, powder beverages, snack seasonings and dairy products (Chegini and Taheri, 2013; Adamson, 2015). Depending on its end use, different characteristics of the powder are important. For applications where the whey powder is dissolved into the product, such as in beverages or sweetened condensed milk, rehydration properties of the whey powder

are very important. Whereas, when the powder is subjected to few further processing steps and is not being hydrated, other characteristics may be of greater importance, such as powder particle morphology, particle size and flowability. Whey powder is the most basic powder that can be produced from liquid whey; and is often used as a low cost substitute for other ingredients (Królczyk *et al*., 2016).

1.5.1 Ready-to-mix beverages

Ready-to-mix (RTM), also known as ready-to-reconstitute, powder beverages are popular convenience products for consumers all over the world (Chaturvedi, Khartad and Chakraborty, 2021). These powder mixes require certain characteristics and functional properties to be suitable for the consumer. It is important that the powder is free-flowing, with large particle size and narrow powder particle distribution, high porosity and has fast rehydration properties (Calton *et al*., 2019). These beverages can be rehydrated by the consumer themselves or can be consumed at the point of production, such as from a vending machine. This is where the powder is rehydrated within the machine, producing different drinks instantly for the consumer, such as hot chocolate or coffee. Whey powder is a popular ingredient in these beverages for a number of reasons, such as being highly stable throughout a wide pH range (pH 2-10), which makes its addition suitable to fruit or acidic drinks (Chavan *et al*., 2015; Kosikowski, 1968). Whey protein is a good source of protein in these beverages, due to its excellent nutritional qualities as well as bland flavour and ease of digestibility (Rittmanic, 2006).

1.5.2. Snack seasonings

As well as whey powder being a popular ingredient in RTM beverages, it is also commonly used in snack applications. Whey powder can be used as a component in snacks such as cookies

or extruded snacks, more popularly, whey powder can be used in a blend in a topical snack seasoning (Johnson, 2000). Seasonings are an important addition to snack foods such as potato crisps as they can aid visually by adding colour as well as enhancing the taste (Hanify, 2001). Whey powder has many functions in snack seasonings, which include improving the nutritional profile of snacks through its protein quality and calcium content, and also acting as a cost-efficient bulking agent and carrier for distributing other concentrated flavours. In particular, whey powder is a popular ingredient for dairy-based flavoured seasonings such as sour cream and cheese, as the whey powder provides background sweet dairy notes (Johnson, 2000).

Snack seasonings can be applied in three different methods; a dry mix dusted onto the snacks, a wet oil slurry sprayed onto the snacks and, for baked products, with an oil layer initially applied onto the snack which acts as a glue, followed by a dry seasoning mix (Johnson, 2000). Flavoured snacks generally contain 6-12% total seasoning (Enggalhardjo and Narsimhan, 2006). The particle size of the seasoning mix is particularly important for dry seasoning mixes as if it is not correct, adhesion issues can arise. This is because it is important to apply the seasoning in a consistent and uniform manner (Hanify, 2001); if all the ingredients in the seasoning mix are not of similar particle size this may cause issues with storing blends as the different ingredients may not remain uniformly suspended, which results in powder/mixture segregation. This can also cause challenges when dusting the seasoning mix onto the snack, as particles of larger size may not adhere sufficiently to the snack and result in drop off of the seasoning from the snack, which will alter the flavour impact if not enough seasoning is on the crisp (Johnson, 2000).

As described by Ermis *et al*. (2009), adhesion of seasoning particles to a snack is a major challenge for industry. If the seasoning does not adhere sufficiently during production of snacks, this can cause financial losses for the company and unsatisfied consumers due to poor flavour performance (Wong *et al*., 2005; Ermis *et al*., 2009). Adhesion of seasoning to a crisp can depend on the specific ingredients in the blend, the powder particle size distribution, and the chemical and physical interactions occurring between the seasoning and the snack. Seasoning adhesion mechanisms to the snack include mechanical interlocking, wetting, electrostatic forces, chemical forces and diffusion (Wong *et al*., 2005; Ermis *et al*., 2009). Snacks generally have an oil film present after frying, or for baked goods, this may be physically applied to the snack. This allows fine particles to wet onto the surface of the snack and form liquid bridges. Van der Waals forces are more common for smaller particles (i.e., less than 60 μm) and stronger electrostatic forces are more prevalent for larger particles in the seasoning (Enggalhardjo and Narsimhan, 2006; Ermis *et al*., 2009). Ermis *et al*. (2009) studied the drop impact force required to detach salt particles from crisps. This was completed with a prototype adhesion tester which dropped salted snacks at different heights. It was demonstrated that the finer particles of salt (53-90 μm) required a larger force to cause drop off of particles, whereas a lower force was required to cause drop-off of larger salt particles (150-300 μm) from the snack. This indicates that the larger particles in the seasoning are more loosely bound to the snack. Ermis *et al*. (2009) also investigated adhesion levels of varying particle size distributions of different flavoured snack seasonings, i.e., salt and vinegar, cheese and onion and prawn cocktail. It was shown that the seasoning with a greater proportion of coarse particles had higher levels of seasoning drop off from the snack. This is as expected, as the detachment rate should be higher with powder systems having larger particle size fractions. When the results from the seasoning drop off studies were compared to the salt drop-off levels, the results were significantly different (Ermis *et al*., 2009). This implies that the amount of drop-off is not

just about the particle size of the powder, but also about the electrostatic load, chemical structure and particle geometry as different ingredients have different adhesive properties.

1.6 Conclusion

In conclusion, whey is a diverse ingredient that can be processed in many different ways to produce a variety of finished products with a wide range of applications. The physical and functional properties of these powders are an integral part of customer quality specifications, due to the impact of these properties on the storage, processing and final use of these powders. Continued research on the processing and functional properties of whey powders is required to support future innovation and value addition.

1.7 References

Adamson, N. (2015). *Whey Processing.* In*:* Bylund, G. (2015). *Dairy processing Handbook.* 5th ed. Available at: <https://dairyprocessinghandbook.tetrapak.com/chapter/whey-processing>

Adel, A., Nadia, M., Mohamed, O. and Abdelhafidh, G. (2008). Study of thermally and chemically unfolded conformations of bovine serum albumin by means of dynamic light scattering. *Materials Science and Engineering,* **28 (5-6)**, pp.594-600.

Alkhatim, H.S., Alcaina, M.I., Soriano, E., Iborra, M.I., Lora, J. and Arnal, J. (1998). Treatment of whey effluents from dairy industries by nanofiltration membranes. *Desalination*, **119 (1-3)**, pp.177-183.

Anandharamakrishnan, C. and Padma Ishwarya, S. (2017). Spray drying. In: Anandharamakrishnan, C. (2017). *Handbook of Drying for Dairy Products*. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.57-95.

Baldasso, C., Barros, T.C. and Tessaro, I.C. (2011). Concentration and purification of whey proteins by ultrafiltration. *Desalination*, **278 (1-3)**, pp.381-386.

Bansal, N. and Bhandari, B. (2016). Functional Milk Proteins: Production and Utilization – Whey-based Ingredients. In: McSweeney, P.L.H. and O'Mahony, J. A. (2016). *Advanced Dairy Chemistry: Volume 1B: Proteins, Applied Aspects*. 4th Ed. New York: Springer, pp.67-98.

Bell, S.J. (2000). Whey protein concentrates with and without immunoglobulins: a review. *Journal of Medicinal Food*, **3 (1)**, pp.1-13.

Bhargava, A. and Jelen, P. (1996). Lactose solubility and crystal growth as affected by mineral impurities. *Journal of Food Science*, **61 (1)**, pp.180-184.

Bord Bia. (2024). *Export performance and prospects report 2023 — 2024* [pdf]. Available at: <https://www.bordbia.ie/globalassets/bordbia.ie/industry/performance-and-prospects/bord-bia-exports-performance-and-prospects-report-2023---2024.pdf> [Accessed on 5th January 2025]

Bozanic, R., Barukcic, I., Lisak, K. and Jakopovic, T.L. (2014). Possibilities of whey utilisation. *Austin Journal of Nutrition and Food Sciences*, **2 (7)**, pp.1036-1042.

Brew, K. (2013). α -Lactalbumin. In: McSweeney, P.L.H and Fox P.F (2013). *Advanced Dairy Chemistry: Volume 1A: Protein: Basic Aspects.* 4th Ed. New York: Springer, pp.261–273.

Brick, T., Ege, M., Boeren, S., Böck, A., Von Mutius, E., Vervoort, J. and Hettinga, K. (2017). Effect of processing intensity on immunologically active bovine milk serum proteins. *Nutrients*, **9 (9)**, p.963.

Brody, E.P. (2000). Biological activities of bovine glycomacropeptide. *British Journal of Nutrition*, **84 (S1**), pp.39-46.

Bronsveld, E. (2015). Milk and Whey Powder In: Bylund, G. (2015). *Dairy processing Handbook.* 5th ed. available at: https://dairyprocessinghandbook.tetrapak.com/chapter/milk-and-whey-powder [Accessed on the 5th January 2025]

Brück, W.M., Redgrave, M., Tuohy, K.M., Lönnerdal, B., Graverholt, G., Hernell, O. and Gibson, G.R. (2006). Effects of bovine α-lactalbumin and casein glycomacropeptide–enriched infant formulae on faecal microbiota in healthy term infants. *Journal of Pediatric Gastroenterology and Nutrition*, **43 (5)**, pp.673-679.

Calton, A., Ma, H., Nordlund, E., Poutanen, K. and Sozer, N. (2019). Instant properties of ingredients used for point of consumption production of high-moisture food structures selectively fortified with protein and dietary fibre. *Journal of Food Engineering*, **263,** pp.204-212.

Cama-Moncunill, R., Casado, M.P., Dixit, Y., Togashi, D., Alvarez-Jubete, L., Cullen, P. and Sullivan, C. (2015). Moisture determination of static and in-motion powdered infant formula utilising multiprobe near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, **23 (4)**, pp.245-253.

Caric, M. and Kalab, M. (1987). Effects of drying techniques on milk powders quality and microstructure: A review. *Food Structure*, **6 (2)**, p.9.

Carić, M., Akkerman, J. C., Milanovi ć, S., Kentish, S. E. and Tamime, A. Y. (2009). Technology of Evaporators, Membrane Processing and Dryers. In: Tamime, A. Y (2009). *Dairy Powders and Concentrated Products*. UK: Wiley- Blackwell, pp.99-142.

Chandan, R.C. (2016). Dairy Processing and Quality Assurance: An overview. In: Chandan, R.C., Kilara, A. and Shah, N.P. (2016). *Dairy Processing and Quality Assurance*. 2nd Ed. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.33–92.

Chatterjee, A., Kanawjia, S.K., Khetra, Y. and Saini, P. (2015). Discordance between in silico & in vitro analyses of ACE inhibitory & antioxidative peptides from mixed milk tryptic whey protein hydrolysate. *Journal of Food Science and Technology*, **52**, pp.5621-5630.

Chaturvedi, S., Khartad, A. and Chakraborty, S. (2021). The potential of non-dairy synbiotic instant beverage powder: Review on a new generation of healthy ready-to-reconstitute drinks. *Food Bioscience*, **42**, p.101195.

Chavan, R.S., Shraddha, R.C., Kumar, A. and Nalawade, T. (2015). Whey based beverage: its functionality, formulations, health benefits and applications. *Journal of Food Processing & Technology*, **6 (10)**, pp.1-8**.**

Chegini, G. and Taheri, M. (2013). Whey powder: process technology and physical properties: a review. *Middle-East journal of Scientific Research*, **13 (10)**, pp.1377-1387.

Córdova-Dávalos, L.E., Jiménez, M. and Salinas, E. (2019). Glycomacropeptide bioactivity and health: a review highlighting action mechanisms and signalling pathways. *Nutrients*, **11 (3)**, p.598.

Courtois, F. (2013). Roller and drum drying for food powder production. In: Bhandari, B., Bansal, N., Zhang, M., & Schuck, P. (2013). *Handbook of Food Powders*. 2nd Ed. Cambridge, UK: Woodhead Publishing, pp.85-104.

Creamer L. K, Loveday S. M and Sawyer L. (2011). β – Lactoglobulin. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.2776- 2783.

Crowley, S.V., Kelly, A.L., Schuck, P., Jeantet, R and O'Mahony, J.A. (2015). Rehydration and Solubility Characteristics of High-Protein Dairy Powders. In: McSweeney, P.L.H. and O'Mahony, J. A. (2015). *Advanced Dairy Chemistry: Volume 1B: Proteins, Applied Aspects.* 4th Ed. New York: Springer, pp.99-132.

Cuartas-Uribe, B., Alcaina-Miranda, M.I., Soriano-Costa, E. and Bes-Pia, A. (2007). Comparison of the behavior of two nanofiltration membranes for sweet whey demineralization. *Journal of Dairy Science*, **90 (3)**, pp.1094-1101.

Cummins, E.J., McDonnell, K.P. and Ward, S.M. (2006). Dispersion modelling and measurement of emissions from the co-combustion of meat and bone meal with peat in a fluidised bed. *Bioresource Technology*, **97 (7)**, pp.903-913.

Cunningham, P., Canty, N., O'Mahony, T., O'Connor, B. and O'Callaghan, D. (2006). System identification of a falling film evaporator in the dairy industry. In: *Proceedings of the UK Automatic Control Conference*. Sheffield, United Kingdom 7-11 August 2006. United Kingdom: UKACC group.

Deeth, H.C. & Hartanto, J. (2009). Chemistry of Milk- Role of Constituents in Evaporation and Drying. In: Tamime, A. Y. (2009). *Dairy Powders and Concentrated Products*. UK: Wiley- Blackwell, pp.1-11.

Demircioglu, M., Kabay, N., Kurucaovali, I. and Ersoz, E. (2003). Demineralization by electrodialysis (ED)—separation performance and cost comparison for monovalent salts. *Desalination*, **153 (1-3)**, pp.329-333.

Deshwal, G.K., Akshit, Kadyan, S., Sharma, H., Singh, A.K., Panjagari, N.R. and Meena, G.S. (2021). Applications of reverse osmosis in dairy processing: an Indian perspective. *Journal of Food Science and Technology*, **58,** pp.1-13.

Dewettinck, K., Rombaut, R., Thienpont, N., Le, T.T., Messens, K. and Van Camp, J. (2008). Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal*, **18 (5)**, pp.436-457.

Ding, H., Li, B., Boiarkina, I., Wilson, D.I., Yu, W. and Young, B.R. (2020). Effects of morphology on the bulk density of instant whole milk powder. *Foods*, **9 (8)**, p.1024.

Dissanayake, M., Ramchandran, L., Donkor, O.N. and Vasiljevic, T. (2013). Denaturation of whey proteins as a function of heat, pH and protein concentration. *International Dairy Journal*, **31 (2)**, pp.93-99.

Doultani, S., Turhan, K.N. and Etzel, M.R. (2004). Fractionation of proteins from whey using cation exchange chromatography. *Process Biochemistry*, **39 (11)**, pp.1737-1743.

Dullius, A., Goettert, M.I. and de Souza, C.F.V. (2018). Whey protein hydrolysates as a source of bioactive peptides for functional foods–Biotechnological facilitation of industrial scale-up. *Journal of Functional Foods*, **42**, pp.58-74.

Enggalhardjo, M. and Narsimhan, G. (2006). Adhesion of dry seasoning particles onto tortilla chip. *Journal of Food Science*, **70 (3)**, pp.215-222.

Ermis, E., Farnish, R.J., Berry, R.J. and Bradley, M.S.A. (2009). Direct measurement of powder flavor adhesion onto crisp surface using a novel adhesion tester. *Particulate Science and Technology*, **27 (4)**, pp.362-372.

Etzel, M.R., (2004). Manufacture and use of dairy protein fractions. *The Journal of Nutrition*, **134 (4)**, pp.996-1002.

Fang, Y., Selomulya, C. and Chen, X.D. (2007). On measurement of food powder reconstitution properties. *Drying Technology*, **26 (1)**, pp.3-14.

Fitzpatrick, J.J., Barry, K., Cerqueira, P.S.M., Iqbal, T., O'Neill, J. and Roos, Y.H. (2007). Effect of composition and storage conditions on the flowability of dairy powders. *International Dairy Journal*, **17 (4)**, pp.383-392.

Fox, P. F. (2011a). Lactose: Chemistry Properties. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.173-181.

Fox, P. F. (2011b). Cheese: Overview. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.534-543.

Fox, P.F., Uniacke-Lowe, T., McSweeney, P.L.H. & O'Mahony, J.A. (2015a). Milk proteins. *Dairy Chemistry and Biochemistry,* 2nd Ed. Cham, Switzerland: Springer International Publishing AG, pp.145-238.

Fox, P.F., Uniacke-Lowe, T., McSweeney, P.L.H. & O'Mahony, J.A. (2015b). Lactose. *Dairy Chemistry and Biochemistry.* 2nd Ed. Cham, Switzerland: Springer International Publishing AG, pp.21-68.

Fox, P.F., Uniacke-Lowe, T., McSweeney, P.L.H. & O'Mahony, J.A. (2015c). Salts of Milk. *Dairy Chemistry and Biochemistry.* 2nd Ed. Cham, Switzerland: Springer International Publishing AG, pp.241-270.

Fox, P.F., Uniacke-Lowe, T., McSweeney, P.L.H. & O'Mahony, J.A. (2015d). Vitamins in Milk and Dairy products. *Dairy Chemistry and Biochemistry.* 2nd Ed. Cham, Switzerland: Springer International Publishing AG, pp.271-298.

Gabites, J.R., Abrahamson, J. and Winchester, J.A. (2008). Design of baghouses for fines collection in milk powder plants. *Powder Technology*, **187 (1)**, pp.46-52.

Gaiani, C., Schuck, P., Scher, J., Desobry, S. and Banon, S. (2007). Dairy powder rehydration: influence of protein state, incorporation mode, and agglomeration. *Journal of Dairy Science*, **90 (2)**, pp.570-581.

Gandhi, G., Amamcharla, J.K. and Boyle, D. (2017). Effect of milk protein concentrate (MPC80) quality on susceptibility to fouling during thermal processing. *LWT-Food Science and Technology*, **81**, pp.170-179.

Gaspard, S.J., Sharma, P., Fitzgerald, C., Tobin, J.T., O'Mahony, J.A., Kelly, A.L. and Brodkorb, A. (2021). Influence of chaperone-like activity of caseinomacropeptide on the gelation behaviour of whey proteins at pH 6.4 and 7.2. *Food Hydrocolloids*, **112**, p.106249.

Gaucheron, F. (2011). Milk Salts: Distribution and Analysis. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.908- 916

Gaucheron, F. (2013). Milk Minerals, Trace Elements and Macroelements. In: Park, Y. W. and Haenlein, G. F. (2013). *Milk and Dairy Products in Human Nutrition: Production, Composition and Health*. Somerset: John Wiley & Sons, pp.172- 200.

Gernigon, G., Schuck, P., Jeantet, R. and Burling, H. (2011). Whey Processing: Demineralization. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.738 – 743.

Ghaly, A.E., Mahmoud, N.S., Rushton, D.G. and Arab, F. (2007). Potential environmental and health impacts of high land application of cheese whey. *Am. J. Agric. Biol. Sci*, **2 (2),** pp.106-117.

Gourdon, M. and Mura, E. (2017). Performance evaluation of falling film evaporators in the dairy industry. *Food and Bioproducts Processing*, **101**, pp.22-31.

Gourdon, M., Innings, F., Jongsma, A. and Vamling, L. (2015). Qualitative investigation of the flow behaviour during falling film evaporation of a dairy product. *Experimental Thermal and Fluid Science*, **60**, pp.9-19.

Gowen, N., Gai, N., O'Mahony, J.A., O'Regan, J. and Goulding, D.A. (2025). Non-protein nitrogen in dairy ingredients: a closer look at its contribution in infant nutritional product formulation. *International Dairy Journal*, p.106201.

Greiter, M., Novalin, S., Wendland, M., Kulbe, K.D. and Fischer, J. (2002). Desalination of whey by electrodialysis and ion exchange resins: analysis of both processes with regard to sustainability by calculating their cumulative energy demand, *Journal of Membrane Science*, **210 (1)**, pp.91-102.

Hammam, A.R., Martínez-Monteagudo, S.I. and Metzger, L.E. (2021). Progress in micellar casein concentrate: Production and applications. *Comprehensive Reviews in Food Science and Food Safety*, **20 (5)**, pp.4426-4449.

Hanify, D.E. (2001). Snack seasoning application. In: Lusas, E.W. and Rooney, L.W (2001). *Snack Foods Processing*. Boca Raton: CRC Press, pp.517-527.

Hazlett, R., Schmidmeier, C. and O'Mahony, J.A., (2021a). Influence of mechanical integrity during pneumatic conveying on the bulk handling and rehydration properties of agglomerated dairy powders. *Journal of Food Engineering*, **288**, p.110103.

Hazlett, R., Schmidmeier, C. and O'Mahony, J.A. (2021b). Approaches for improving the flowability of high-protein dairy powders post spray drying– A review. *Powder Technology*, **388**, pp.26-40.

Hill, T. R. (2022). Vitamins and Minerals in Milk: Levels and Effects of Dairy Processing. In: McSweeney, P.L.H., O'Mahony, J.A. and Kelly, A.L. (2022). *Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts and Minor Constituents.* 4th Ed. New York: Springer, pp.297– 327.

Hoffman, P. (2004). Plate evaporators in food industry—theory and practice. *Journal of Food Engineering*, **61 (4)**, pp.515-520.

Hollar, C.M., Parris, N., Hsieh, A. and Cockley, K.D. (1995). Factors affecting the denaturation and aggregation of whey proteins in heated whey protein concentrate mixtures. *Journal of Dairy Science*, **78 (2)**, pp.260-267.

Huppertz, T. (2013). Chemistry of the Caseins. In: McSweeney, P.L.H and Fox P.F. (2013) *Advanced Dairy Chemistry: Volume 1A: Protein: Basic Aspects.* 4th Ed. New York: Springer, pp.135-161.

Huppertz, T. and Gazi, I. (2016). Lactose in dairy ingredients: Effect on processing and storage stability. *Journal of Dairy Science*, **99 (8)**, pp.6842-6851.

IMARC, 2024. Whey Powder Pricing Report 2024: Price Trend, Chart, Market Analysis, News, Demand, Historical and Forecast Data. [online] Available at: <https://www.imarcgroup.com/whey-powder-pricing-report> [Accessed on 2nd of January 2025]

Jeewanthi, R.K.C., Lee, N.K. and Paik, H.D. (2015). Improved functional characteristics of whey protein hydrolysates in food industry. *Korean Journal for Food Science of Animal Resources*, **35(3)**, p.350.

Jelen, P. (2022). Lactose: Occurrence, Properties, Reactions, and Significance. In: McSweeney, P.L.H., O'Mahony, J.A. and Kelly, A.L. (2022). *Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts and Minor Constituents.* 4th Ed. New York: Springer, pp.1-19.

Jelen, P. (2011). Whey Processing: Utilization and Products. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.731-737.

Jelen. P. (2009). Dried Whey, Whey Proteins, Lactose and Lactose Derivative Products. In: Tamime, A. Y (2009). *Dairy Powders and Concentrated Products*. UK: Wiley-Blackwell, pp.255- 267.

Jensen, R.G., Ferris, A.M. and Lammi-Keefe, C.J. (1991). The composition of milk fat. *Journal of Dairy Science*, **74 (9)**, pp.3228-3243.

Johnson, B. (2000). US whey products in snack and seasonings. *US Dairy Export Council*. pp.1-8.

Kailasapathy, K. A. (2016). Chemical Composition, Physical, and Functional Properties of Milk and Milk Ingredients. In: Chandan, R.C., Kilara, A. and Shah, N.P (2016). *Dairy Processing and Quality Assurance*. 2nd Ed. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.156-202.

Kelly, A. L. and Fox, P. F. (2016). Manufacture and Properties of Dairy Powders In: McSweeney, P.L.H. and O'Mahony, J. A. (2016). *Advanced Dairy Chemistry: Volume 1B: Proteins, Applied Aspects.* 4th Ed. New York: Springer, pp.1-33.

Kelly, P. (2019). Manufacture of whey protein products: concentrates, isolate, whey protein fractions and microparticulate. In: Deeth, H.C and Bansal, N. (2019). *Whey proteins*. Oxford: Academic Press, pp.97-122.

Keshani, S., Daud, W.R.W., Nourouzi, M.M., Namvar, F. and Ghasemi, M. (2015). Spray drying: An overview on wall deposition, process and modelling. *Journal of Food Engineering*, **146**, pp.152-162.

Kilara. A. (2016). Whey and Whey Products. In: Chandan, R.C., Kilara, A. and Shah, N.P. (2016). *Dairy Processing and Quality Assurance*. 2nd Ed. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.609-638.

Korhonen, H. and Marnila, P. (2011). Milk Proteins: Lactoferrin. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp. 801-806.

Kosikowski, F.V. (1968). Nutritional beverages from acid whey powder. *Journal of Dairy Science*, **51 (8)**, pp.1299-1301.

Królczyk, J.B., Dawidziuk, T., Janiszewska-Turak, E. and Sołowiej, B. (2016). Use of whey and whey preparations in the food industry–A review. *Polish Journal of Food and Nutrition Sciences*, **66 (3)**, pp.157-165.

Kumar, P., Sharma, N., Ranjan, R., Kumar, S., Bhat, Z.F. and Jeong, D.K. (2013). Perspective of membrane technology in dairy industry: A review. *Asian-Australasian Journal of Animal Sciences*, **26 (9)**, p.1347.

Lewis, M.J. (2011). The measurement and significance of ionic calcium in milk–a review. *International Journal of Dairy Technology*, **64 (1)**, pp.1-13.

Li, Y.H., Wang, W.J., Guo, L., Shao, Z.P. and Xu, X.J. (2019). Comparative study on the characteristics and oxidation stability of commercial milk powder during storage. *Journal of Dairy Science*, **102 (10)**, pp.8785-8797.

Lindmark Månsson, H. (2008). Fatty acids in bovine milk fat. *Food & Nutrition Research*, **52 (1)**, p.1821.

Lönnerdal, B. and Suzuki, Y. A. (2013). Lactoferrin. In: McSweeney, P.L.H and Fox P.F. (2013). *Advanced Dairy Chemistry: Volume 1A: Protein: Basic Aspects.* 4th Ed. New York: Springer, pp.295-317.

Lucey, J. A and Horne, D. S. (2022). Milk Salts: Technological Significance. In: McSweeney, P.L.H., O'Mahony, J.A. and Kelly, A.L. (2022). *Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts and Minor Constituents.* 4th Ed. New York: Springer, pp.297-327.

Macwan, S.R., Dabhi, B.K., Parmar, S.C. and Aparnathi, K.D. (2016). Whey and its utilization. *International Journal of Current Microbiology and Applied Sciences*, **5 (8)**, pp.134-155.

Malafronte, L., Ahrné, L., Schuster, E., Innings, F. and Rasmuson, A. (2015). Exploring drying kinetics and morphology of commercial dairy powders. *Journal of Food Engineering*, **158**, pp.58-65.

Marella, C., Salunke, P., Biswas, A.C., Kommineni, A. and Metzger, L.E., (2015). Manufacture of modified milk protein concentrate utilizing injection of carbon dioxide. *Journal of Dairy Science*, **98 (6)**, pp.3577-3589.

Mclouchlin, P.T., Bernhart, F.W. and Tomarelli, R.M. (1963). Demineralization of Whey: Use of Its Protein in Infant Feeding. *Clinical Pediatrics*, **2 (6)**, pp.302-306.

McSweeney, D.J., Maidannyk, V., O'Mahony, J.A. and McCarthy, N.A. (2021). Rehydration properties of regular and agglomerated milk protein concentrate powders produced using nitrogen gas injection prior to spray drying. *Journal of Food Engineering*, **305**, p.110597.

Meunier-Goddik, L. and Sandra, S. (2011). Liquid Milk Products: Pasteurised Milk. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.274-280.

Milovanovic, B., Djekic, I., Miocinovic, J., Djordjevic, V., Lorenzo, J.M., Barba, F.J., Mörlein, D. and Tomasevic, I. (2020). What is the color of milk and dairy products and how is it measured?. *Foods*, **9 (11)**, p.1629.

Mohammad, A.W., Teow, Y.H., Ang, W.L., Chung, Y.T., Oatley-Radcliffe, D.L. and Hilal, N. (2015). Nanofiltration membranes review: Recent advances and future prospects. *Desalination*, **356**, pp.226-254.

Morr, C.V. and Ha, E.Y.W. (1993). Whey protein concentrates and isolates: processing and functional properties. *Critical Reviews in Food Science & Nutrition*, **33 (6)**, pp.431-476.

Mulvihill, D. and Donovan, M. (1987). Whey proteins and their thermal denaturation-a review. *Irish Journal of Food Science and Technology*, **11 (1)**, pp.43-75.

Mustafa, L., Alsaed, A.K. and Al-Domi, H. (2014). Drying of sweet whey using drum dryer technique and utilization of the produced powder in French-type bread and butter cookies. *Pakistan Journal of Biological Sciences*, **17 (6)**, pp.812-820.

Muuronen, K., Partanen, R., Heidebrecht, H.J. and Kulozik, U. (2021). Effects of conventional processing methods on whey proteins in production of native whey powder. *International Dairy Journal*, **116**, p.104959.

Neff, E. and Morris, H.A.L. (1968). Agglomeration of milk powder and its influence on reconstitution properties. *Journal of Dairy Science*, **51 (3)**, pp.330-338.

O'Mahony, J. A. and Fox, P. F. (2013). Milk Proteins: Introduction and Historical Aspects. In: McSweeney, P.L.H and Fox P.F. (2013). *Advanced Dairy Chemistry: Volume 1A: Protein: Basic Aspects.* 4th Ed. New York: Springer, pp.43-85.

O'Donoghue, L.T., Haque, M.K., Kennedy, D., Laffir, F.R., Hogan, S.A., O'Mahony, J.A. and Murphy, E.G. (2019). Influence of particle size on the physicochemical properties and stickiness of dairy powders. *International Dairy Journal*, **98**, pp.54-63.

O'Sullivan, J.J., Drapala, K.P., Kelly, A.L. and O'Mahony, J.A. (2018). The use of inline high-shear rotor-stator mixing for preparation of high-solids milk protein-stabilised oil-in-water emulsions with different protein: fat ratios. *Journal of Food Engineering*, **222**, pp.218-225.

O'Sullivan, J.J., Norwood, E.A., O'Mahony, J.A. and Kelly, A.L. (2019). Atomisation technologies used in spray drying in the dairy industry: A review. *Journal of Food Engineering*, **243**, pp.57-69.

Ozel, B., McClements, D.J., Arikan, C., Kaner, O. and Oztop, M.H. (2022). Challenges in dried whey powder production: Quality problems. *Food Research International*, **160**, p.111682.

Pan, K., Song, Q., Wang, L. and Cao, B. (2011). A study of demineralization of whey by nanofiltration membrane. *Desalination*, **267 (2-3)**, pp.217-221.

Patel, K., Chen, X.D., Jeantet, R. and Schuck, P., 2010. One-dimensional simulation of co-current, dairy spray drying systems–pros and cons. *Dairy Science & Technology*, **90 (2-3)**, pp.181-210.

Patil, M.H., Tanguy, G., Floch-Fouéré, C.L., Jeantet, R. and Murphy, E.G. (2021). Energy usage in the manufacture of dairy powders: advances in conventional processing and disruptive technologies. *Drying Technology*, **39 (11)**, pp.1595-1613.

Park, C.W. and Drake, M.A. (2014). The distribution of fat in dried dairy particles determines flavor release and flavor stability. *Journal of Food Science*, **79 (4)**, pp.452-459.

Prosekov, A.J., Ul'rih, E.V., Budrik, V.G., Botina, S.G. and Agarkova, E.J. (2013). On ways demineralization of whey. *International Journal of Applied and Fundamental Research*, **1**, p.3.

Prost, J.S., Gonzalez, M.T. and Urbicain, M.J. (2006). Determination and correlation of heat transfer coefficients in a falling film evaporator. *Journal of Food Engineering*, **73 (4)**, pp.320-326.

Rebouillat, S. and Ortega-Requena, S. (2015). Potential applications of milk fractions and valorization of dairy by-products: A review of the state-of-the-art available data, outlining the innovation potential from a bigger data standpoint. *Journal of Biomaterials and Nanobiotechnology*, **6 (3)**, p.176.

Refstrup, E and Bonke, J. (2011). Milk Dryers: Drying Principles. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.3320 – 3327.

Reig, M., Vecino, X. and Cortina, J.L. (2021). Use of membrane technologies in dairy industry: An overview. *Foods*, **10 (11)**, p.2768.

Rittmanic, S. (2006). US whey proteins in ready-to-drink beverages. *US Dairy Export Council*, pp.7-8.

Rocha-Mendoza, D., Kosmerl, E., Krentz, A., Zhang, L., Badiger, S., Miyagusuku-Cruzado, G., Mayta-Apaza, A., Giusti, M., Jiménez-Flores, R. and García-Cano, I. (2021). Invited review: Acid whey trends and health benefits. *Journal of Dairy Science*, **104 (2)**, pp.1262-1275.

Ruska, D. and Jonkus, D. (2014). Crude protein and non-protein nitrogen content in dairy cow milk. *Rural Sustainability Research*, **32 (1)**, pp.36-40.

Ryan, M.P. and Walsh, G. (2016). The biotechnological potential of whey. *Reviews in Environmental Science and Bio/Technology,* **15**, pp.479-498.

Sánchez, J., Ruiz, Y., Raventós, M., Auleda, J.M. and Hernández, E. (2010). Progressive freeze concentration of orange juice in a pilot plant falling film. *Innovative Food Science & Emerging Technologies*, **11 (4)**, pp.644-651.

Sawyer, L. (2013). β -Lactoglobulin. In: McSweeney, P.L.H and Fox P.F (2013) *Advanced Dairy Chemistry: Volume 1A: Protein: Basic Aspects.* 4th Ed. New York: Springer, pp.211-261.

Schuck, P. (2011a). Lactose: Crystallization. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.2171-2184.

Schuck, P. (2002). Spray drying of dairy products: state of the art. *Le Lait*, **82 (4)**, pp.375-382.

Schuck, P. (2011b). Dehydrated Dairy Products: Milk Powder: Physical and Functional Properties of Milk Powders. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.1141-1147.

Schuck, P., Jeantet, R., Tanguy, G., Méjean, S., Gac, A., Lefebvre, T., Labussière, E. and Martineau, C. (2015). Energy consumption in the processing of dairy and feed powders by evaporation and drying. *Drying Technology*, **33 (2)**, pp.176-184.

Seo, C.W. (2022). Improved flowability and wettability of whey protein-fortified skim milk powder via fluidized bed agglomeration. *Food Science of Animal Resources*, **42 (6)**, p.915.

Singh, P.K. and Singh, H. (2016). Dry Milk Products. In: Chandan, R.C., Kilara, A. and Shah, N.P. (2016). *Dairy Processing and Quality Assurance*. 2nd Ed. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.582-608.

Sivakumar, R., Saravanan, R., Perumal, A.E. and Iniyan, S. (2016). Fluidized bed drying of some agro products–A review. *Renewable and Sustainable Energy Reviews*, **61**, pp.280-301.

Skanderby, M. (2011). Plant and Equipment Milk Dryers: Dryer Design. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.3328-3345.

Skanderby, M., Westergaard, V., Partridge, a., and Muir, D. (2009). Dried Milk Products In: Tamime, A. Y. (2009). *Dairy Powders and Concentrated Products*. UK: Wiley-Blackwell, pp.180-233.

Szulc, K. and Lenart, A. (2010). Effect of agglomeration on flowability of baby food powders. *Journal of Food Science*, **75 (5)**, pp.276-284.

Taylor, M. W. and MacGibbon, A. K. H. (2011). Milk Lipids: General Characteristics. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.2638-2643.

Turchiuli, C., Eloualia, Z., El Mansouri, N. and Dumoulin, E. (2005). Fluidised bed agglomeration: Agglomerates shape and end-use properties. *Powder Technology*, **157 (1-3)**, pp.168-175.

Wang, C., Reis, M.G., Waterhouse, G.I.N., Hemar, Y. and Reis, M.M. (2021). Prediction of dairy powder functionality attributes using diffuse reflectance in the visible and near infrared (Vis-NIR) region. *International Dairy Journal*, **117**, p.104981.

Wong, D.C.Y., Adams, M.J., Seville, J.P.K. and Fryer, P.J. (2005). A computational model of flavour deposition onto food surfaces. *Food and Bioproducts Processing*, **83 (2)**, pp.99-106.

Wong, S.Y. and Hartel, R.W. (2014). Crystallization in lactose refining—A review. *Journal of Food Science*, **79 (3)**, pp.257-272.

Xia, X., Tobin, J.T., Fenelon, M.A., Mcsweeney, P.L. and Sheehan, J.J. (2022). Production, composition and preservation of micellar casein concentrate and its application in cheesemaking: A review. *International Journal of Dairy Technology*, **75 (1)**, pp.46-58.

Zahedirad, M., Asadzadeh, S., Nikooyeh, B., Neyestani, T.R., Khorshidian, N., Yousefi, M. and Mortazavian, A.M. (2019). Fortification aspects of vitamin D in dairy products: A review study. *International Dairy Journal*, **94**, pp.53-64.

Zhang, Y., Munir, M.T., Udugama, I., Yu, W. and Young, B.R. (2018). Modelling of a milk powder falling film evaporator for predicting process trends and comparison of energy consumption. *Journal of Food Engineering*, **225**, pp.26-33.

Zhou, Z. and Langrish, T. (2022). Color formation and Maillard reactions during the spray drying process of skim milk and model systems. *Journal of Food Process Engineering*, **45 (1)**, p.13936.

Zotta, T., Solieri, L., Iacumin, L., Picozzi, C. and Gullo, M. (2020). Valorization of cheese whey using microbial fermentations. *Applied Microbiology and Biotechnology*, **104 (7)**, pp.2749-2764.

Chapter 2

Effects of in-line shearing of liquid whey concentrate on physicochemical and rehydration properties of whey powder

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Declaration

This chapter was written by Emma J. Gordon (E.J.G) and reviewed by all co-authors. E.J.G co-designed the study with the co-authors and performed all of the experimental work.

Abstract

The objective of this study was to investigate the impact of in-line shearing of liquid whey concentrate on physicochemical and rehydration properties of the resultant powder. Three times throughout a year, June (Trial 1), September (Trial 2) and November (Trial 3), a sample of cooled, crystallised, liquid whey concentrate was subjected to three different treatments: low shear (LS), which involved passing the liquid whey once through a high-pressure pump with an integrated mesh; medium shear (MS), which involved applying an in-line shear mixing process to the crystallised whey and high shear (HS), which involved passing the crystallised whey through the high-pressure pump with an integrated mesh three times. The resulting treated concentrates, along with a control (CP), were dried on a pilot-scale spray dryer with the physicochemical and rehydration properties of the resulting powders analysed. For all treatments, the powder particle size was smaller than for the CP powder. The CP powder had the largest volume-weighted mean diameter (D[4,3]) value of 184.78 ± 29.98 μm across all trials, with the LS, MS and HS treated powders having D[4,3] values of 162.67 ± 43.00, 155.44 ± 28.45 and 147.00 ± 24.95 μm respectively. The morphology and shape of the powder particles were also modified, with classic tomahawk-shaped lactose crystals, being the dominant solids constituent of the powder particles, which became more rounded with increasing intensity of shear. Powder density values also increased with increasing extent of shear. All other properties tested remained generally unchanged and shearing did not affect the rehydration properties of the whey powder. Overall, the LS, MS and HS pre-drying treatments were successful in altering the particle size and shape of the resulting powder, without negatively affecting the physicochemical and rehydration properties of the whey powders. These findings provide new insights into the impact of in-line shear mixing of pre-crystallised liquid whey concentrate on physicochemical and rehydration properties of the resulting whey powders.

2.1 Introduction

Whey is generally produced as a co-product of cheese and casein production in the dairy industry. Liquid whey can be processed into a variety of different whey-based products, the simplest of which is whey powder, as it requires the least amount of processing. Such whey powder has a wide range of applications, including animal feed, soups, bakery, confectionary, powder beverages, snack seasonings and dairy products (Adamson, 2015). Globally, approximately 121 million tons of liquid whey is processed each year (Ozel *et al*., 2022) of which, approximately 60% is converted into value-added whey products through additional processing (Macwan *et al*., 2016). These products include whey powder, lactose, whey permeate, demineralised whey powder, whey protein concentrate and whey protein isolate (Ozel *et al*., 2022). The other 40% is generally used as animal feed or disposed of (Macwan *et al.,* 2016).

The production of whey powder follows several basic steps. It typically begins with the separation of casein from the liquid whey stream, which can be completed enzymatically through rennet and/or starter culture addition, utilizing microfiltration technology or through the addition of an acidogen to decrease the pH (Jelen, 2011). This whey stream contains approximately 5-6% solids and is generally concentrated to higher solids contents by the removal of water. This is traditionally completed using evaporation and now more commonly through membrane concentration technologies such as nanofiltration (NF) and/or reverse osmosis (RO) (Kumar *et al*., 2013; Reig, Vecino and Cortina., 2021). NF not only removes water but also removes some of the lower molecular weight monovalent ions, such as sodium and chloride (Kumar *et al*., 2013). These technologies are advantageous compared to other concentrating technologies (e.g.vacuum evaporation) as they use less energy (e.g. steam, with

associated water and electricity consumption) and are operated at lower temperatures (and thereby preserves product functionality by limiting denaturation of whey proteins) (Gandhi, Amamcharla and Boyle, 2017).

To further remove water from the feed, whey must be brought up to higher temperatures to remove water through evaporation, most commonly, using a falling-film vacuum evaporator (Cunningham *et al.,* 2006). This can be done under vacuum to allow operation at lower temperatures, usually 30-70 °C (Zhang *et al*., 2018), while protecting the naturally heat sensitive whey proteins (Gandhi, Amamcharla and Boyle, 2017). Once the whey feed has been concentrated sufficiently to approximately 60% total solids (TS), it is rapidly cooled to roughly 30°C using a plate heat exchanger and transferred to whey crystallisation tanks to initiate the lactose crystallisation process (Kilara, 2016). In these tanks, the whey concentrate is constantly agitated and is further cooled down to 15-20°C over a period of 12-16 h to promote the growth of uniformly sized small crystals, that typically have the classic tomahawk shape of a lactose crystal. This crystallisation step is essential in the production of whey powder and impacts on the finished powder quality. If the lactose is not crystallised sufficiently (75-80%) (O'Donoghue *et al*., 2019), and remains in the glassy state prior to drying, this can result in detrimental effects such as product sticking to equipment during drying and caking of the powder during storage (Huppertz and Gazi, 2016).

It is important for dairy scientists and processors to continually develop powders with novel and interesting physical and functional properties to meet the adapting needs of industrial and retail end-users and consumers. One of the possible ways this can be done is to intervene at the wet phase stage prior to drying and modify targeted physicochemical properties of the feed

(e.g., particle size, viscosity), such that the spray drying properties of the feed (e.g. size of atomised droplets), and therefore the physical properties of the resultant powders (e.g. particle size, bulk density) will be modified in an intentional way. In recent years, there have been a number of innovative pre-treatments investigated that alter the functional and physiochemical properties of the resultant feed and/or powder. This includes using an inline high-shear mixing method to produce fat-filled milk emulsions (O'Sullivan *et al*., 2018), injection of carbon dioxide before and during ultrafiltration to reduce mineral content and viscosity for more optimal functionality (Marella *et al*., 2015), and utilizing a twin screw extrusion porosifcation technology (EPT^{TM}) to reduce viscosity of the feed and improve rehydration properties of the powder (Patil *et al*., 2021).

From these previous studies, it has been shown that it is possible to modify the physiochemical and functional properties of the resultant powder when intervening at the wet-stage pre-drying. The main objective of the study reported in this chapter was to understand the impacts on the physical and functional characteristics of the whey powder of applying various extents of shearing to the crystallised whey concentrate pre-drying. The findings from this study will benefit dairy scientists when developing innovative dairy powders with optimized physicochemical and functional properties.

2.2 Materials and methods

2.2.1 Materials

Three different shearing treatments were applied to cooled, crystallised, rennet whey concentrate to obtain three different particle size distributions of feed for subsequent powder production using a pilot-scale spray drier. The cooled, crystallised rennet whey concentrate was obtained from a crystallisation tank in the whey plant in Kerry Ingredients (Tralee Road., Listowel, Co. Kerry). Three different trials were conducted at three different points throughout the dairy season (November 2023, June 2024 and September 2024) to take seasonality into account. For each trial, one batch of whey was sampled to produce the 4 different powders. The crystallised rennet whey concentrate was approximately 60% total solids and between 11.4 -17.0% protein across the three trials. Post-shearing, these rennet whey concentrates were subsequently dried on a pilot-scale drier to produce three finished powders with different powder particle size distributions. A control whey concentrate with no treatment applied was also dried under identical conditions. The three different treatments included passing the whey through a pump with a mesh once, using a shearing mixer to agitate and shear the cooled crystallised rennet whey concentrate and passing the concentrate through a pump with mesh three times to achieve varying particle size distributions in the finished powder which is described in further detail in Section 2.2.2

2.2.2 Preparation of powders impacted by in-line shearing of whey concentrate

Various preliminary trials were conducted to determine what treatments could be completed on the cooled crystallised rennet whey concentrate pre-drying to alter the particle size distribution of the powder post drying. It was shown that passing the concentrate through a Silverson pump with mesh size 2.4 mm^2 caused a disruption to the crystallised concentrate,

resulting in smaller powder particles once dried. Passing the concentrate through the Silverson pump multiple times resulted in an even further reduction in finished powder particle size. Another method to alter the particle size of the cooled, crystallised rennet whey concentrate was to use a pilot scale shear mixer (Stephan model K721.465.01) to shear and mix the whey concentrate.

2.2.2.1 Control pilot plant powder

The control pilot plant (CP) powder was produced by obtaining cooled, crystallised rennet whey concentrate at approximately 60% TS from a crystallisation tank from the whey production plant in Kerry Ingredients. This concentrate was subsequently dried on the pilot plant dryer to create the CP powder. The pilot-plant spray dryer is a NIRO (GEA Group, Aktiengesellschaft, Petter-Müller-Str. 12 40468 Düsseldorf, Germany), model FU11.DA single stage dryer with a cyclone attached. This dryer utilizes a nozzle atomizer with 2 bar pressure and has approximately 5 L per hour capacity. All powders were dried using an inlet air temperature of 220°C and an outlet temperature of 90-95 °C. Two different powder samples were obtained (from the cyclone and the bed) and these were recombined post drying.

2.2.2.2 Low shear powder

The low shear (LS) powder was produced by obtaining cooled crystallised rennet whey concentrate and passing this concentrate through a Silverson in-line pump, model 700LS (Waterside, Chesham, HP5 1PQ) with an integrated mesh (size 2.4 mm^2). Approximately 20 kg of concentrate was placed in a 100 L balance tank attached to the pump. This concentrate was passed once through the pump and exited into another balance tank to ensure treatment

was conducted on all the crystallised whey concentrate being used. This LS concentrate was then dried using the same pilot scale dryer and under the same conditions as previously described in Section 2.3.2.1.

2.2.2.3 Medium shear powder

The medium shear (MS) powder was produced by placing approximately 20 kg of cooled, crystallised whey from the crystallisation tank into a Stephan mixer model K721.465.01 (Stephan, Deeside Industrial Park, Delta House, Tenth Ave, Deeside CH5 2UA). The Stephan mixer was set to the medium shear setting for 10 min and subjected the whey concentrate to a blade agitator which spun in the bottom of the bowl while a mixing baffle circulated around the edges of the bowl to remove any product build-up. Cooled water was attached and circulated in the jacket of the mixer to maintain low temperatures. This concentrate was subsequently dried on the pilot scale dryer as previously described in Section 2.3.2.1.

2.2.2.4 High shear powder

The high shear (HS) powder was produced in a similar way to the LS powder discussed in Section 2.2.2.2; however, the whey was passed three times though the same high-pressure pump with integrated mesh to further shear the concentrated crystallised rennet whey concentrate. After this treatment, the whey concentrate was dried under the same conditions as previously described in Section 2.3.2.1.

2.2.3 Powder composition

Protein content was calculated by determining the nitrogen content using the Kjeldahl method adapted from IDF Standard 020-1/2:2001. Ash content of the powder was determined using the method from the IDF Standard 89:1979 and IDF Standard 90:1979. Moisture was measured using IDF Standard 26:2004 (E)/ ISO Standard 5537:2004.

2.2.4 Powder physical properties

2.2.4.1 Particle size analysis

The particle size distribution (PSD) of the powders was measured using the Malvern Mastersizer 3000, which uses a laser diffraction technique to analyse the size of individual powder particles. The Mastersizer 3000 was equipped with an automated Aero S dry powder disperser cell (Malvern Instruments, Worcestershire, UK). Approximately 10 g of each sample was placed in the feed hopper of the Aero S cell. The feed hopper was set at a height of 3 mm, a feed rate of 30% was set and a dispersion pressure of 1.5 bar was used so that the optimal obscuration level was achieved for all powder samples while they were being analysed. The background and measurement durations of 20 s were used, and the particle refractive index was set at 1.45. Analysis of each powder sample was completed in triplicate. The particle size distribution of the powders were reported as the Dv(10), Dv(50) and Dv(90). These parameters describe a point in the PSD where 10, 50 and 90%, respectively, of the measured volume of the sample has a powder particle size of that value or smaller. The Dv[4,3] was also reported, which is indicative of the sample's particle mean diameter, also known as the volume weighted mean.

2.2.4.2 Particle morphology

Powder particle morphology of each sample was determined using a scanning electron microscope (SEM) (JOEL- 5510 Scanning Electron Microscope, Joel Ltd, Tokyo, Japan). The powder was applied to double-sided adhesive carbon tape and stuck to aluminium SEM stubs. A sputter coater (Quorum Q150R ES Sputter Coating Unit, Emitech K550X, Ashford, UK) was using to apply a thin (5nm) coating of 80:20 gold: palladium to the samples prior to image analysis. This coating was used to avoid charging while the sample was being analysed. The stubs were then placed in the SEM and micrographs were taken (5 kV accelerating voltage) for each, using magnifications between 35 -300x.

2.2.4.3 Particle shape

The powder particle shape of each sample was analysed in triplicate using a Qicpic™ dynamic image analyser (Sympatec GmbH, Clausthal-Zellerfeld, Germany) with the RODOS attachment. For all powder samples, the same settings were used; a feed rate of 35%, bar pressure of 1.0 bar and a hopper height of 2.5 mm. Approximately 10 g of each sample was placed into the hopper for each test which was conducted in triplicate. For a result to be utilized, it was necessary that the optical density had to remain below 3% throughout the test and a minimum of 100,000 particles must have passed through the system to get an accurate reading.

Four parameters were focused on in this study: aspect ratio (AR), convexity (C), sphericity (S) and roundness (R). The dynamic imaging analysis method takes the silhouette/perimeter of each of the powder particles and calculates values for each descriptor as mentioned above and by Gaiani *et al*., (2011) and Nikolova *et al*., (2014).

The AR value is indicative of how elongated the powder particles are, and calculated by taking the minimum diameter value and dividing this by the maximum diameter of the particle. The closer the AR value is to 0, the more elongated the powder particle is. The C value illustrates how compact the particle is and is a ratio of the area of the open concave ("hollow") region (Gaiani *et al.,* 2011; Nikolova *et al.*, 2014); the smaller the value, the more hollow areas in the surface of the powder particle are present. The S value relates to the smoothness of the powder particle, with the values ranging from rough (0) to smooth (1) (Gaiani *et al.,* 2011; Nikolova *et al.*, 2014). The R value relates to how round the powder particle is; the closer the number is to 1, the more round it is. The R value is calculated as the ratio of the average radius of curvature of all convex regions to the circumscribed circle of the particle (Sympatec, 2024).

2.2.4.4 Colour

Colour of the powders were measured using a Konica Minolta Chroma Meter CR-410 (Minolta Ltd., Milton Keynes, UK). This instrument was calibrated using a white tile prior to analysis to ensure accurate colour readings. Approximately 20 g of sample powder was placed in a transparent petri dish to contain the powder and to ensure the Chroma meter was only analysing the powder itself. Prior to analysis, the powder was packed into the petri dish by pushing the lid of the petri dish down on the powder to ensure that there was no air bubbles/surface unevenness present that could alter colour readings. Three different L*A*B* colour readings were taken in three different areas of the petri dish to ensure the most accurate result for each powder. L* represents darkness to lightness where values range from 0 to 100, A* represents greenness to redness and B* represents blueness to yellowness, with both A* and B* values spanning from -128 to +127 (Chudy *et al*., 2020).

Delta E (ΔE) was another parameter measured for colour analysis of the powder. Equation 2.1 calculates the colour-difference between two powders (Lee, 2005). This was calculated by comparing the L*A*B* values for the control in each trial with each treated powder.

$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 (\Delta b^*)^2]^{1/2}$…….…….. Equation 2.1

2.2.4.5 Powder density

Two different density values were obtained: bulk and tapped density. This was completed by pouring 100 g of a test portion of the powder sample into a 250 ml plastic graduated cylinder, graduated in 1 ml increments. The volume was then read from the graduated cylinder. This was noted as the bulk volume which was expressed in grams per millilitre. This powder-filled graduated cylinder was then placed into the JEL STAV II Jolting Volumeter (J. Engelsmaan Apparatebau, Ludwigshafen, Germany). This tapping device was set to perform 1250 taps followed by an automatic stop. After the tapping was completed, the volume was read from the graduated cylinder, and this was noted as the tapped volume. The equation below was used to convert from poured/tapped bulk volume to poured/tapped bulk density (Equation 2.2; Wang *et al*., 2021). Each sample was tested in triplicate to ensure accurate readings.

$$Poured/\ tapped\ density = \frac{powder\ weight(g)}{final\ volume\ (ml)} \text{…….. Equation 2.2}$$

2.2.4.6 Flowability

The flow index of each powder sample was determined using a Brookfield Powder Flow Tester (PFT) (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA). Each powder sample was tested in triplicate using a method previously described by Crowley *et al*. (2014). A 6” vane-shaped lid and 6” trough was used for all samples. The trough was manually filled

with powder using a spoon and, once the trough was filled, a curved shaping blade was circled around the powder filled trough to get a uniform, curved powder surface. The flow function was determined by plotting the unconfined failure strength data versus the major principal consolidating stress data (Alonso-Miravalles *et al*., 2020); a trendline was plotted, and the inverse of the slope obtained was indicative of the flow index.

2.2.5 Colloidal stability of reconstituted whey powder

2.2.5.1 Viscosity of reconstituted powder

Each powder from Trial 1 was rehydrated to 25% w/w total solids using room temperature deionized water while stirring for 60 min. Apparent viscosity of rehydrated samples was measured immediately after rehydration under rotational conditions using an Anton Paar MCR 102e Rheometer (Unit 21, Grattan Business Park, Clonshaugh Business and Technology Park, Dublin, D17 H526) equipped with a concentric cylinder geometry (bob length 40.00 mm, bob diameter 26.66 mm, cup diameter 28.92 mm) at 25°C. Samples were pre-sheared from 0 to 100 s^{-1} over 30s; apparent viscosity was reported as the average viscosity measured during 30s at a constant shear rate of $100s^{-1}$.

2.2.5.2 Stability of reconstituted whey powder

Each powder from Trial 1 was rehydrated as described previously in Section 2.3.5.1. The stability of the solutions was measured using a Turbiscan LAB –Optical Analyser (Microtrac Formulaction Inc., Toulouse, France) with 800 nm near infrared (NIR) light. A "SMART scan" was completed on each sample, at a temperature of 25°C, which allowed the instrument to decide how many measurements to perform over time, based on the stability of each sample.

The parameter reported was the Turbiscan Stability Index (TSI) which gives an indication of the stability of the solution.

2.2.6 Protein profile, denaturation and aggregation of whey powder

2.2.6.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

The proteins present in the whey powder were characterized using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) under non-reducing and reducing conditions using a mini-gel electrophoresis unit (Bio-Rad Laboratories, Copenhagen, Denmark) which was placed over ice. Analysis was performed on pre-cast mini-protein Tetra Cell TGX 4-20% acrylamide gradient gels (Bio-Rad Laboratories, Copenhagen, Denmark). For non-reducing conditions, 100 µL of whey powder solution which was made up to a protein concentration of approximately 4 mg/ml was combined with l00 µl of Laemmli sample buffer. For reducing conditions, 100 µL whey powder solution at the same concentration was mixed with 95 µL of Laemmli sample buffer and 5 µL of β-mercaptoethanol. For each sample, a 12 µL aliquot of the solution was placed into the well of the gel along with 5 µL of SDS-PAGE broad range protein standard (Bio-Rad Laboratories, Copenhagen, Denmark). The gel was run at 200 V for ~ 40 min to separate the proteins. Once sufficient separation had occurred, the gel was removed and stained for 1 h with Comassie Brilliant blue R-250 and destained by submerging the gel in distilled water which was frequently changed for ~ 48 h until a clear background was obtained.

2.2.6.2 Measurement of total protein, pH 4.6-soluble protein and non-protein nitrogen

Total protein content was calculated by determining the nitrogen content using the Kjeldahl method adapted from IDF Standard 020-1/2:2001. The pH 4.6-soluble fractions of samples were prepared following the method of O'Kennedy and Mounsey (2006) with some modifications, detailed as follows. Each whey powder was made up to a 1% protein solution (60 mL). Acetic acid (6 mL of 10%, w/v) was added to each whey powder solution (60 mL), which was then heated at 40 °C for 10 min, after which 6 mL of sodium acetate (1mol L^{-1}) was added to the mixture and held at 40 °C for a further 10 min, after which the solutions were allowed to cool to 22 °C. The pH of the whey powder solution was then adjusted to pH 4.6 with either 10% (w/v) acetic acid or 1 mol L^{-1} if necessary. The solutions were then divided into 3 centrifuge tubes and were centrifuged at 10,000 g at 22 °C for 20 min. Whatman no. 542 filter paper was used to separate the filtrate from the supernatant. The extent of total whey protein denaturation was determined using Kjeldahl nitrogen analysis of the initial samples and the respective pH 4.6-soluble fractions.

The non-protein-nitrogen (NPN) fraction of samples were prepared following the AOAC Official Method 991.21 with some modification, detailed as follows. The various whey powders were rehydrated to 3.5 % protein for 1 h in a 40 °C water bath. The whey powder solutions (10 mL ± 0.1 mL) were pipetted into an Erlenmeyer flask followed by the addition of (40 mL ± 0.5 mL) 15 % trichloroacetic acid (TCA) solution which was then swirled to mix the two solutions together. The precipitate was let to settle for 20 min, and followed by filtration of the solution (Whatman No. 1 paper, 15 cm). The total NPN content was determined by Kjeldahl nitrogen analysis of the initial samples and the respective NPN fractions.

2.2.5 Powder functional properties

2.2.5.1 Rehydration process

The dispersibility of each powder was analysed using a Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS) unit. This is a system that can monitor the gas release from powder in a solution and thus provides information on the rehydration process of dissolving a powder. The unit consists of a water-filled glass vessel with a magnetic stirring bar, above which a weighing boat with a defined amount of powder is placed on a mechanism which tips the powder into the water. Once the test begins, the powder is poured into the glass vessel and it begins to wet and dissolve. The magnetic stirring bar impacts on the inside wall of the glass vessel and releases sound which is recorded by a microphone fixed above the glass vessel. As the powder is dissolving and gas is being released from the powder in the water, the frequency of the sound being emitted changes until the powder is fully dissolved, when the frequency returns to a steady state. This is then displayed as a graph of time (seconds) vs frequency (Hz) (Shaozong *et al*., 2019).

For all powder samples, 500 mg of powder was weighed into a weigh boat and the glass vessel was filled with 25 mls of ultrapure water at room temperature, to give a powder concentration of 0.2%. The length of each test was 800 s, which included 30 s at the beginning of the test to stabilize the system and obtain a clear steady-state frequency. This procedure was adapted from that of Wu *et al*. (2019). The BARDS unit was cleaned using $NaCO_3$ between different treated powders and the glass vessel was rinsed with deionized water in between replicates to ensure the system remained clean. For all samples, time was plotted against frequency picked up by the microphone, which gave a spectrum from which a variety of different parameters could be

obtained. This included the minimum frequency (f_{min}) achieved during the test, the time the f_{min} occurs (t_m), and the volume of gas released during the test.

2.2.6 Study design and statistical data analysis

For this study, three individual trials were conducted throughout the year (June, September and November). From each trial, four different powders were produced; (1) the control pilot plant (CP) powder which had no treatment applied to the cooled crystallised rennet whey concentrate and was then dried on the pilot-scale spray dryer; (2) the low shear (LS) powder, whereby the concentrate for this powder was passed through a pump with an integrated mesh once and subsequently dried on the pilot plant dryer; (3) the medium shear (MS) powder, where the concentrate was subjected to shearing using a shear mixer and then dried on the pilot-scale dryer and (4) the high shear (HS) powder, for which the concentrate was passed through the pump with integrated mesh three times. For each trial, all concentrate was sampled from the same crystallisation tank once the rennet whey reached a sufficient temperature to ensure that adequate lactose crystallisation had occurred.

Data were analysed using a Univariate General Linear Model (GLM) with SPSS Version 29.0.0 software (IBM, Armonk, New York, USA). The GLM procedure allowed analysis of the effects of multiple independent variables on one dependent variable as well as the examination of any interaction effects. Prior to running statistical analysis, assumptions of data normality, homogeneity of variance (Levene's test), and independence of errors were assessed; appropriate data transformations were applied, where necessary. *Post-hoc* multiple comparison tests (Tukey's test) were conducted to explore statistically significant interactions further. A statistical significance level, α, of 0.05 was used throughout. The effects of pre-drying shearing

treatment and trial on particle size, particle shape, colour, flowability, bulk volume, tapped volume, reconstituted viscosity, total protein, denatured protein and non-protein nitrogen were statistically analysed. All analyses were carried out in triplicate and for all experimental data, three trials were performed and the effect of trial on all parameters measured was statistically analysed. The effect of shearing treatment on viscosity of the reconstituted powders was carried out on a single trial. Unless otherwise stated, results are expressed as mean ± standard deviation from triplicate analysis.

2.3 Results and discussion

2.3.1 Composition

The composition of the four powders across three trials are listed in Table 2.1. Small differences in compositions were identified between powders from the same trial with different treatments applied. The average protein results across the three trials for CP, LS, MS and HS powders were 14.30 ± 1.74, 14.10 ± 2.64, 14.10 ± 2.63 and 14.00 ± 2.77 %, respectively. Statistical analysis demonstrated a significant difference ($p < 0.05$) in protein values between the CP powder sample and all other samples. However, the difference in protein values between the LS, MS and HS powders were not shown to be significant ($p > 0.05$). This indicates that the shearing treatments may have affected the protein contents in the resulting powder when compared to the CP powder.

For each trial, all whey concentrate was sampled from the same crystallisation tank implying that the differences in protein and ash between the dried powder samples from the same trial may be due to discrepancies in testing or effects of the treatments applied on the powders.

Statistically significant differences in protein values were shown across all trials ($p < 0.05$), which confirms that the protein values were significantly affected by Trial. Differences in composition between the three trials are most likely due to the effects of seasonality, Trial 1 took place in June; Trial 2 took place in September and Trial 3 took place in November. Protein content increased throughout the year which is similar to the results shown in the study conducted by Bernabucci *et al*. (2015), as protein contents were lowest during the summer and highest throughout the winter. Whey powder is found to generally have between 11- 14.5% protein depending on when throughout the dairy season it was produced (Bronsveld, 2015).

Sweet whey tends to have ash contents of approximately 8% (Chandan, 2016). In this current study, ash contents of approximately 5% were reported. This is most likely due to the nanofiltration (NF) membrane technology used to concentrate the liquid whey pre-evaporation. NF not only removes water but is also used to remove organic compounds with molecular weight less than 300 Da, which includes some monovalent and small divalent minerals (Pan *et al*., 2011, Mohammad *et al.,* 2015) which would thus result in lower contents of ash being present in the powder.

It is important to keep moisture content of whey powders below 4.5% to ensure stability during storage (Chandan, 2016). Lower contents of moisture seen in this study may be due to conditions used on the pilot-scale spray dryer.

Table 2.1: Composition of Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powders.

Treatment	Trial	Protein	Moisture (%)	Ash
CP	Trial 1	12.60 ± 0.02	1.13	5.07
	Trial 2	14.40 ± 0.15	1.54	6.25
	Trial 3	16.00 ± 0.20	1.59	5.80
	Average	**14.30** ± 1.74	**1.36 ± 0.36**	**5.46 ± 1.01**
LS	Trial 1	11.20 ± 0.09	0.99	4.86
	Trial 2	14.40 ± 0.08	0.68	5.64
	Trial 3	16.50 ± 0.12	2.10	4.72
	Average	**14.10** ± 2.64	**1.26 ± 0.75**	**5.07 ± 0.50**
MS	Trial 1	11.30 ± 0.09	1.13	5.07
	Trial 2	14.50 ± 0.05	2.00	6.19
	Trial 3	16.50 ± 0.12	1.57	5.08
	Average	**14.10** ± 2.63	**1.57 ± 0.44**	**5.45 ± 0.64**
HS	Trial 1	11.20 ± 0.21	1.34	5.13
	Trial 2	14.10 ± 0.03	2.29	4.86
	Trial 3	16.80 ± 0.05	2.28	5.12
	Average	**14.00** ± 2.77	**1.97 ± 0.55**	**5.04 ± 0.15**

2.3.2 Powder physical properties

2.3.1.1 Particle size analysis

The particle size distribution values are displayed in Table 2.2. In all three trials, the CP powder sample had the largest volume-weighted mean diameter (D[4,3]), the average of which being 185 ± 30.0 μm when compared to the D[4,3] of the average of the treated powder samples; LS, MS and HS, which had D[4,3] values of 163.00 ± 43.00, 155.00 ± 28.40 and 147.00 ± 25.00

μm, respectively. This agrees with the hypothesis that shearing the cooled crystallised concentrated whey prior to drying (as described in section 2.3.2) results in powder particles once dried. This is most likely due to breakage of the large crystals that were formed during the lactose crystallisation process into smaller crystals. The D[4,3] values of the CP powder sample varied across the 3 trials; however, when applying the treatments, the particle size reduced consistently across all trials. D[4,3] values for CP samples when compared to LS and MS samples were demonstrated to be statistically different ($p < 0.05$); however, the interaction between CP and MS D[4,3] value was not significantly different ($p > 0.05$). This may be due to the different shearing equipment used for the MS powder when compared to the LS and HS powders.

The D[4,3] value for the HS powder sample was the lowest value across the three trials. This is due to the HS sample obtaining the largest amount of shearing treatment, which can be reflected in the particle size results. The reduction in particle size following the shearing treatments resulted in a subsequent increase in specific surface area (SSA) of each sample, starting with CP of 108 ± 9.63 m^2/g, which was lower compared to 115.00 ± 22.90, 118.00 ± 12.40 and 120.00 ± 16.30 m^2/g for LS, MS and HS treatments respectively. As the particles are broken down this in turn results in an increase in the surface area of the particles (Crowley *et al*., 2014). Statistical analysis demonstrated a significant difference ($p < 0.05$) for all samples across all trials.

Table 2.2: Particle size distribution values of Control pilot plant (C) powder, Low shear (LS) powder, Medium shear (MS) powder and High shear (HS) whey powder.

		Dv10	Dv50	Dv90	D[4,3]	D[3,2]	Span	SSA
				(µm)			-	m^2/g
	Trial 1	19.40 ± 0.15	152.00 ± 0.58	302.00 ± 2.52	157.00 ± 1.53	50.30 ± 0.33	1.86 ± 0.01	119.00 ± 0.91
CP	Trial 2	23.40 ± 0.32	176.00 ± 0.00	469.00 ± 3.79	216.00 ± 1.15	58.80 ± 0.40	2.54 ± 0.02	102.00 ± 0.70
	Trial 3	21.90 ± 0.47	155.00 ± 1.53	375.00 ± 5.69	181.00 ± 1.53	58.20 ± 0.89	2.27 ± 0.06	103.00 ± 1.57
	Average	**21.60 ± 2.03**	**161.00 ± 13.10**	**382.00 ± 84.10**	**185.00 ± 30.00**	**55.80 ± 4.72**	**2.22 ± 0.34**	**108.00 ± 9.63**
	Trial 1	17.40 ± 0.21	144.00 ± 0.58	290.00 ± 1.53	148.00 ± 1.15	45.70 ± 0.46	1.89 ± 0.00	132.00 ± 1.36
LS	Trial 2	29.00 ± 0.31	160.00 ± 1.15	459.00 ± 4.73	211.00 ± 2.00	67.80 ± 0.49	2.69 ± 0.01	88.50 ± 0.63
	Trial 3	19.20 ± 2.15	125.00 ± 6.08	257.00 ± 7.77	129.00 ± 1.15	48.70 ± 4.34	1.91 ± 0.05	124.00 ± 10.50
	Average	**21.90 ± 6.20**	**143.00 ± 17.40**	**335.00 ± 108.10**	**163.00 ± 43.00**	**54.10 ± 12.0**	**2.16 ± 0.46**	**115.00 ± 22.90**
	Trial 1	18.80 ± 0.40	138.00 ± 1.53	278.00 ± 4.58	143.00 ± 2.00	47.70 ± 0.46	1.89 ± 0.00	126.00 ± 1.21
MS	Trial 2	22.00 ± 1.71	143.00 ± 2.31	414.00 ± 21.50	188.00 ± 8.54	57.70 ± 2.21	2.73 ± 0.09	104.00 ± 3.93
	Trial 3	18.50 ± 0.44	129.00 ± 2.31	264.00 ± 8.72	135.00 ± 4.04	47.90 ± 1.01	1.90 ± 0.03	125.00 ± 2.69
	Average	**19.80 ± 1.93**	**137**.00 **± 7.04**	**319.00 ± 83.10**	**155.00 ± 28.50**	**51.10 ± 5.70**	**2.18 ± 0.48**	**118.00 ± 12.40**
	Trial 1	17.50 ± 0.10	135.00 ± 0.58	266.00 ± 1.00	137.00 ± 0.58	44.80 ± 0.15	1.85 ± 0.01	134.00 ± 0.38
HS	Trial 2	24.00 ± 0.15	148.00 ± 1.00	361.00 ± 3.79	175.00 ± 1.53	58.70 ± 0.56	2.27 ± 0.02	102.00 ± 0.91
	Trial 3	19.10 ± 1.05	123.00 ± 4.00	248.00 ± 8.50	128.00 ± 4.51	48.30 ± 2.25	1.86 ± 0.00	124.00 ± 5.80
	Average	**20.20 ± 3.37**	**135.00 ± 12.50**	**291.00 ± 60.40**	**147.00 ± 25.00**	**50.60 ± 7.25**	**1.99 ± 0.24**	**120.00 ± 16.30**

Dv10, Particle size below which 10% of material volume exists.
Dv50, Particle size below which 50% of material volume exists.
Dv90, Particle size below which 90% of material volume exists.
D[4,3], volume-weighted mean particle diameter.
D[3,2] surface-weighted mean particle diameter
Span -measurement of the width of the distribution calculated as (Dv90)- (Dv10)/ (Dv50)
SSA- Specific surface area

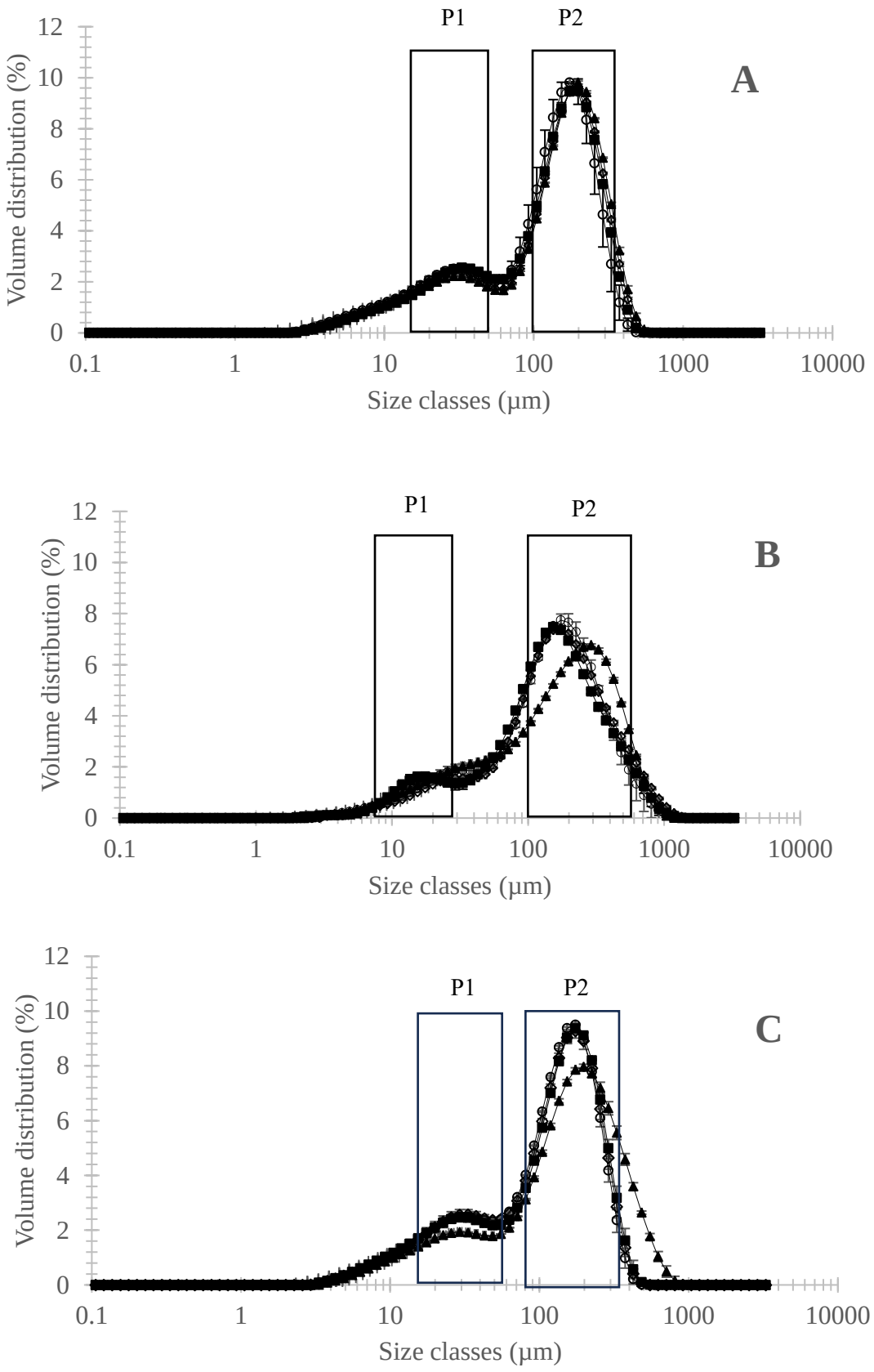


Figure 2.1. Particle size distribution of (A) Trial 1 (B) Trial 2 (C) Trial 3 of Control pilot plant (CP) —▲—, Low shear (LS) —◇—, Medium shear (MS) —■— and High shear (HS) —○— whey powder

The mean particle size (Dv50) reflected similar results to that of the D[4,3]. The CP sample for the average of the 3 trials had the largest value at 161.00 ± 13.10 µm, compared to the average value for the Dv50 of the 3 treated powders, reducing as higher shearing treatments were applied, i.e., 142.80 ± 17.40, 136.80 ± 7.04 and 135.00 ± 12.50 µm for HS, MS and LS respectively. The interaction between the Dv50 value for the CP powder and HS powder was statistically significant ($p < 0.05$). This is most likely due to the HS powder receiving the most severe shearing treatment. The particle size below which 90 % of material volume exists (Dv90) also reflected this result, as again the average CP sample had the largest Dv90 value, at 382.00 ± 84.10 µm and as the intensity of the shearing treatments increased, the Dv90 value was lower, at 335.00 ± 108.00, 319.00 ± 83.10 and 291.00 ± 60.40 µm, respectively. Similar to that of the D[4,3] value, the interaction between CP and MS Dv90 value was not significantly different ($p > 0.05$); however, a reduction in particle size was still demonstrated for all treatments (Table 2.2).

The particle size below which 10% of the material volume exists (Dv10) was not hugely affected by these shearing treatments. For the average of the 3 trials, the Dv10 was 21.60 ± 2.03, 21.90 ± 6.20, 19.80 ± 1.93 and 20.20 ± 3.37 µm for CP, LS, MS and HS powder samples This may be due to the smaller lactose crystals not being affected by the shearing treatments chosen due to their small size escaping the effects of the shearing treatments. This may also be due to the fact the smaller powder particles tend to be more rich in protein and contain lower proportions of lactose (O'Donoghue *et al*., 2019) and thus the Dv10 remained generally unchanged between treatments. Statistical analysis indicated that the Dv10 values for the CP and MS samples and the LS and HS samples were not significantly different ($p > 0.05$). This may be due to the same shearing equipment used for the LS and HS samples. The MS shearing

equipment may not have affected the Dv10 value and thus was similar to the Dv10 value for the CP powder.

The D[4,3] values across the three trials and were statistically significant ($p < 0.05$). This may be due to the varying composition of the powders, in particular protein contents, due to seasonality across the year as the same drying parameters were used for all three trials.

The span is the measurement of the width of the distribution and the average value for span for the CP powders had a value of 2.22 ± 0.34 which was lower for the three shearing treatments. This indicates that the HS treatment was most effective at reducing the number of larger particles while not disrupting the smaller particles, and thus reducing the span.

Figure 2.1 displays the particle size distribution graphs for the three trials. For all plots, (A), (B) and (C), there were two clear populations of particles (P1 and P2). This is most likely due to the pilot scale dryer producing two different powder samples, one sample from the cyclone (~30%) and one sample from the bed (~70%) which were later recombined as described in Section 2.3.2.1. P1 is probably mostly the cyclone powder sample while P2 is mostly the main chamber (bed) sample which is rich in larger lactose crystals.

The plot from Trial 1 (A) does not show a clear difference between the control samples and the sheared samples, which may be due to the more minimal change in particle sizes between the controls and the treated powders. The particle size distribution plot for Trial 2 (B) and Trial 3 (C), exhibit a clear difference between the control and the treated powders (LS, MS and HS).

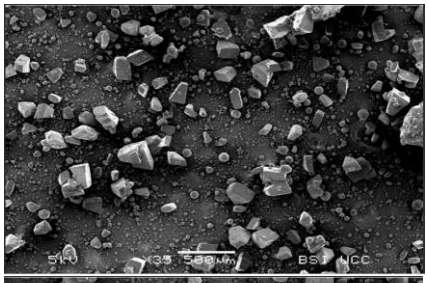
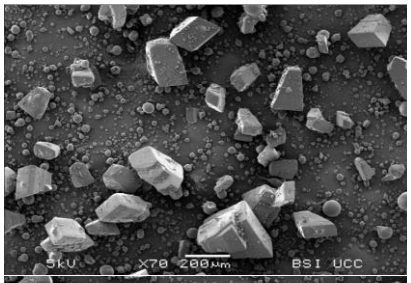
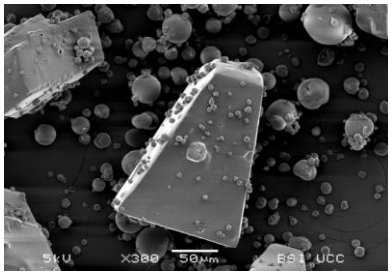
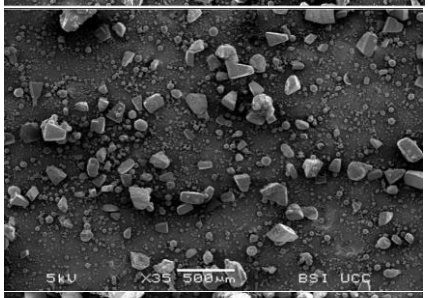
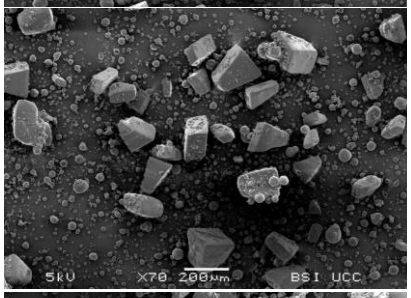
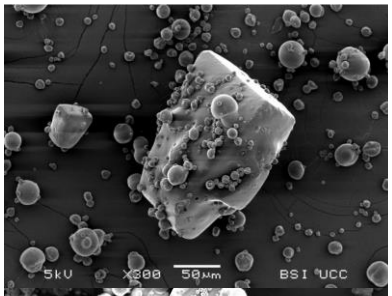
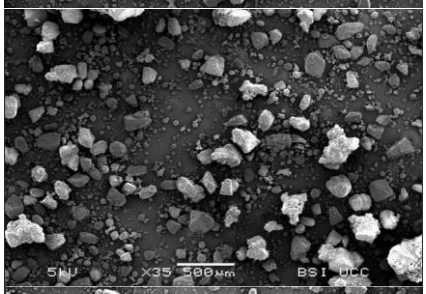
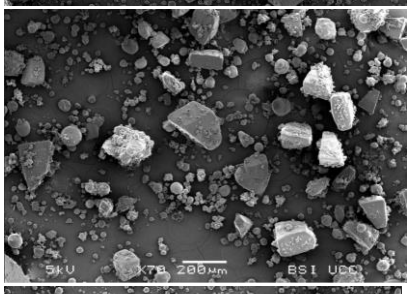
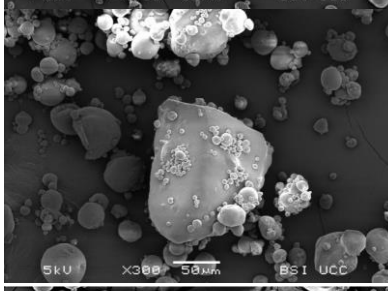
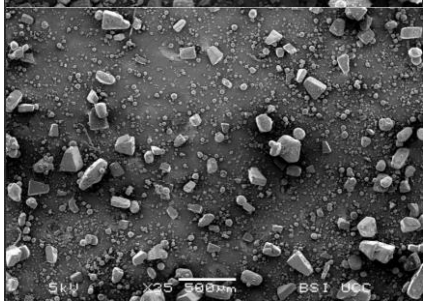
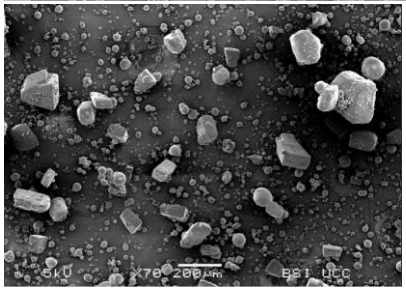
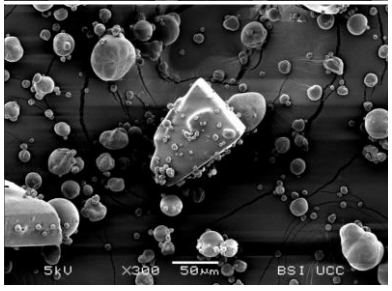
2.3.1.2 Morphology of whey powder particles

It is evident from the SEM images displayed in Table 2.3 that the shearing treatments influenced the size and shape of the powder particles, with such particles dominated by the presence of lactose crystals. These micrographs allow clear visualization of the lactose crystals in the powders. Under all magnifications, the CP powder sample appeared to have the largest particles, followed by LS, MS and HS particles which had larger amounts of smaller particles. This was reflective of the results from particle size distribution analysis. As well as smaller particles evident in the SEM images of the treated powders, there were less tomahawk-shaped crystals in the CP sample when compared to the sheared samples. This can be clearly seen when comparing the CP powder image at 35x and 70x magnification to the images of the LS, MS and HS powder images at the same magnifications.

The 300x magnification for the CP powder showed the clear tomahawk shape associated with lactose crystals. From the 300x magnification images in Table 2.3, it was evident that the shearing processes for LS, MS and HS altered the shape of the lactose crystal as they appeared more rounded compared to the classic tomahawk-shaped lactose crystal seen in the CP powder.

The round components surrounding the lactose crystals are the other components of the whey solids such as amorphous lactose, ash and protein which was similarly seen in SEM images of pre-crystallised whey powder taken by Saito (1988).

Table 2.3: Scanning electron microscopy (SEM) images of Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powder under magnifications of 35, 70 and 300x.

Sample	35x	70x	300x
Trial 1 CP			
Trial 1 LS			
Trial 1 MS			
Trial 1 HS			

2.3.1.3 Particle shape analysis

The particle shape of the CP, LS, MS and HS powders were analysed using dynamic image analysis which analyses the perimeters shape of > 100,000 individual powder particles and calculates an average value of each descriptor based on these silhouettes. The descriptors of interest in this study were aspect ratio (AR), convexity (C), sphericity (S) and roundness (R).

For all trials and all powders, the AR values ranged from 0.77 to 0.81 (Table 2.4). Statistical analysis demonstrated a significant difference ($p < 0.05$) between the CP powder sample and all other samples. However, the paired multiple comparison test demonstrated no significant difference between the HS when compared with the LS sample and the HS sample compared with the MS sample ($p > 0.05$). This indicates there is a difference in elongation of the powder samples when comparing the CP powder with the shear-treated powders (LS, MS and HS).

The C values, which illustrate how compact the particle is, were similar for all samples, with a mean value of 0.93 ± 0.00. From this result, it is clear the convexity of the powder particles is not a point of difference when comparing the extent of shearing treatment of different samples. The S value of all samples of powders analysed were 0.83 to 0.85. Statistical analysis indicated that there was a significant difference ($p < 0.05$) when comparing all samples against the CP sample, which indicates that the shearing treatments does affect the smoothness of the dried powder particles. The paired multiple comparison test showed no significant difference between the HS when compared with the LS sample ($p > 0.05$), which may be due to the same shearing equipment used for both samples, even though different extents of shearing took place.

The R values varied the most out of all the descriptors of interest for this study. For all powders analysed, LS, MS and HS all had larger R values compared to their respective control sample R value. This can be seen in Table 2.4, as the average R value for the CP powder was 0.47 ± 0.05 compared to the R value for the LS, MS and HS powders i.e., 0.50 ± 0.03, 0.51 ± 0.04 and 0.50 ± 0.03, respectively. Statistical analysis indicated that there was a significant difference ($p < 0.05$) when comparing all samples against the CP sample. Similar to the C value, the paired multiple comparison test for the R value demonstrated no significant difference between the HS and LS sample ($p > 0.05$), which is most likely due to the same shearing equipment being used for these samples. The R value relates to how round the particle is and indicates that the shearing treatments applied have create round particles when compared to the CP powder. This is most likely due to rounding the edge of the lactose crystals in the concentrate.

Table 2.4: Particle shape descriptor analysis of Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powder. The table includes the average (50%) results for aspect ratio (AR), sphericity (S), convexity (C) and roundness (R).

Treatment	Trial	Aspect ratio	Convexity	Sphericity	Roundness
	Trial 1	0.77 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.49 ± 0.00
CP	Trial 2	0.77 ± 0.00	0.93 ± 0.00	0.83 ± 0.00	0.42 ± 0.00
	Trial 3	0.80 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.51 ± 0.00
	Average	**0.78 ± 0.02**	**0.93 ± 0.00**	**0.83 ± 0.01**	**0.47 ± 0.05**
	Trial 1	0.77 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.50 ± 0.00
LS	Trial 2	0.79 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.49 ± 0.01
	Trial 3	0.80 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.52 ± 0.00
	Average	**0.79 ± 0.02**	**0.93 ± 0.00**	**0.84 ± 0.00**	**0.50 ± 0.03**
	Trial 1	0.78 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.51 ± 0.00
MS	Trial 2	0.78 ± 0.01	0.93 ± 0.00	0.83 ± 0.00	0.47 ± 0.02
	Trial 3	0.81 ± 0.00	0.94 ± 0.00	0.85 ± 0.00	0.56 ± 0.00
	Average	**0.79 ± 0.02**	**0.93 ± 0.00**	**0.84 ± 0.01**	**0.51 ± 0.04**
	Trial 1	0.77 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.51 ± 0.00
HS	Trial 2	0.79 ± 0.01	0.93 ± 0.00	0.83 ± 0.01	0.47 ± 0.03
	Trial 3	0.80 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.52 ± 0.01
	Average	**0.79 ± 0.01**	**0.93 ± 0.00**	**0.84 ± 0.00**	**0.50 ± 0.03**

2.3.1.4 Colour

In terms of colour, the L* value represents the samples brightness ranging from black (0) to white (100). This value was relatively consistent between the 4 powder samples for each individual trial (Table 2.5). Trial 2 samples had the lowest L* values at 91.20 ± 0.02 (CP), 90.74 ± 0.03 (LS), 91.06 ± 0.14 (MS) and 91.39 ± 0.12 (HS), which may be due to the fact Trial 2 samples had the largest particle sizes of the powder as the L* value tends to decrease with increasing particle size (Haas *et al*., 2019). This can be attributed to increased light-scattering of samples with smaller particle size and thus causing an increased brightness of the powder and L* value (Haas *et al*., 2019). Statistical analysis indicated that there was no

significant difference ($p > 0.05$) between CPP and HS, CPP and MS or between HS and MS samples.

The a* values represent redness (>0) to greenness (<0). The average of the 3 trials CP powder has an a* value of -5.68 ± 0.43 compared to -6.08 ± 0.78, -5.91 ± 0.42 and -6.01 ± 0.61 for LS, MS and HS respectively. This indicates the sheared samples were slightly redder in colour compared to the control. Statistical analysis indicated that there was a significant difference ($p < 0.05$) when comparing all samples, indicating that the extent of shearing treatment influenced the a* value of the resultant powders. This was similarly seen for the b* value representing blueness (<0) to yellowness (>0); for the average of the 3 trials, CP powder had a b* value of 21.81 ± 0.30 compared to 23.11 ± 1.00, 22.22 ± 0.48 and 22.58 ± 0.99 for LS, MS and HS, respectively, which indicated the sheared powders were slightly more yellow in colour compared to the control. The b* values for all samples had the same significant difference ($p <0.05$) as the a* value. The increase in the a* and b* values can be recognized with the treated powders gaining a slightly more saturated colour when compared to the control (Haas *et al*., 2019).

Across all L*, a* and b* values, the difference between trials were statistically significant ($p <0.05$), indicating that Trial has an effect on powder colour. As previously mentioned, this is most likely due to seasonality.

The ΔE value was also calculated to determine the colour difference between control powder and sheared powders (LS, MS and HS) for each individual trial. It is reported that a colour difference of ΔE = >3 can be perceived by an average observer (Ghidouche *et al*., 2013). As

seen in Table 2.5, all ΔE values were < 3 which indicates there is little visible colour difference between the sheared powders and the control.

All powders in each trial were produced from the one batch of whey and were subjected to the same processing conditions, and so it may be concluded that the small difference in colour between powders is not due to composition, but may be because of the shearing treatments applied.

2.3.1.5 Powder density

The treated powder samples (LS, MS, HS) had higher densities than the control powder sample (CP), which was evident in both the bulk and tapped density values. The average CP value for bulk density (ρ_{bulk}) was 0.70 ± 0.08 g/ml compared to 0.74 ± 0.01 g/ml for the LS powder sample, 0.73 ± 0.02 g/ml for the MS powder sample and 0.72 ± 0.06 g/ml for the HS powder sample. For tapped density (ρ_{tapped}), the average CP value was 0.90 ± 0.08 g/ml compared to 0.94 ± 0.03 g/ml for the LS powder sample, 0.92 ± 0.03 for the MS powder sample and 0.92 ± 0.07 g/ml for the HS powder sample. Statistical analysis for both ρ_{bulk} and ρ_{tapped} indicated that there were a significant difference ($p < 0.05$) when comparing all samples against the CP sample, which indicates that the shearing treatments affect the densities. These differences in density may be due to the smaller particles occupying the spaces between the larger particles and thus giving larger density values. These findings agree with reports by Hazlett, Schmidmeier and O'Mahony (2021a) and Zhang *et al*. (2023) that an increase in density is seen with a decrease in powder particle size. This is because decreasing the size of the particles increases their exposed surface areas, which allows for an increase in inter-particle interactions to occur, influencing the density properties of the powder. This is an important consideration

for bulk-handling properties such as transport and storage (Hazlett, Schmidmeier and O'Mahony., 2021a).

Both ρ_{bulk} and ρ_{tapped} values for all Trials were shown to be significantly different ($p < 0.05$). It is interesting to note that for Trial 2 powders, the density values were much lower, in particular for the CP trial 2 powder sample and the HS trial 2 sample when comparing these values to the same powders from a different trial. The trial had the largest particle size when compared to the other trials. This is most likely what influenced the density values demonstrated (Hazlett, Schmidmeier and O'Mahony *et al*., 2021a; Zhang *et al*., 2023). Seasonality may have also influenced these statistically different ($p < 0.05$) results between trials.

2.3.1.6 Flowability

The flowability of the powders was determined using the flow index (i) which is the inverse of the flow function slope; the larger the i value, the more free-flowing the powder sample is (Fitzpatrick *et al*., 2004). These values are then classified using the Jenike classification to describe flowability. This states that, for i values 0 to 1, the sample is hardened, samples with i values between 1-2 are very cohesive, samples with i values between 2 and 4 are cohesive those with i values between 4 and 10, are easy flowing and, for i values > 10, a sample is free flowing (Fitzpatrick *et al*., 2004).

The average of the i values for all 3 trials classify the powder as easy flowing with values of 5.05 ± 1.43, 5.03 ± 0.54, 4.54 ± 0.65 and 5.36 ± 1.69 (Table 2.5) for CP, LS, MS and HS powder samples respectively. There was little change in flowability between control samples

and sheared samples across the 3 trials. Statistical analysis concluded there was not a significant difference ($p > 0.05$) between CP sample and all other samples. This indicates that shearing the whey concentrate did not affect the flowability of the resultant powders.

Trial 2 samples had significantly lower i values, indicative of more cohesive powder samples when compared to the other two trials. From statistical analysis, it can be concluded that data for Trial 2 are statistically different ($p < 0.05$) from Trials 1 and 3; however, Trial 1 and Trial 3 are not statistically different ($p > 0.05$). These differences may be explained as Trial 2 has the widest particle size distribution, which can be reflected in the largest values for span as seen in Table 2.2. The fine particles may fill gaps between the larger particles which in turn results in increased inter-particle cohesive forces and interlocking between particles (Shi *et al*., 2018) and may explain the decrease in flowability for Trial 2 when compared to Trials 1 and 3.

Table 2.5: Commission Internationale d'Eclairage (CIE) colour space values, *ΔE,* flow index (i), Jenike classification, bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) of Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powder.

Treatment	Trial	Colour space values			ΔE	Density		Flow index	Jenike
		L*	a*	b*		ρ_{bulk}	ρ_{tapped}	(i)	classification (-)
	Trial 1	93.30 ± 0.04	-6.06 ± 0.02	22.00 ± 0.05	-	0.76 ± 0.01	0.97 ± 0.01	6.10 ± 0.70	Easy flowing
CP	Trial 2	91.20 ± 0.02	-5.21 ± 0.03	22.00 ± 0.04	-	0.61 ± 0.01	0.81 ± 0.01	3.43 ± 0.34	Cohesive
	Trial 3	93.10 ± 0.05	-5.78 ± 0.04	21.50 ± 0.04	-	0.74 ± 0.01	0.93 ± 0.01	5.63 ± 0.40	Easy flowing
	Average	**92.50 ± 1.15**	**-5.68 ± 0.43**	**21.80 ± 0.30**	**-**	**0.70 ± 0.08**	**0.90 ±0.08**	**5.05 ± 1.43**	**Easy flowing**
	Trial 1	93.40 ± 0.03	-6.13 ± 0.02	22.10 ± 0.06	0.16	0.75 ± 0.01	0.97 ± 0.01	5.47 ± 0.12	Easy flowing
LS	Trial 2	90.70 ± 0.03	-5.28 ± 0.01	23.30 ± 0.06	1.39	0.73 ± 0.01	0.91 ± 0.00	4.43 ± 0.57	Easy flowing
	Trial 3	92.90 ± 0.06	-6.83 ± 0.03	24.00 ± 0.05	2.72	0.74 ± 0.00	0.94 ± 0.03	5.20 ± 0.26	Easy flowing
	Average	**92.30 ± 1.44**	**-6.08 ± 0.78**	**23.10 ± 1.00**	**1.37**	**0.74 ± 0.01**	**0.94 ± 0.03**	**5.03 ± 0.54**	**Easy flowing**
	Trial 1	93.40 ± 0.08	-6.12 ± 0.03	21.70 ± 0.04	0.32	0.71 ±0.01	0.90 ±0.01	3.94 ± 0.04	Cohesive
MS	Trial 2	91.10 ± 0.14	-5.43 ± 0.01	22.70 ± 0.04	0.74	0.75 ± 0.01	0.92 ± 0.00	4.46 ± 0.67	Easy flowing
	Trial 3	93.70 ± 0.07	-6.19 ± 0.01	22.20 ± 0.04	0.93	0.74 ± 0.00	0.92 ± 0.01	5.23 ± 0.52	Easy flowing
	Average	**92.60 ± 1.33**	**-5.91 ± 0.42**	**22.20 ± 0.48**	**0.47**	**0.73 ± 0.02**	**0.92 ± 0.01**	**4.54 ± 0.65**	**Easy flowing**
	Trial 1	93.40 ± 0.08	-5.98 ± 0.01	21.60 ± 0.01	0.42	0.78 ± 0.00	0.97 ± 0.01	6.52 ± 0.61	Easy flowing
HS	Trial 2	91.40 ± 0.12	-5.42 ± 0.02	22.70 ± 0.01	0.76	0.66 ± 0.02	0.85 ± 0.01	3.42 ± 0.42	Cohesive
	Trial 3	92.80 ± 0.05	-6.64 ± 0.06	23.50 ± 0.08	2.20	0.73 ± 0.01	0.96 ± 0.01	6.13 ± 0.47	Easy flowing
	Average	**92.50 ± 1.03**	**-6.01 ± 0.61**	**22.60 ± 0.99**	**0.87**	**0.72 ± 0.06**	**0.92 ± 0.07**	**5.36 ± 1.69**	**Easy flowing**

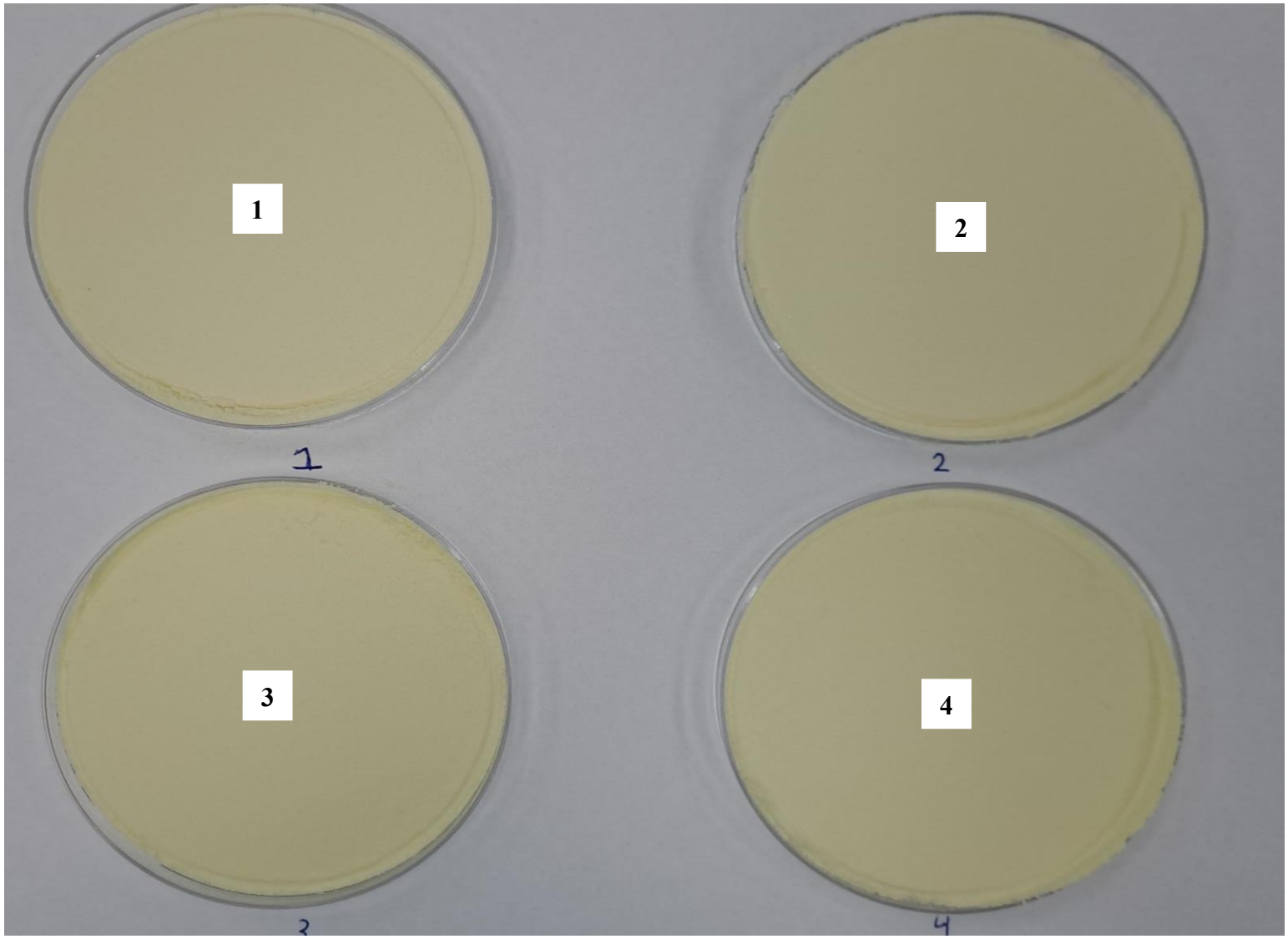


Figure 2.2 Trial 1 Whey powder samples (1) Control pilot plant (CP) powder (2) Low shear (LS) powder, (3) Medium shear (MS) powder and (4) High shear (HS) whey powder

2.3.2 Powder functional properties

2.3.2.1 Rehydration process

Broadband acoustic resonance dissolution spectroscopy (BARDS) monitors gas release from powders in solution. It does this by recording the change in acoustic resonance, which is identified and monitored as a change in frequency, as gas-exchange occurs between powder and solution during the dispersion of powder in the solution (Wu *et al*., 2019), enabling BARDS to be used as a suitable technique for monitoring rehydration properties of powder.

Plot A in Figure 2.3, Figure 2.4 and Figure 2.5 compares the rehydration behaviour of a sample of CP powder versus a sample of LS, MS and HS powders from each of the three trials. The change in frequency, exhibited by the fundamental curve on the plot, is due to the change in the volume of gas present in the water (Wu *et al*., 2019; Vos *et al*., 2016), with the fundamental curve of each powder can be used to compare powder rehydration abilities (Vos *et al*., 2016).

There were small differences between rehydration profiles of the individual trials. In Figure 2.3 A, the four powders displayed very similar rehydration profiles, all reaching a minimum frequency (f_{min}) of approximately 3 kHz. whereas Figure 2.4 A, which displays the rehydration profile for Trial 2, shows more variation between powders. The HS powder had the shortest time at which the minimum frequency of the curve occurred (t_m). A faster initial gas release/ faster t_m indicates better wetting and dispersion ability of the powder (Wu *et al*., 2019). The MS powder produced a slightly altered profile, and so it took a similar amount of time to reach its f_{min}; however, it took a longer time to return to steady state compared to the HS powder sample. In Trial 2, the f_{min} for all powders varied significantly. The CP and LS powder showed very poor wettability, with little change in frequency captured due to little/no gas release from

the powders (Wu *et al*., 2019). This may be linked to the poor flowability of these powders (Table 2.5). Cohesive powders tend to form clumps on initial surface contact of the liquid, which can result in layer forming on the surface of the liquid which can prevent/slow down the solution from penetrating the rest of the powder particles (Hazlett *et al*., 2021b). Figure 2.5 Plot A shows the HS powder to have the shortest t_m at approximately 50 s, compared to the other powders. The MS powder had the longest t_m, at about 100 s and took the longest amount of time to return to steady state indicating that the MS powder had the poorest rehydration ability in this trial.

These findings can be reflected in Figures 2.3 B, 2.4 B and 2.5 B which display the gas volume profile. On these plots, the up-slope of the curve is due to the generation of gas being greater than the gas elimination rate, which is reversed when the down-slope begins. The gas volume maximum indicates where the two rates are equal (Wu *et al*., 2019). This generation of gas occurs as the powder particles transfer gas to the water, which is then being eliminated as the gas escapes from the surface of the water (Wu *et al*., 2019). When little gas is produced as the powder enters the water, this can be interpreted as the powder having poor dispersibility and, the slower the gas release, the slower the rehydration process (Wu *et al*., 2019).

From the rehydration profiles of the different powders, it is evident that the shearing treatments applied to the LS, MS and HS powders had little to no effect on the rehydration properties of the powders when compared to the control powder sample, as there was not a clear trend shown.

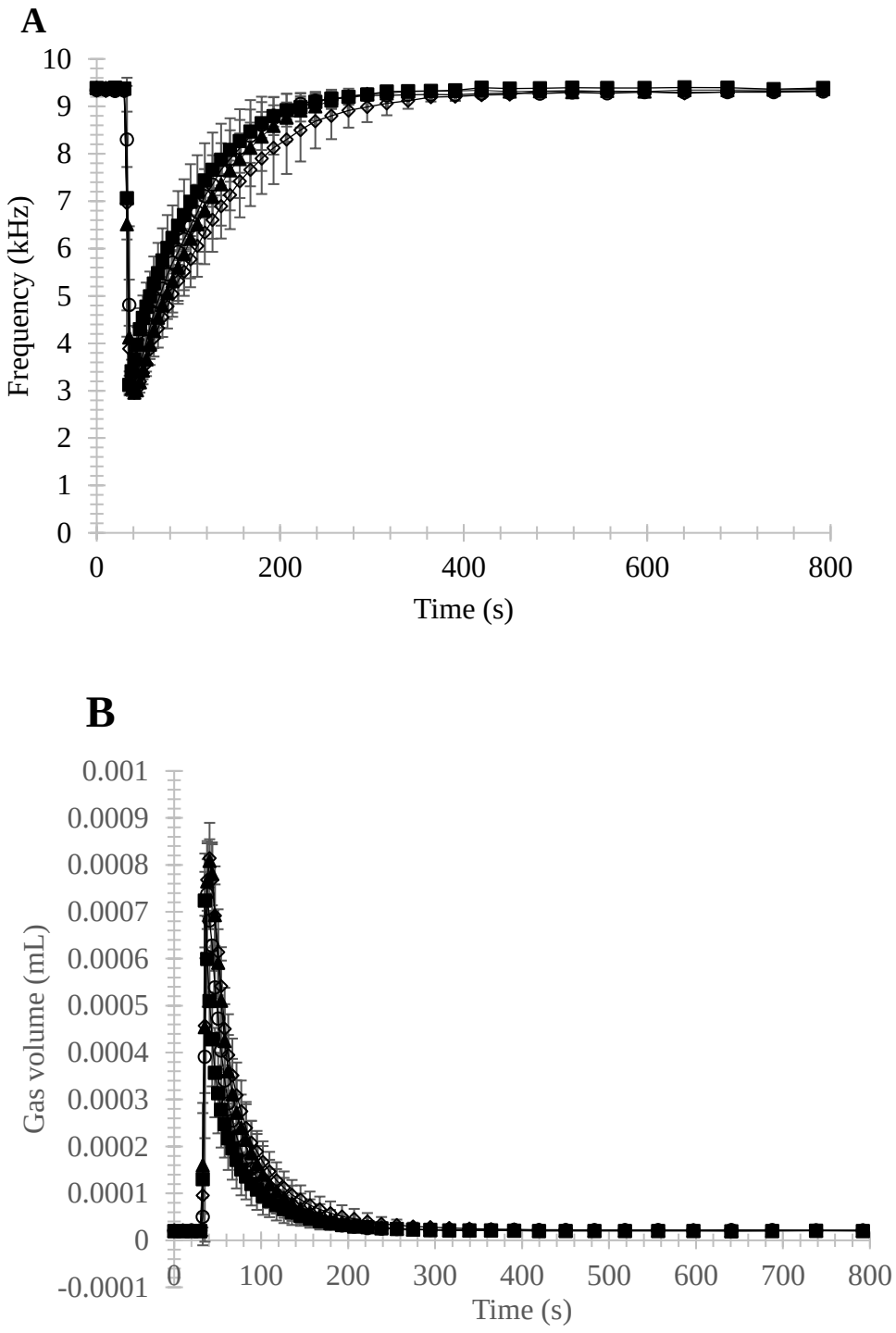


Figure 2.3 (A) BARDS frequency spectrum and (B) gas volume profile of Trial 1 Control pilot plant (CP) —▲—, Low shear (LS) —◇—, Medium shear (MS) —■— and High shear (HS) —o— whey powder, all at 0.2% concentration.

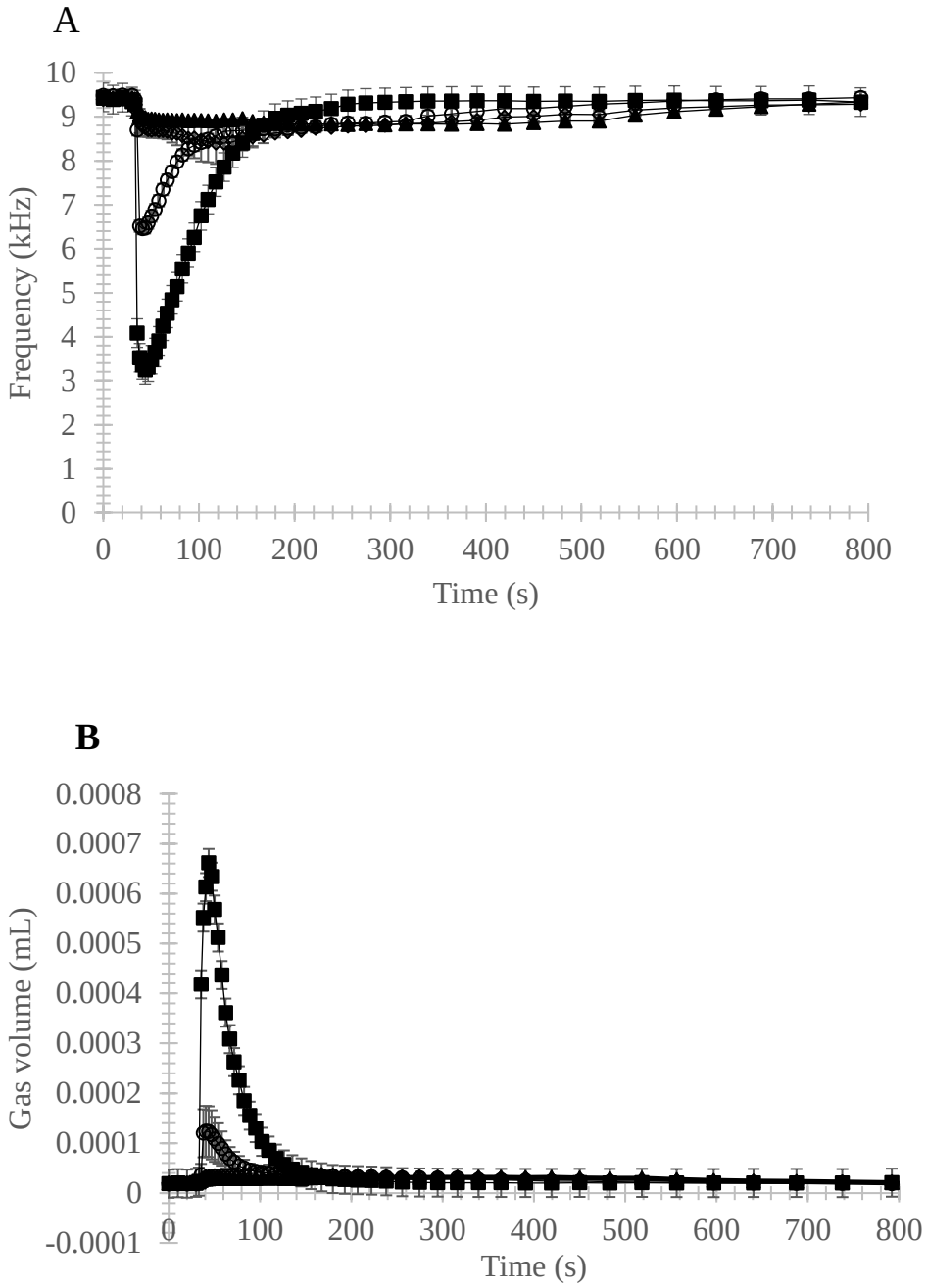


Figure 2.4. (A) BARDS frequency spectrum and (B) gas volume profile of Trial 2 Control pilot plant (CP) —▲—, Low shear (LS) —◇—, Medium shear (MS) —■— and High shear (HS) —o— whey powder, all at 0.2% concentration.

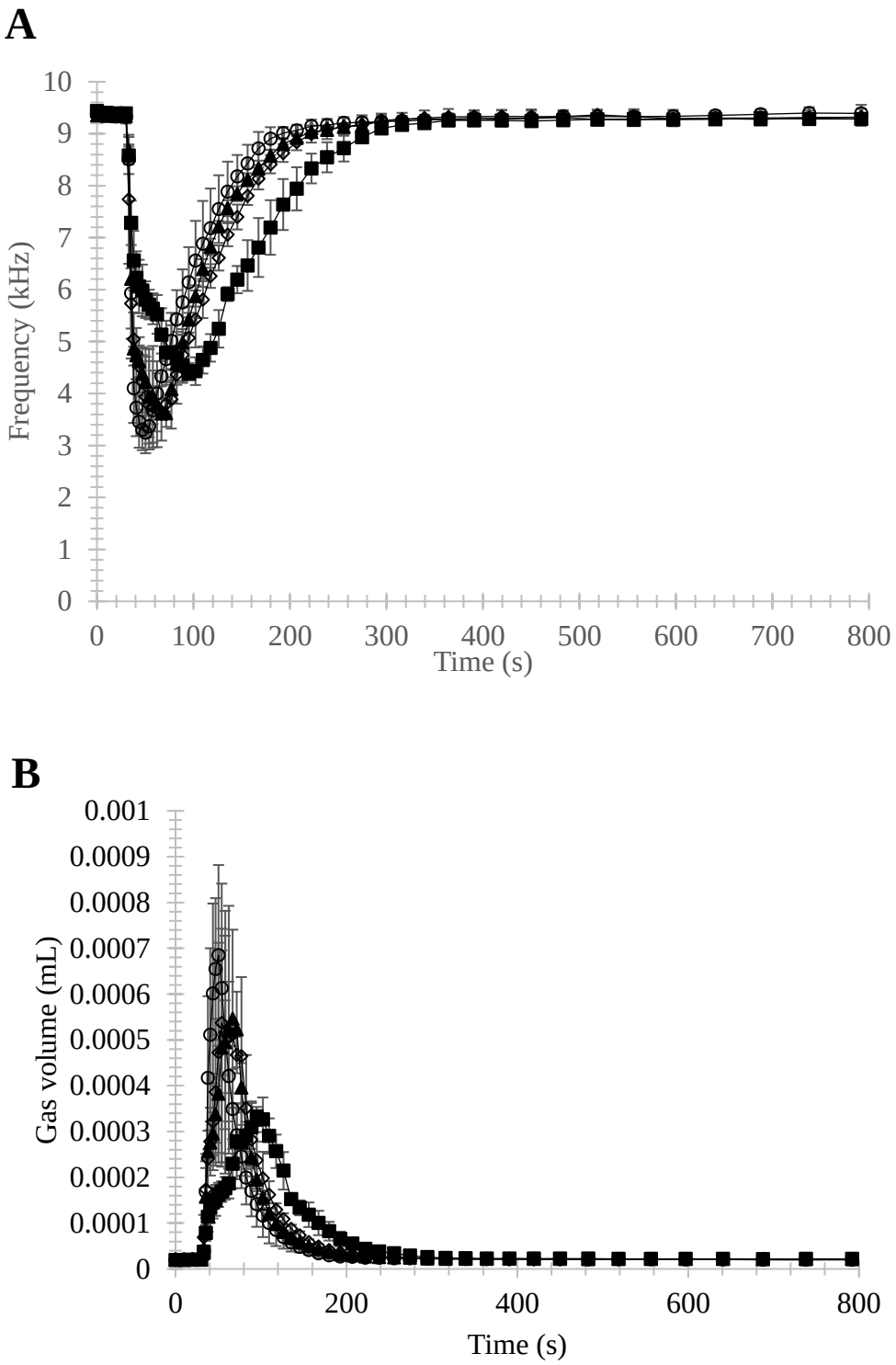


Figure 2.5. (A) BARDS frequency spectrum and (B) gas volume profile of Trial 3 Control pilot plant (CP) —▲—, Low shear (LS) —◇—, Medium shear (MS) —■— and High shear (HS) —o— whey powder, all at 0.2% concentration.

2.3.3 Colloidal stability of reconstituted whey powder

2.3.3.1 Viscosity and stability of reconstituted whey powder

The whey powder samples from Trial 1 were reconstituted to understand the impact, if any, of the shearing treatments applied on the viscosity of the reconstituted whey powders in an attempt to extrapolate back to the effect that shearing treatments may have had on the viscosity and colloidal stability of whey concentrate prior to spray drying. The results (Table 2.6) showed a decrease in viscosity with increasing shearing treatment. Statistical analysis demonstrated a significant difference ($p < 0.05$) between the CP sample and HS sample; while there was no significant difference ($p > 0.05$) between the other samples, there was a notable decrease in viscosity with increasing intensity of shear.

Table 2.6: Apparent viscosity of rehydrated Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powder from Trial 1 reconstituted to 25%, w/w, total solids and measured at a constant shear rate of 100 1/s

Sample	Apparent Viscosity (mPa.s)
CP	3.25 ± 0.02^{a}
LS	$3.01 \pm 0.18^{a,b}$
MS	$2.89 \pm 0.15^{a,b}$
HS	2.55 ± 0.26^{b}

Values not showing a common superscript are significantly different ($p<0.05$)

Figure 2.6 shows the stability profiles of the reconstituted CP, LS, MS and HS powders from Trial 1. The delta backscattering (ΔBS) value was obtained periodically throughout analysis to calculate the Turbiscan stability index (TSI) of the samples (Wu, Guo and Lin., 2020), which is then used to understand the rate of sedimentation of the sample; the lower the TSI value, the

more stable the sample is. From this analysis, it is clear that the reconstituted CP sample was the most stable when compared to the other reconstituted samples throughout the entire 120 min.

Stokes' law describes how the viscosity of a fluid, the diameter of a particle and the density of the particle (compared to the density of the continuous phase) all impact on the drag force (sedimentation rate) of the particle (Holkar *et al*., 2019). Shearing reduced the D[4,3] values from 157.00 ± 1.53 μm for the CP sample down to 148.00 ± 1.15, 143.00 ± 2.00 and 137.00 ± 0.58 μm for LS, MS and HS samples respectively. In parallel, the viscosity of the sheared samples was lower compared to the CP sample as the viscosity of the CP sample was 3.25 ± 0.02 mPa.s compared to 3.01 ± 0.18, 2.89 ± 0.15 and 2.55 ± 0.26 mPa.s for LS, MS and HS samples. Interestingly, increasing the shear led to higher TSI values, indicative of lower colloidal stability of the reconstituted powders. These results suggest that the lower colloidal stability of the sheared samples (even though they had smaller particles than the control) was due to the reduced viscosity post-shearing.

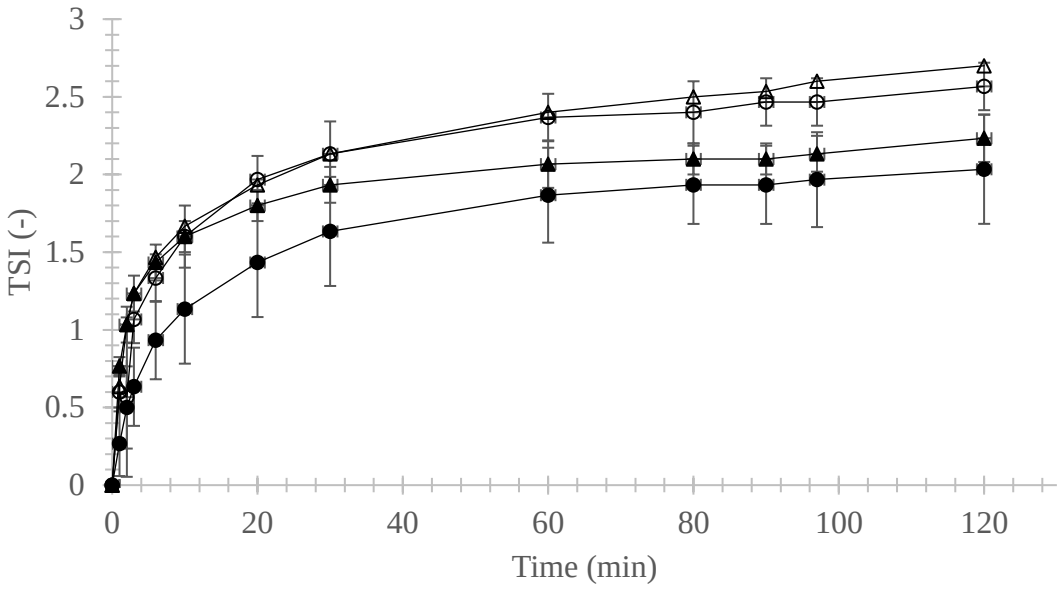


Figure 2.6. Turbiscan Stability Index (TSI) values vs. time (min) of reconstituted Control pilot plant (CP) —●—, Low shear (LS) —○—, Medium shear (MS) —▲— and High shear (HS) —△— whey powder from Trial 1 reconstituted to a 25%, w/w, total solids

2.3.4 Protein profile, denaturation and aggregation

2.3.4.1 Protein profile

The protein profile of the whey powders from the three trials are displayed in the SDS-PAGE gel images in Figures 2.7 and 2.8. Whey proteins are made up of a combination of β-lactoglobulin (β-lg), α-lactalbumin (α-lac), bovine serum albumin (BSA), immunoglobulins (Igs) and some other minor proteins such as lactoferrin (LF) (Dissanayake and Vasiljevic, 2009). The main whey protein bands on the SDS-PAGE gels from the three trials were identified by comparison with a study conducted recently by Gai *et al*. (2025) and their relative molecular weights.

2.3.4.2 Whey protein denaturation and aggregation

The CP powders from Trial 1, 2 and 3 are displayed on the SDS-PAGE gel image in Figure 2.7 showing little difference between the non-reducing bands (lanes 2-4) for the 3 trials. This indicates that the protein profile of the samples across the three trials are not noticeably different. Adding β-mercaptoethanol to the whey powder solutions resulted in reduction of disulphide bonds between whey proteins thus returning whey protein aggregates that formed during denaturation to their original monomeric forms (Gai *et al*., 2025). This resulted in larger band volumes for the whey powders under reducing conditions (lanes 5-7), which indicates that the whey proteins were partially denatured, which was particularly evident for the β-lg, BSA and LF protein bands. There were no major noticeable differences between trials.

However, in Table 2.7, a greater level of whey protein denaturation was shown for Trial 3 powders (~ 40 %) when compared to denaturation values for powders from Trial 1 and Trial 2 (~ 20 %). Statistical analysis demonstrated a significant difference ($p < 0.05$) for denatured protein values between trials. This indicates that the Trial affects the denatured protein value, which is most likely due to seasonality.

Figure 2.8 displays the typical SDS-PAGE gel images of the CP, LS, MS and HS rennet whey powders from the three trials. From Fig 2.8 A, there is little difference between the non-reducing bands (lanes 2-5) of the CP, LS, MS and HS Trial 1 powders. The reducing bands (lanes 6-9) also do not display any noticeable changes between shearing treatments; this is also similarly seen in image B for Trial 2. The difference between reducing and non-reducing bands can be seen most significantly in, Figure 2.8 C for Trial 3, which is also reflected in Table 2.8,

due to higher denaturation levels demonstrated for Trial 3 when compared to Trial 1 and Trial 2.

The effect of shearing treatments on whey protein denaturation can also be evaluated from the images in Fig 2.8. In all images, it appears there is little difference between CP, LS, MS and HS reducing whey powder samples (lanes 6-9). However, statistical analysis of the denatured protein values (Table 2.7) indicates that there is a statistical difference ($p < 0.05$) when comparing the CP sample with the LS, MS and HS samples. This indicates that the shearing treatment influences whey protein denaturation levels in the resultant powder. However, when comparing statistically the LS, MS and HS samples against each other the differences are not significant ($p > 0.05$). This indicates that the extent of shearing does not have an influence on the level of protein denaturation in the samples.

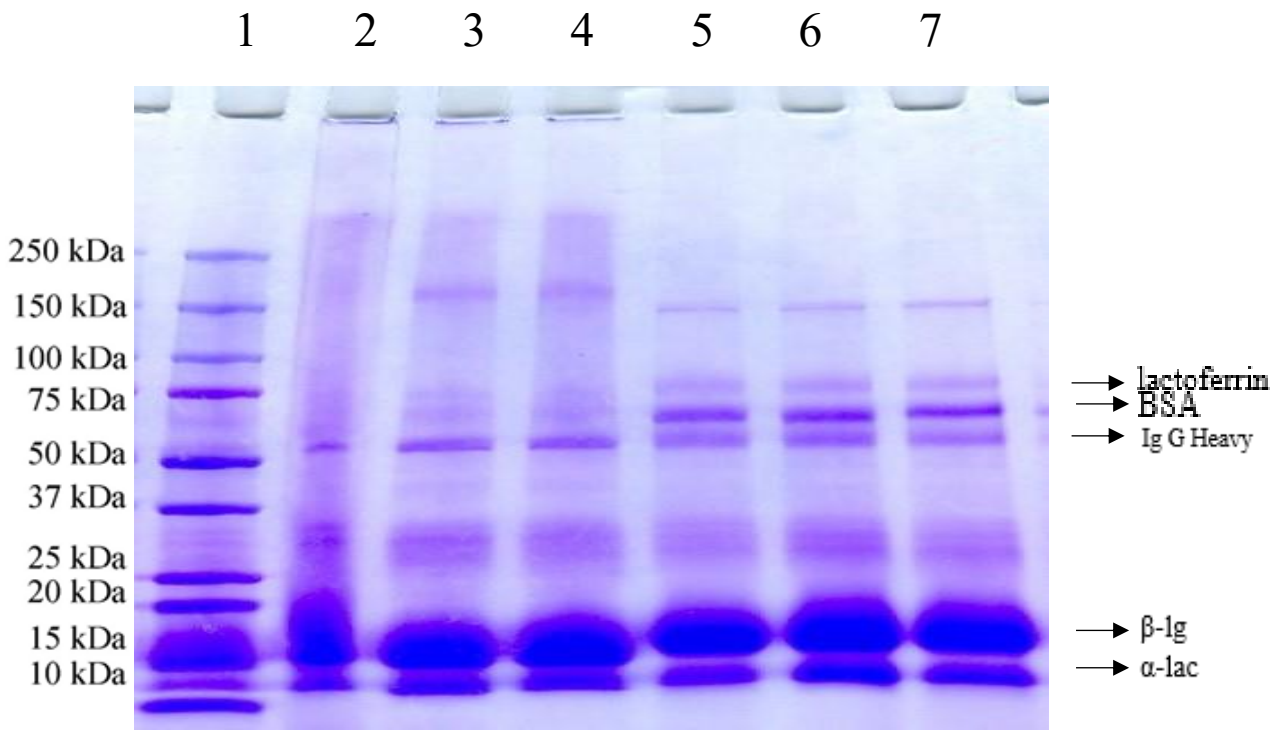


Fig 2.7: SDS-PAGE electrophoretograms of whey powder. Lane 1, protein standard; lane 2-4 Control pilot plant (CP) whey powder trial 1, trial 2 and trial 3 under non-reducing conditions; lane 5-7 Control pilot plant (CP) whey powder trial 1, trial 2 and trial 3 under reducing conditions.

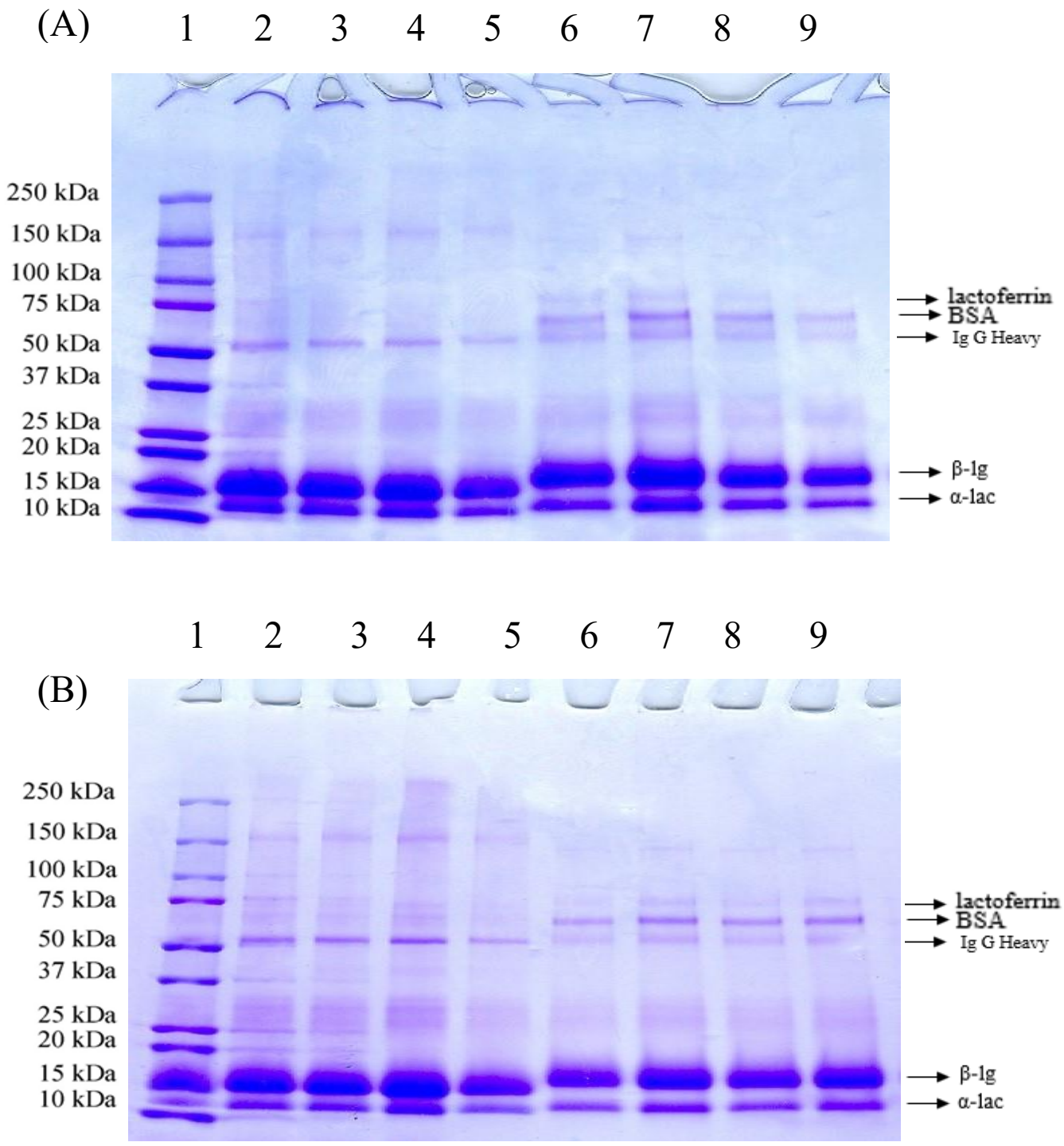


Fig 2.8: SDS-PAGE electrophoretograms of whey powder from Trial 1 (A), Trial 2 (B) and Trial 3 (C). Lane 1, protein standard; lanes 2-5 Control pilot plant (CP); Low shear (LS), Medium shear (MS) and High shear (HS) whey powders under non-reducing conditions, lanes 6-9; Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powders under reducing conditions.

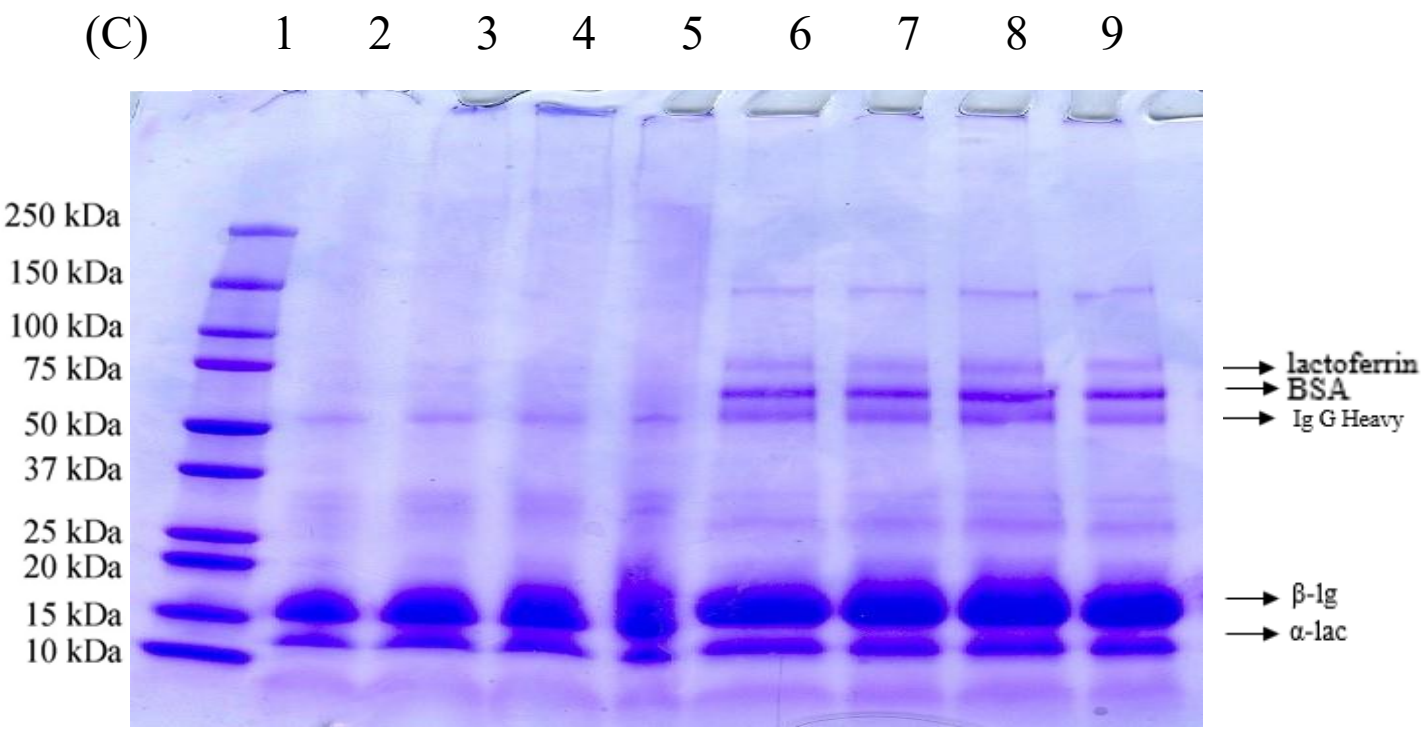


Fig 2.8 continued: SDS-PAGE electrophoretogram.

2.3.4.4 Non-protein-nitrogen

The average NPN values, expressed as a % of total protein, for CP, LS, MS and HS powders were 16.30 ± 1.82 %, 15.90 ± 1.61 %, 16.10 ± 1.92 % and 16.40 ± 2.42 % respectively. Statistical analysis of the paired multiple comparison test showed no significant difference between all samples ($p > 0.05$). This indicates that the shearing treatments did not influence the NPN values. However, a statistically significant difference ($p < 0.05$) was demonstrated between trials, which may be due to a seasonal effect of the NPN values.

Non-protein nitrogen (NPN) is a fraction constituted of nitrogen containing compounds such as urea, creatine, α-amino nitrogen, uric acid, ammonia and creatinine that are not part of the true protein (O'Mahony and Fox, 2013). They are mostly produced from protein breakdown in

the cow and are present in milk from the animal's blood (Ruska and Jonkus, 2014). NPN generally makes up 25 % of the total nitrogen in whey. However, today, most whey powders go through some membrane filtration prior to drying to increase the total solids of the liquid whey prior to drying. In the production of the whey powder in this study, prior to evaporation, the liquid stream went through a nanofiltration process to increase the total solids. During this process there is a loss of some on the NPN proteins through the permeate. From a study conducted by Kelly and Kelly (1995) nanofiltration led to a NPN protein loss of approximately 25 %. This agrees with the NPN values demonstrated in this study.

Table 2.7: The concentration (%) of total protein, denatured protein and non-protein nitrogen values of Control pilot plant (CP), Low shear (LS), Medium shear (MS) and High shear (HS) whey powder. Denatured protein and Non-protein Nitrogen values are expressed as a % of total protein.

Treatment	Trial	Total protein (%)	Denatured protein (% of total protein)	Non-protein Nitrogen (% of total protein)
CP	Trial 1	12.60 ± 0.02	11.50 ± 0.63	15.20 ± 0.60
	Trial 2	14.40 ± 0.15	13.70 ± 0.86	18.40 ± 1.16
	Trial 3	16.00 ± 0.20	40.80 ± 0.74	15.30 ± 0.19
	Average	**14.30 ± 1.74**	**22.00 ± 16.30**	**16.30 ± 1.82**
LS	Trial 1	11.20 ± 0.09	11.70 ± 0.61	16.30 ± 0.46
	Trial 2	14.40 ± 0.08	11.80 ± 0.84	17.20 ± 0.64
	Trial 3	16.50 ± 0.12	39.80 ± 0.72	14.10 ± 0.03
	Average	**14.10 ± 2.64**	**21.10 ± 16.20**	**15.90 ± 1.61**
MS	Trial 1	11.30 ± 0.09	11.40 ± 0.92	16.10 ± 0.32
	Trial 2	14.50 ± 0.05	13.00 ± 0.86	18.00 ± 0.33
	Trial 3	16.50 ± 0.12	41.20 ± 1.70	14.20 ± 0.02
	Average	**14.10 ± 2.63**	**21.90 ± 16.80**	**16.10 ± 1.92**
HS	Trial 1	11.20 ± 0.21	12.30 ± 0.25	16.00 ± 0.31
	Trial 2	14.10 ± 0.03	12.20 ± 0.26	19.00 ± 0.35
	Trial 3	16.80 ± 0.05	40.50 ± 0.80	14.20 ± 0.02
	Average	**14.00 ± 2.77**	**21.70 ± 16.30**	**16.40 ± 2.42**

2.4 Conclusions

The objective of this study was to investigate the impact of shearing crystallised whey concentrate pre-drying on the physical and functional properties of the resultant dried whey powder. It was hypothesized, that in-line shearing of the crystallised whey concentrate would reduce the particle size of the resultant powder due to a reduction in the size of the lactose crystals. The results demonstrated that shearing the liquid whey concentrate pre-drying alters the particle size distribution of the resultant whey powder. This, in turn, affected some of the physicochemical properties of the whey powder such as morphology, colour, density and levels of protein denaturation. However, the pre-drying shearing treatments did not show any evidence of influencing the rehydration properties of the powders. These findings will enhance the scientific understanding among dairy researchers and processors of how shearing whey concentrate before drying influences the physical and functional characteristics of the final powder. These insights can be applied to tailor whey powders with specific particle size distributions for targeted uses, such as in a snack seasoning system. From an industry perspective, this method offers a practical solution for modifying particle size, as it involves an in-line process prior to drying. This technology could be simply integrated into existing production lines with minimal disruption and risk of microbial contamination, since the shearing process occurs before any thermal treatment.

2.5 References

Adamson, N. (2015). *Whey Processing.* In*:* Bylund, G. (2015). *Dairy processing Handbook.* 5th ed. Available at: <https://dairyprocessinghandbook.tetrapak.com/chapter/whey-processing> [Accessed on 5th January 2025]

Alonso-Miravalles, L., Zannini, E., Bez, J., Arendt, E.K. and O'Mahony, J.A. (2020). Physical and flow properties of pseudocereal-based protein-rich ingredient powders. *Journal of Food Engineering*, **281**, p.109973.

Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Cappelli, F.P. and Calamari, L. (2015). Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science*, **98 (3)**, pp.1815-1827.

Bronsveld, E. (2015). Milk and Whey Powder In: Bylund, G. (2015). *Dairy processing Handbook.* 5th ed. available at: https://dairyprocessinghandbook.tetrapak.com/chapter/milk-and-whey-powder [Accessed on the 5th January 2025]

Chandan, R.C. (2016). Dairy Processing and Quality Assurance: An overview. In: Chandan, R.C., Kilara, A. and Shah, N.P. (2016). *Dairy Processing and Quality Assurance*. 2nd Ed. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.33–92.

Chudy, S., Bilska, A., Kowalski, R. and Teichert, J. (2020). Colour of milk and milk products in CIE L* a* b* space. **76 (2)**, pp.77-81.

Crowley, S. V., Gazi, I., Kelly, A.L., Huppertz, T., and O'Mahony, J.A. (2014). Influence of protein concentration on the physical characteristics and flow properties of milk protein concentrate powders. *Journal of Food Engineering,* **135**, 31–38.

Cunningham, P., Canty, N., O'Mahony, T., O'Connor, B. and O'Callaghan, D. (2006). System identification of a falling film evaporator in the dairy industry. In *Proceedings of the UK Automatic Control Conference*. Sheffield, United Kingdom 7-11 August 2006. United Kingdom: UKACC group.

Dissanayake, M. and Vasiljevic, T. (2009). Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *Journal of Dairy Science*, **92 (4)**, pp.1387-1397.

Fitzpatrick, J.J., Iqbal, T., Delaney, C., Twomey, T. and Keogh, M.K. (2004). Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. *Journal of Food Engineering*, **64 (4)**, pp.435-444.

Gai, N., Uniacke-Lowe, T., O'Regan, J., Goulding, D.A. and Kelly, A.L. (2025). Influence of β-lactoglobulin and κ-casein genotype on whey protein denaturation in heated bovine milk and rennet whey. *International Dairy Journal*, **160**, p.106107.

Gaiani, C., Boyanova, P., Hussain, R., Pazos, I.M., Karam, M.C., Burgain, J. and Scher, J. (2011). Morphological descriptors and colour as a tool to better understand rehydration properties of dairy powders. *International Dairy Journal*, **21 (7)**, pp.462-469.

Gandhi, G., Amamcharla, J.K. and Boyle, D. (2017). Effect of milk protein concentrate (MPC80) quality on susceptibility to fouling during thermal processing. *LWT-Food Science and Technology*, **81**, pp.170-179.

Ghidouche, S., Rey, B., Michel, M. and Galaffu, N. (2013). A Rapid tool for the stability assessment of natural food colours. *Food Chemistry*, **139 (1-4)**, pp.978-985.

Haas, K., Obernberger, J., Zehetner, E., Kiesslich, A., Volkert, M. and Jaeger, H. (2019). Impact of powder particle structure on the oxidation stability and color of encapsulated

crystalline and emulsified carotenoids in carrot concentrate powders. *Journal of Food Engineering*, **263**, pp.398-408.

Hazlett, R., Schmidmeier, C. and O'Mahony, J.A., (2021a). Influence of mechanical integrity during pneumatic conveying on the bulk handling and rehydration properties of agglomerated dairy powders. *Journal of Food Engineering*, **288**, p.110103.

Hazlett, R., Schmidmeier, C. and O'Mahony, J.A. (2021b). Approaches for improving the flowability of high-protein dairy powders post spray drying– A review. *Powder Technology*, **388**, pp.26-40.

Huppertz, T. and Gazi, I. (2016). Lactose in dairy ingredients: Effect on processing and storage stability. *Journal of Dairy Science*, **99 (8)**, pp.6842-6851.

Jelen, P. (2011). Whey Processing: Utilization and Products. In: J. W. Fuquay, P. F. Fox, & P. L. J. McSweeney. (2011). *Encyclopedia of Dairy Sciences*. 2nd Ed. Oxford: Academic Press, pp.731-737.

Kelly, J. and Kelly, P. (1995). Nanofiltration of whey: quality, environmental and economic aspects. *International Journal of Dairy Technology*, **48 (1)**, pp.20-24.

Kilara. A. (2016). Whey and Whey Products. In: Chandan, R.C., Kilara, A. and Shah, N.P (2016). *Dairy Processing and Quality Assurance*. 2nd Ed. Chichester, UK; Hoboken, NJ: John Wiley & Sons, pp.609 – 638.

Kumar, P., Sharma, N., Ranjan, R., Kumar, S., Bhat, Z.F. and Jeong, D.K. (2013). Perspective of membrane technology in dairy industry: A review. *Asian-Australasian Journal of Animal Sciences*, **26 (9)**, p.1347.

Laemmli, U.K., (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227 (5259)**, pp.680-685.

Lee, Y.K. (2005). Comparison of CIELAB ΔE* and CIEDE2000 color-differences after polymerization and thermocycling of resin composites. *Dental Materials*, **21 (7)**, pp.678-682.

Macwan, S.R., Dabhi, B.K., Parmar, S.C. and Aparnathi, K.D. (2016). Whey and its utilization. *International Journal of Current Microbiology and Applied Sciences*, **5 (8)**, pp.134-155.

Marella, C., Salunke, P., Biswas, A.C., Kommineni, A. and Metzger, L.E., (2015). Manufacture of modified milk protein concentrate utilizing injection of carbon dioxide. *Journal of Dairy Science*, **98 (6)**, pp.3577-3589.

Mohammad, A.W., Teow, Y.H., Ang, W.L., Chung, Y.T., Oatley-Radcliffe, D.L. and Hilal, N. (2015). Nanofiltration membranes review: Recent advances and future prospects. *Desalination*, **356**, pp.226-254.

Nikolova, Y., Petit, J., Sanders, C., Gianfrancesco, A., Desbenoit, N., Frache, G., Francius, G., Scher, J. and Gaiani, C. (2014). Is it possible to modulate the structure of skim milk particle through drying process and parameters?. *Journal of Food Engineering*, **142**, pp.179-189.

O'Kennedy, B.T. and Mounsey, J.S. (2006). Control of heat-induced aggregation of whey proteins using casein. *Journal of Agricultural and Food Chemistry*, **54 (15)**, pp.5637-5642.

O'Mahony, J. A. and Fox, P. F. (2013). Milk Proteins: Introduction and Historical Aspects. In: McSweeney, P.L.H and Fox P.F (2013) *Advanced Dairy Chemistry: Volume 1A: Protein: Basic Aspects.* 4th Ed. New York: Springer, pp.43-85.

O'Donoghue, L.T., Haque, M.K., Kennedy, D., Laffir, F.R., Hogan, S.A., O'Mahony, J.A. and Murphy, E.G. (2019). Influence of particle size on the physicochemical properties and stickiness of dairy powders. *International Dairy Journal*, **98**, pp.54-63.

O'Sullivan, J.J., Drapala, K.P., Kelly, A.L. and O'Mahony, J.A. (2018). The use of inline high-shear rotor-stator mixing for preparation of high-solids milk protein-stabilised oil-in-water emulsions with different protein: fat ratios. *Journal of Food Engineering*, **222**, pp.218-225.

Ozel, B., McClements, D.J., Arikan, C., Kaner, O. and Oztop, M.H. (2022). Challenges in dried whey powder production: Quality problems. *Food Research International*, **160**, p.111682.

Pan, K., Song, Q., Wang, L. and Cao, B. (2011). A study of demineralization of whey by nanofiltration membrane. *Desalination*, **267 (2-3)**, pp.217-221.

Patil, M.H., Tanguy, G., Floch-Fouéré, C.L., Jeantet, R. and Murphy, E.G. (2021). Energy usage in the manufacture of dairy powders: advances in conventional processing and disruptive technologies. *Drying Technology*, **39 (11)**, pp.1595-1613.

Reig, M., Vecino, X. and Cortina, J.L. (2021). Use of membrane technologies in dairy industry: An overview. *Foods*, **10 (11)**, p.2768.

Ruska, D. and Jonkus, D. (2014). Crude protein and non-protein nitrogen content in dairy cow milk. *Rural Sustainability Research*, **32 (1)**, pp.36-40.

Saito, Z. (1988). Lactose crystallization in commercial whey powders and in spray-dried lactose. *Food Structure*, **7 (1)**, p.9.

Shaozong, W., Fitzpatrick, J., Cronin, K., Ahmed, M.R., Fitzpatrick, D. and Miao Song, M.S., (2019). Application of broadband acoustic resonance dissolution spectroscopy

(BARDS) to the gas release behaviour during rehydration of milk protein isolate agglomerates. *Journal of Food Engineering*, **253**, pp.14-20.

Shi, H., Mohanty, R., Chakravarty, S., Cabiscol, R., Morgeneyer, M., Zetzener, H., Ooi, J.Y., Kwade, A., Luding, S. and Magnanimo, V. (2018). Effect of particle size and cohesion on powder yielding and flow. *KONA Powder and Particle Journal*, **35,** pp.226-250.

Sympatec. (2024). *Particle Shape*. Available at: <https://www.sympatec.com/en/particle-measurement/glossary/particle%20shape/> [Accessed on 4th December 2024]

Vos, B., Crowley, S.V., O'sullivan, J., Evans-Hurson, R., McSweeney, S., Krüse, J., Ahmed, M.R., Fitzpatrick, D. and O'Mahony, J.A. (2016). New insights into the mechanism of rehydration of milk protein concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS). *Food Hydrocolloids*, **61**, pp.933-945.

Wang, C., Reis, M.G., Waterhouse, G.I.N., Hemar, Y. and Reis, M.M. (2021). Prediction of dairy powder functionality attributes using diffuse reflectance in the visible and near infrared (Vis-NIR) region. *International Dairy Journal*, **117**, p.104981

Wu, C.S., Guo, J.H. and Lin, M.J. (2020). Stability evaluation of pH-adjusted goat milk for developing ricotta cheese with a mixture of cow cheese whey and goat milk. *Foods*, **9 (3)**, p.366.

Wu, S., Fitzpatrick, J., Cronin, K., Ahmed, M.R., Fitzpatrick, D. and Miao, S. (2019). Application of broadband acoustic resonance dissolution spectroscopy (BARDS) to the gas release behaviour during rehydration of milk protein isolate agglomerates. *Journal of Food Engineering*, **253**, pp.14-20.

Zhang, Y., Munir, M.T., Udugama, I., Yu, W. and Young, B.R. (2018). Modelling of a milk powder falling film evaporator for predicting process trends and comparison of energy consumption. *Journal of Food Engineering*, **225**, pp.26-33.

Zhang, F., O'Mahony, J.A., Miao, S. and Cronin, K. (2023). An Experimental Study on the Dilute Phase Pneumatic Conveying of Fat-Filled Milk Powders: Particle Breakage. *Powders*, **2 (1)**, pp.124-134.

Chapter 3

Influence of altering particle size post-drying on physical and functional characteristics of whey powder

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Declaration

This chapter was written by Emma J. Gordon (E.J.G.) and reviewed by all co-authors. E.J.G. co-designed the study with the co-authors and performed all of the experimental work.

Abstract

The objective of this study was to investigate the impact of modifying powder particle size on the physicochemical and rehydration properties of whey powder. Three times throughout a year, June (Trial 1), September (Trial 2) and November (Trial 3), a sample of rennet whey powder was subjected to three different treatments: agglomeration (AG), using a pilot-scale fluid bed dryer to increase the particle size of the powder through agglomeration; single milling (SM) which involved passing the whey powder once through a mill with an integrated mesh and double milling (DM) where the whey powder was passed twice through the same mill. Physicochemical and rehydration properties of the resulting powders, along with a control (CP) powder were analysed. For all treatments, powder particle size was altered in comparison with the CP powder; in all three trials, the AG sample had the largest volume-weighted mean diameter (D[4,3]) of 314.00 ± 64.50 μm, followed by the CP, SM and DM powder samples, with values of 142.00 ± 5.10, 86.00 ± 7.83 and 58.10 ± 9.18 μm. The morphology and shape of the powder particles was significantly modified following the post-drying treatments. Colour and flowability of the powder were also altered, with SM and DM powders being whiter and more cohesive, and the AG powder being darker and more easy flowing. The more extensive the milling, the poorer the rehydration properties of the powder. Analysis also revealed that the DM milling process resulted in some denaturation of whey proteins. Overall, agglomeration did not negatively affect the physicochemical and rehydration properties of the whey powders, although SM and DM powders displayed some significant changes. However, when these powders were compared in a snack-seasoning application test, the AG powder performed the worst, due to issues with adhesion and thus had the highest drop-off value. These novel results provide new insights into the impact of post-drying particle size alteration on physicochemical and rehydration properties of the resulting whey powders.

3.1 Introduction

Whey powder has a wide range of applications due to its versatile nature and relatively low cost. It is growing in popularity, as evidenced by the production of 1.89 million tons of whey powder in the European Union in 2019, with production projected to reach 2.44 million tons annually by 2030 (Ozel *et al*., 2022). It is important for dairy scientists and processors to continually develop powders with novel and interesting physical and functional properties to meet the evolving needs of industrial and retail end-users and consumers. This can be done not only by targeting interventions in the wet phase (e.g., in-line shearing, as studied in Chapter 2), but by altering the physicochemical and functional properties of powders post-drying through various process technology interventions. For example, poor flowability of high protein powders is a challenge for dairy processors due to their strong interparticle interactions and thus cohesive nature (Hazlett, Schmidmeier and O'Mahony, 2021b). Efforts have been made to improve this through novel post-drying agglomeration methods, e.g., shear, extrusion, roller compaction and steam jet agglomeration, as well as modifications to the surface of the particles (dry and wet coating), to reduce interparticle interactions and thus improve the flowability of the powders (Hazlett, Schmidmeier and O'Mahony, 2021b).

Some dairy powders can have difficulties with rehydration (due to for example, powder density and particle surface composition). It is common practice in the dairy industry to utilise mechanical methods (e.g., agitation) and chemical additives (e.g., addition of calcium chelators for casein powders) to facilitate more rapid/extensive rehydration (Wu *et al*., 2022). However, the powder itself can also be altered post-drying, through agglomeration or coating with a surfactant (e.g., lecithin), to aid the rehydration ability of the powder (Sharma, Jana and Chavan, 2012; Wu *et al*., 2022). As well as increasing the particle size through agglomeration,

superfine grinding is an emerging technique that can be implemented post-drying during food powder processing (Gao *et al*., 2020). These powders can have altered physicochemical properties, such as density and flowability, as well as optimising the functional properties of the powder, including enhanced bioavailability (Gao *et al*., 2020). A variety of different superfine grinding techniques can be utilised including; jet milling, ball milling or vibration milling, as well as some novel techniques such as cryogenic grinding, which embrittles the material through low temperatures so that the particles can fracture more easily (Gao *et al*., 2020).

Particle size distribution is a very important property of dairy powders. In previous studies, it has been shown that dairy powders with high lactose contents (i.e., whey permeate and demineralised whey powder) often have wider particle size distributions than other types of dairy powders (O'Donoghue *et al*., 2019). In a study conducted by O'Donoghue *et al*. (2019) it was shown that, upon fractionation of whey permeate and demineralised whey powder into different particle size classes, smaller particles had higher protein and lower lactose contents when compared to larger size fractions. This is important as powders with wider particle size distributions have greater propensity for segregation (Gausemel *et al*., 2022). During storage of such powder, segregation of the powder can occur based on differences in particle size, which can subsequently result in different size fractions having different bulk chemical composition. This was evident in a recent study by Gausemel *et al*. (2022), which studied how different powder sampling techniques can result in different chemical compositions, i.e., a sample taken from the top of the sack may have different properties compared to a sample taken at the bottom of the sack.

From these previous studies, it has been shown that it is possible to modify the physiochemical and functional properties of powders post-drying, and that there is a complex relationship between composition, particle size distribution and propensity for segregation. The main objective of the study reported in this chapter was to understand the impact of altering particle size post-drying on physical and functional characteristics of whey powder. The findings of this study will enable a greater understanding of the impact of post-drying particle size alteration on relevant physical attributes of whey powder and how these modifications can be targeted to create powders fit for purpose based on the needs of particular applications.

3.2 Materials and methods

3.2.1 Materials

Three different treatments were applied to dried rennet whey powder to obtain 3 different particle size distributions, along with a control powder to which no treatment was applied. The original rennet whey powder was obtained from Kerry Ingredients Charleville (Kilmallock road., Charleville, Co. Cork). Three different trials were conducted at three different points throughout the dairy season (June, September and November) to take seasonality into consideration. For each trial, one batch of whey powder was sampled to produce the 3 different powders and one control. The rennet whey powder contained between 11.40 -17.00% protein over the course of the three trials. The three different treatments including (1) subjecting the powder to an agglomerating process (AG) to produce larger powder particles, (2) putting the powder through a mill once (SM) and (3) putting the powder through a mill twice (DM) to produce finer particles.

3.2.2 Preparation of powders with altered particle size distributions

Various preliminary trials were conducted to determine what treatments could be applied to the powder post-drying to alter the particle size distribution of the finished powder. The objective was to alter the particle size of the powder post-drying. The powder particle size was increased through agglomeration using a pilot scale fluid bed with an integrated nozzle. The powder particle size was reduced by passing the powder through a mill with a perforated screen (0.5 mm^2). These treatments are described in more detail in Section 3.2.2.1- 3.2.2.3.

3.2.2.1 Control powder

The control rennet whey powder (CP) was obtained from Kerry Ingredients Charleville three times throughout the dairy season (November, June and September). This powder was produced in the whey plant in Listowel and dried using a large-scale industrial spray dryer. It was used as a control throughout this experiment and was also used as the base for production of the treated powders.

3.2.2.2 Agglomerated powder

The agglomerated (AG) powder was produced by placing approximately 6 kg of whey powder into a Niro atomozier combi coata pilot scale fluid bed dryer (GEA Group, Aktiengesellschaft, Petter-Müller-Str. 12 40468 Düsseldorf, Germany) as shown in Figure 3.1. A 20% (w/v) whey solution was prepared by rehydrating whey powder in water for 30 min at 60 °C while continuously stirring. This solution was then placed in a water bath at 60 °C, where plastic tubing, 6.35 mm in diameter, was wrapped with insulated foil and linked between the whey solution and the spray atomizer of the dryer. This whey solution was pumped through the plastic tubing using a Masterflex L/S easy-load economy drive peristaltic pump (Masterflex SE, Willy-Brandt-Allee 300, 45891 Gelsenkirchen, Germany). The inlet air temperature was set at 70 °C and the outlet air temperature was maintained between 50-55°C. The powder was placed in the base of the dryer and it was powered on to heat the powder for approximately 5 min. The nozzle was then opened and the pump turned on and the whey solution sprayed onto the powder for 30 s, which distributed ~123 g of whey solution onto the powder as it was fluidised in the drier, which caused powder particles to stick together. The powder was allowed to dry for an additional 5 min before the drier was opened and socks cleaned down to minimise

losses of powder as well as mechanically break down any large agglomerates. The drier was then turned on again for a further 5 min until the powder was sufficiently dried.

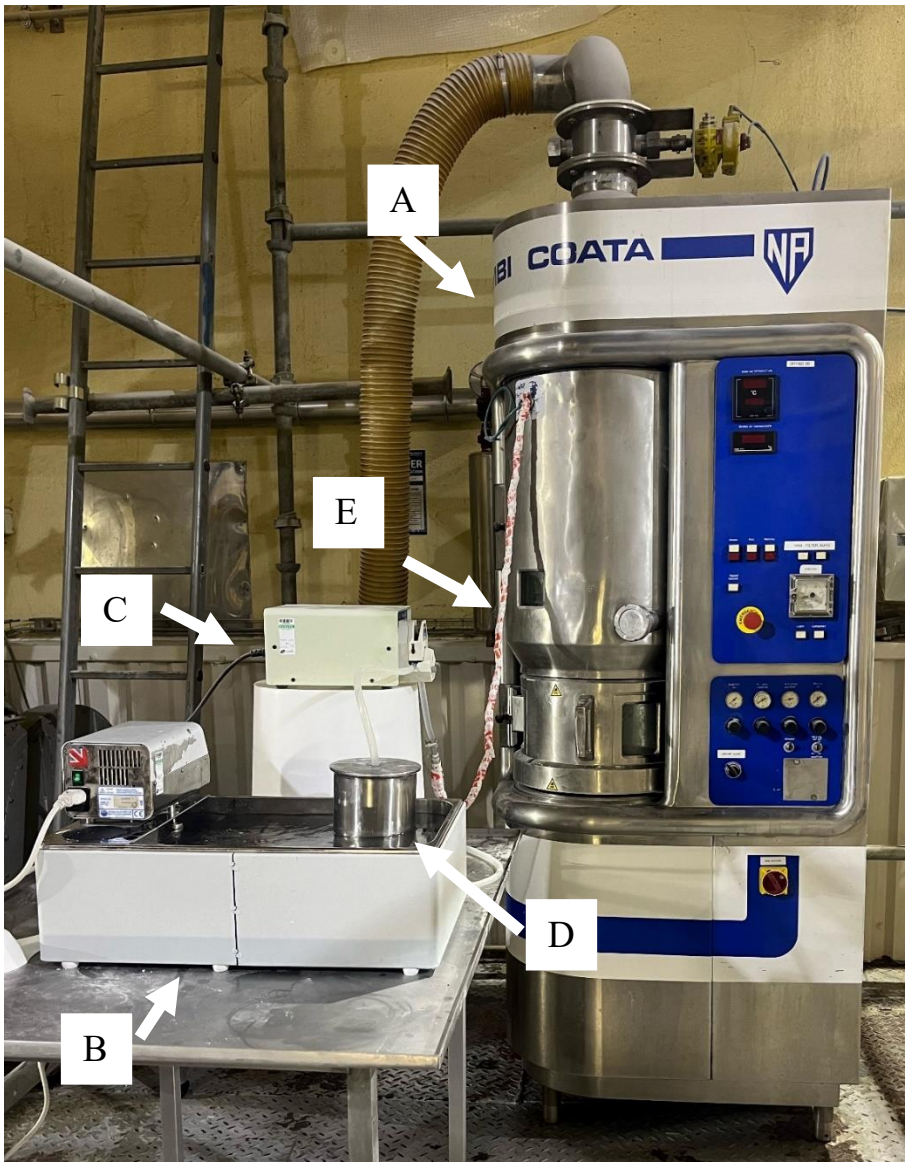


Fig 3.1: Pilot-scale fluid bed dryer with attachments to produce the agglomerated (AG) whey powder. (A) Niro atomozier combi coata pilot scale fluid bed dryer, (B) Water bath at 60 °C, (C) Masterflex L/S easy-load economy drive peristaltic pump, (D) 20% whey solution, (E) plastic tubing wrapped with insulated foil to transport whey solution to the atomiser.

3.2.2.3 Milled powder

The single-milled (SM) powder was produced by taking 6 kg of regular whey powder and using the smallest perforated screen size (0.5 mm^2) on the Christy and Norris 8" lab mill (Christy Turner Ltd, Knightsdale Road, Ipswich, Suffolk, England). The rotating beater within the mill operates at 8000 rpm which forces powder through the stationary perforated screen and thus reduces powder particle size. The powder was collected in the bag underneath the mill. The double-milled (DM) powder was produced similarly to that of the SM powder; however, the powder was passed twice through the perforated screen within the mill, resulting in the powder particle size being reduced even further.

3.2.3 Analysis of powder composition, physical and functional properties

Protein, moisture and ash analysis was completed on all powders produced as described in Section 2.3.3. Powder physical properties, including particle size, morphology, particle shape, density, colour and flowability were analysed as described in Section 2.3.4 of Chapter 2. The rehydration process was analysed as detailed in Section 2.3.5 of Chapter 2.

The protein profile, pH 4.6-soluble protein and non-protein nitrogen (NPN) contents of powders were analysed as described in Section 2.3.7 of Chapter 2.

3.2.4 Analysis of whey powder performance in selected applications

3.2.4.1 Snack seasoning application analysis

The following ingredients, all sourced from Kerry, were used at specified inclusion rates to develop a model seasoning system to evaluate whey powders (only completed using samples from Trial 1, for a preliminary study): CP/AG/DM whey powder (62%), cheese powder (25%), umamex yeast extract (3%), onion powder (1%), garlic powder (0.5%), citric acid (1%), salt (7.2%), turmeric oleoresin (0.3%) and paprika oleoresin (0.2%). This seasoning system was then applied to Walkers (Walkers Crisps, Leicester, UK) Salt and Shake potato crisps.

Three different variations of the recipe above were made using the CP, AG or DM whey powder. The paprika and turmeric oleoresin were plated on to the salt. All the other ingredients in the recipe were gently mixed using a benchtop mixer (Hobart, Model no. N-506, South Ridge Avenue, Trou, Ohio, 45374, USA) followed by sieving, to ensure they were all combined sufficiently. An oven was preheated to 100 °C and 45.00 g (± 0.20 g) of potato crisps were placed into the oven on a baking tray and heated for 5 min to release the natural oils from the crisps. Then, 7.50 g of the seasoning mix was weighed out into a labelled sample cup describing which type of whey powder (CP/AG/DM) was used in the seasoning system, and the warmed crisps were then placed into a large plastic mixing bag, along with the sample cup with seasoning mix, and gently shaken in circular motions for 1 min to allow the seasoning to sufficiently coat the crisps. The crisps were then gently removed from the bag to avoid breaking them, and any seasoning left in the bottom of the bag was weighed (residue in bag). The crisps were then placed on a sieve which was shaken gently back and forth 30 times and any seasoning that dropped off was weighed.

3.2.4.2 Beverage application analysis

The following ingredients, all sourced from Kerry, were used in developing a model beverage system to evaluate the performance of whey powder with different particle sizes: CP/AG/DM Whey powder (57%), full cream milk powder (10.5%), instant milk powder (21%), silicon dioxide powder (1%) icing sugar (10.5%).

Three different variations of the recipe above were made using the CP, AG and DM powder. Rehydration properties of the recipe were tested as described in Section 2.3.5.1 of Chapter 2

3.2.6 Shelf life stability

3.2.6.1 Materials

Nine plastic-lined brown paper bags were filled with 500 g of CP powder and sealed by sewing the bag opening closed. Three of these bags were stored in a Binder constant climate chamber incubator (Binder Gmbh, Im Mittleren Osch 5, 78532 Tuttlingen, Germany) at 40 °C and 40% relative humidity (RH), another three bags were stored in a fridge at 4 °C and the final three bags were stored at ambient conditions (10°C ± 5°C). Every 6 weeks (± 4 d), the powders were removed from their storage conditions and colour and rehydration properties of the powders were analysed.

3.2.6.2 Analysis of colour and rehydration properties

The colour of the powder was analysed as described in Section 2.3.4.5 of Chapter 2. The rehydration properties of the powder were analysed as described in Section 2.3.5.1 of Chapter 2.

3.2.7 Study design and statistical data analysis

For this study, three individual trials were conducted throughout the year (June, September and November). From each trial, four different powders were analysed: (1) the control plant (CP) powder: (2) the agglomerated (AG) powder, where the particle size was increased through agglomeration, (3) the single milled (SM) powder, where the whey powder was passed once through a mill with integrated mesh and (4) the double mill (DM) powder, where the powder was passed twice through the mill with integrated mesh. For each trial, all powders were sampled from the same powders batch.

Data were analysed using a Univariate General Linear Model (GLM) with SPSS Version 29.0.0 software (IBM, Armonk, New York, USA). The GLM procedure allowed analysis of the effects of multiple independent variables on one dependent variable as well as the examination of any interaction effects. Prior to running statistical analysis, assumptions of data normality, homogeneity of variance (Levene's test), and independence of errors were assessed; appropriate data transformations were applied, where necessary. A *Post-hoc* multiple comparison test (Tukey's test) was conducted to explore statistically significant interactions further. A statistical significance level, a, of 0.05 was used throughout.

The effects of post-drying alterations of particle size and trial on particle size, particle shape, colour, flowability, bulk volume, tapped volume, total protein, denatured protein, non-protein nitrogen, snack seasoning application and shelf-life colour were statistically analysed.

All analyses were carried out in triplicate. For all experimental data, three trials were performed and the effect of trial on all parameters measured was statistically analysed. The effect of post-drying alterations of particle size on snack seasoning application was carried out on a single trial and shelf-life colour was carried out on two trials.

3.3 Results and discussion

3.3.1 Powder composition

The composition of the four powders across the three trials is provided in Table 3.1. The whey powder used to produce the powder for each trial came from the same batch, which implies that any differences in protein and ash between the dried powder samples from the same trial may be due to discrepancies in testing or effect of the treatments applied to the powders.

Throughout the three trials, the AG powder sample consistently had lower protein content the CP, SM and DM powders in the same trial, with average protein contents for CP, AG, SM and DM powders, being 13.50 ± 2.60, 11.80 ± 1.40, 13.40 ± 3.10 and 13.60 ± 2.10 %. Statistically, there was a significant difference ($p < 0.05$) when comparing the protein content of the AG powder with all other samples. The lower protein content for the AG powder sample is most likely due to losses of fine, protein rich powder through the exhaust air in the pilot scale fluid bed dryer. All efforts were made to prevent this loss during the processing of the AG powder, ensuring any powder build up on the socks was recombined with the finished powder. A significant difference ($p < 0.05$) was reported for between the CP and SM sample; however, no significant difference ($p > 0.05$) was reported for between the CP and DM sample. This indicates that the agglomeration process utilized in this study most likely impacted the protein contents, whereas the milling process did not.

Statistical analysis demonstrated that there was a significant difference ($p < 0.05$) in the protein contents between trials. The large variation in protein contents between trials is most likely associated with the protein contents increasing throughout the year due to seasonality in milk

(Bernabucci *et al*., 2015). Whey powder is generally known to have 11.00 – 14.50 % protein throughout the dairy season (Bronsveld, 2015).

The ash contents generally reduced throughout the year as the ash values for the CP powder were 6.23, 5.82 and 5.02 % for Trial 1,2 and 3 respectively. As previously discussed in section 2.4.1, rennet whey powder generally contains ~ 8.00 % ash (Bronsveld, 2015). These lower than normal ash values seen in this study were due to the NF process that took place as a method of concentration that removed smaller compounds, such as minerals, when producing the standard whey powder (Pan *et al*., 2011, Mohammad *et al.,* 2015).

Moisture contents for the CP, AG, SM and DM powders were 1.57 ± 0.14, 1.33 ± 0.21, 1.69 ± 0.53 and 1.92 ± 0.55 % respectively. The AG powder samples had slightly lower moisture contents, this is most likely due to the additional drying to which the powder was subjected in the pilot scale fluid bed dryer when it was being produced. There was a slight increase in moisture contents for the SM and DM powder samples, which may have been due to powder absorbing additional moisture when subjected to the milling process in a non humidity controlled room.

Table 3.1: Composition of Control plant (CP), Agglomerated (AG), Single mill (SM) and Double mill (DM) whey powders.

		Protein	Moisture (%)	Ash
CP	Trial 1	11.20 ± 0.02	1.66	6.23
	Trial 2	13.20 ± 0.11	1.64	5.82
	Trial 3	16.30 ± 0.07	1.40	5.02
	Average	**13.50 ± 2.60**	**1.57 ± 0.14**	**5.69 ± 0.62**
AG	Trial 1	10.30 ± 0.08	1.39	6.62
	Trial 2	12.10 ± 0.06	1.50	5.48
	Trial 3	13.00 ± 0.14	1.09	4.00
	Average	**11.80 ± 1.40**	**1.33 ± 0.21**	**5.37 ± 1.31**
SM	Trial 1	10.80 ± 0.08	2.23	6.70
	Trial 2	12.50 ± 0.04	1.18	6.00
	Trial 3	16.80 ± 0.27	1.67	5.32
	Average	**13.40 ± 3.10**	**1.69 ± 0.53**	**6.01 ± 0.69**
DM	Trial 1	11.60 ± 0.06	2.06	5.11
	Trial 2	13.40 ± 0.13	1.31	6.00
	Trial 3	15.80 ± 0.07	2.39	5.30
	Average	**13.60 ± 2.10**	**1.92 ± 0.55**	**5.43 ± 0.40**

3.3.1 Powder physical properties

3.3.1.1 Particle size analysis

The particle size distribution values for the whey powder are displayed in Table 3.2. In all three trials, as expected, the AG powder sample had the largest volume weighted mean diameter (D[4,3]), while the CP, SM and DM powder samples had much lower D[4,3] values. Statistical analysis reported a significant difference ($p < 0.05$) between D[4,3] values for all samples. These values prove the hypothesis that agglomerating and milling the whey powder post-drying can alter the particle size distribution. The higher the D[4,3] value for the AG sample is most

likely due to the agglomeration process applied. The reduction in D[4,3] value for the SM and DM powders is due to the milling action physically breaking down the powder particles to smaller particles. By milling the powder twice, the whey powder is even further reduced in size to create the DM powder. The D[4,3] values across the three trials are significantly different ($p > 0.05$), which may be due to a seasonal effect of the variations in composition of the powder.

Altering the particle size of the powder also influenced the specific surface area (SSA). As anticipated, the AG sample had the smallest SSA, with an average value of 74.00 ± 1.66 m^2/g, followed by CP, SM and DM with average SSA values of 109.00 ± 8.46, 321.00 ± 97.40 and 633.00 ± 86.50 m^2/g respectively. The SSA values for all samples were significantly different ($p < 0.05$). The smallest particles had the largest surface area due to an increase in the level of exposed surface area due to the breaking of particles (Crowley *et al*., 2014). The particle size below which 90 % of material volume exits (Dv90) and the particle size below which 10% of the material volume exists (Dv10) also reflected the same pattern as the D[4,3] values as, again the AG sample's average of the three trials had the largest Dv90 and Dv10 value followed by the CP, SM and DM samples. This indicates that, on altering the particle size of the powder post-drying all parts of the particle size distribution are affected. A statistically significant difference ($p < 0.05$) was demonstrated when comparing the Dv10 values of all samples; however, when comparing the Dv90 values, the CP sample was not significantly different ($p > 0.05$) to the SM sample but was significantly different ($p < 0.05$) to the DM and AG samples. This indicates that all processes significantly affect the Dv10 value, while the AG process and DM process significantly affected the Dv90 value.

Figure 3.2 displays the particle size distribution graphs for the three trials. All of the plots, (A), (B) and (C), have a similar trend with three clear populations (P1, P2 and P3). The CP powder is made up of two different populations, with the majority of the volume in P2, which is most likely made up of particle rich in lactose crystals, whereas the smaller population in P1 is likely made up of the smaller fine protein-rich powders. The impact of milling on particle size distribution curves were clearing displayed as the volume distribution curves for the SM and DM powders were shifted to the left and displayed as a single population visible in P1, due to the milling treatment reducing the particle size of the powder. In all three trials, the AG powder showed two populations (P2 and P3), with P3 most likely made up of the larger agglomerates that were formed during the pilot scale agglomeration process. The distribution profiles displayed in Figure 3.2 reflect the values in Table 3.2 for the effect of agglomerating and milling whey powders post-drying.

Table 3.2: Particle size distribution values of Control plant (CP), Agglomerated (AG), Single mill (SM) and Double mill (DM) whey powder.

		Dv10	Dv50	Dv90 (µm)	D[4,3]	D[3,2]	Span -	SSA m^2/g
	Trial 1	31.00 ± 1.03	138.00 ± 2.65	274.00 ± 2.00	146.00 ± 2.00	60.300 ± 1.65	1.76 ± 0.02	99.60 ± 2.73
CP	Trial 2	24.80 ± 0.30	120.00 ± 1.00	271.00 ± 1.20	136.00 ± 0.60	52.50 ± 0.40	2.05 ± 0.02	114.00 ± 0.82
	Trial 3	24.50 ± 0.26	127.00 ± 0.58	292.00 ± 1.53	144.00 ± 0.00	52.50 ± 0.29	2.10 ± 0.01	114.00 ± 0.64
	Average	**26.80 ± 3.64**	**128.00 ± 3.05**	**279.00 ± 11.10**	**142.00 ± 5.10**	**55.10 ± 4.48**	**1.97 ± 0.18**	**109.00 ± 8.46**
	Trial 1	40.50 ± 0.70	172.00 ± 3.61	1310.00 ± 147.00	385.00 ± 28.60	82.70 ± 1.81	7.35 ± 0.71	72.60 ± 1.64
AG	Trial 2	35.80 ± 0.50	157.00 ± 5.00	664.00 ± 203.00	297.00 ± 31.50	73.10 ± 1.14	5.29 ± 0.35	73.40 ± 0.89
	Trial 3	41.90 ± 0.66	174.00 ± 0.58	386.00 ± 18.60	259.00 ± 25.60	79.10 ± 0.49	1.99 ± 0.10	75.80 ± 0.48
	Average	**39.40 ± 3.18**	**168.00 ± 8.99**	**786.00 ± 472**.00	**314.00 ± 64.50**	**78.30 ± 4.81**	**4.86 ± 2.70**	**74.00 ± 1.66**
	Trial 1	10.60 ± 0.06	65.30 ± 0.26	197.00 ± 4.16	87.40 ± 1.21	18.70 ± 0.53	2.85 ± 0.06	327.00 ± 8.26
SM	Trial 2	12.50 ± 0.50	73.00 ± 4.00	204.00 ± 4.50	93.10 ± 3.10	27.60 ± 3.46	2.63 ± 0.09	220.00 ± 29.90
	Trial 3	9.83 ± 0.02	58.60 ± 0.95	176.00 ± 6.43	77.60 ± 2.15	14.50 ± 0.12	2.83 ± 0.00	415.00 ± 3.70
	Average	**11.00 ± 1.40**	**65.60 ± 7.22**	**192.00 ± 14.80**	**86.00 ± 7.83**	**20.20 ± 6.69**	**2.77 ±0.12**	**321.00 ± 97.40**
	Trial 1	6.16 ± 0.11	41.50 ± 0.68	138.00 ± 1.00	58.70 ± 0.56	9.14 ± 0.10	3.19 ± 0.04	657.00 ± 7.07
DM	Trial 2	7.30 ± 0.10	46.80 ± 0.40	154.00 ± 1.50	66.90 ± 2.10	11.20 ± 0.10	3.14 ± 0.04	537.00 ± 4.83
	Trial 3	5.49 ± 0.04	33.00 ± 0.20	117.00 ± 1.73	48.60 ± 0.64	8.72 ± 0.10	3.22 ± 0.03	702.00 ± 6.01
	Average	**6.30 ± 0.89**	**40.40 ± 6.97**	**136.00 ± 18.70**	**58.10 ± 9.18**	**9.70 ± 1.32**	**3.18 ± 0.04**	**633.00 ± 86.50**

Dv10, Particle size below which 10% of material volume exists
Dv50, Particle size below which 50% of material volume exists
Dv90, Particle size below which 90% of material volume exists
D[4,3], volume-weighted mean particle diameter.
D[3,2] surface-weighted mean particle diameter
Span- measurement of the width of the distribution calculated as (Dv90)- (Dv10)/ (Dv50)
SSA- specific surface area

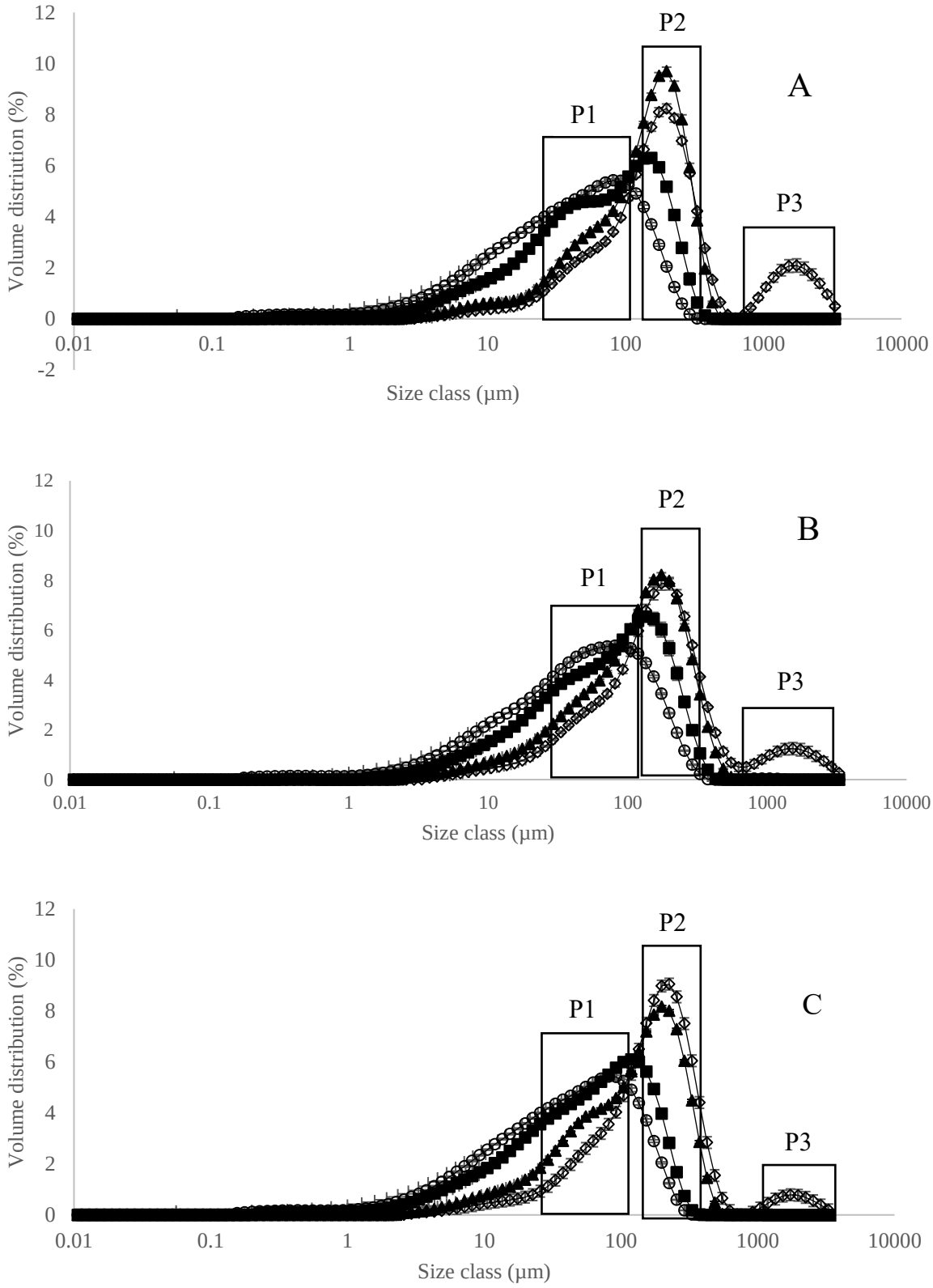


Figure 3.2. Particle size distribution of (A) Trial 1 (B) Trial 2 (C) Trial 3 of Control plant (CP) —▲— , Agglomerated (AG) —◇— , Single mill —■— (SM) and Double milled —○— (DM) whey powder

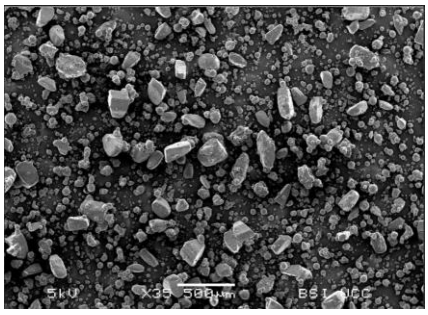
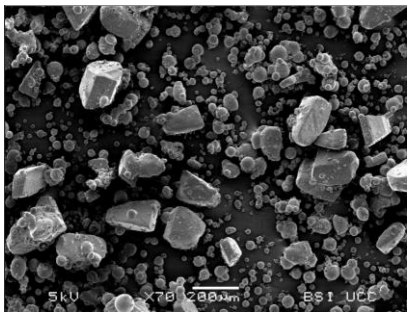
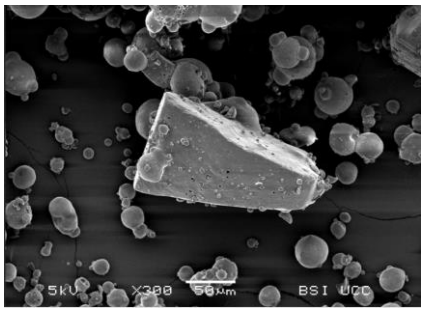
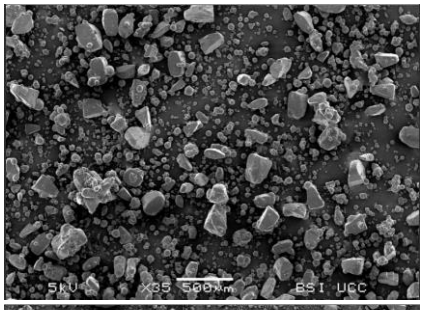
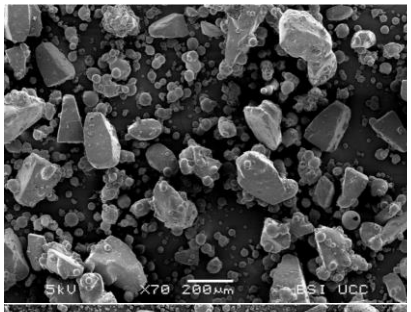
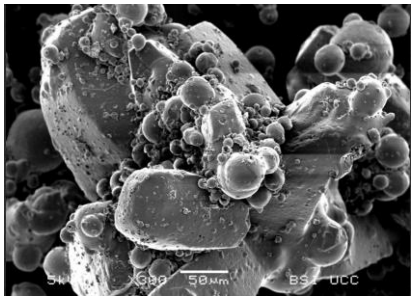
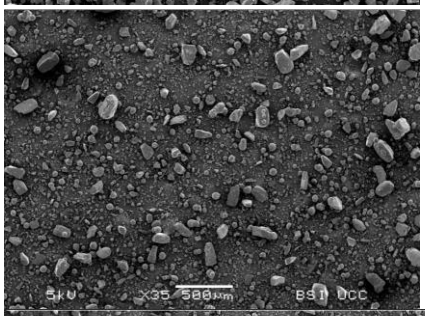
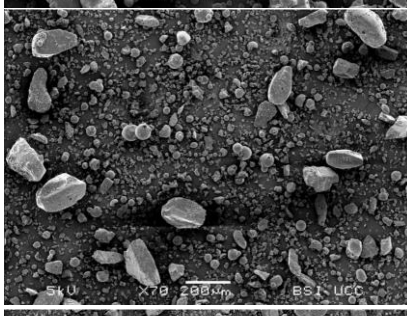
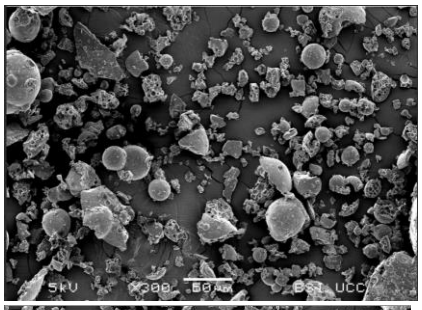
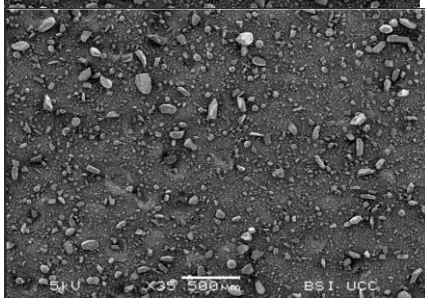
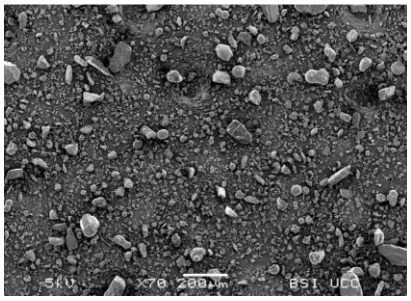
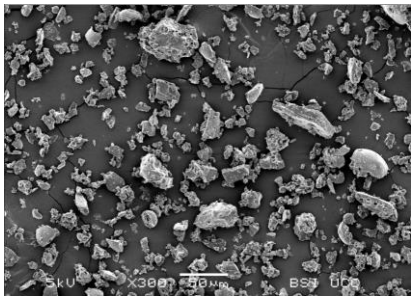
3.3.1.2 Morphology

Images from the SEM analysis, which were conducted on Trial 1 powders at 35x, 70x and 300x magnifications, are shown in Table 3.3. It is clear that there is a change to the particle structure when the agglomerating and milling treatments were applied to the whey powder. The 300x magnification images most clearly highlight the differences in particle structure between the different powders. The CP sample displays a clearly identifiable tomahawk shaped lactose crystal surrounded by smaller particles, most likely made up of other whey solids such as protein and ash. Similar SEM images for whey powder can be seen in a study conducted by Saito (1988) of lactose and whey powder structure.

In the 300x image for the AG sample, an agglomerate can be seen which probably consists of a lactose crystal joined together with other whey powder particles. The SM and DM powders at the 300x magnification display much smaller sheared and fragmented particles when compared to the CP powder and the sheared solid lactose crystals can be seen to be surrounded by broken-down whey solid particles. These can be differentiated as the lactose crystal particles are solid, whereas the broken-down whey solids particles (made up of ash, protein and amorphous lactose) have hollow vacuoles within the powder particles which can be seen in the 300x images of samples SM and DM, with an increase of these evident in the 300x DM sample due to more milling occurring. The vacuoles are present as they were originally filled with enclosed air within powder particles when they were formed into droplets in the dryer and are visible now during to the milling breaking down these particles (Salunke *et al*., 2023).

From both the 35x and 70x magnifications images, it is evident that milling causes the particles to become smaller (SM and DM powders) and that the agglomeration process causes particles to stick together which in turn results in production of larger particles.

Table 3.3: Scanning electron microscopy (SEM) images of Control plant (CP), Agglomerated (AG), Single milled (SM) and Double milled (DM) whey powder under magnifications of 35, 70 and 300x

Sample	35x	70x	300x
Trial 1 CP			
Trial 1 AG			
Trial 1 SM			
Trial 1 DM			

3.3.1.3 Particle shape

Dynamic image analysis was used to analyse the particle shape of the CP, AG, SM and DM powder samples. Each chosen descriptor calculates a value based on the silhouette of more than 100,000 individual particles. The descriptors of interest in this study were aspect ratio (AR), convexity (C), sphericity (S) and roundness (R). The average AR value for the CP and AG powders were not significantly different ($p > 0.05$) at 0.77 ± 0.01 and 0.77 ± 0.02, respectively. However, the average AR values for the SM sample was 0.76 ± 0.01 and for the DM sample was 0.73 ± 0.01 and these were significantly different ($p > 0.05$) when compared to the AR value for the CP powder. This indicates that increased milling caused an increase in elongation of the powder particles which can also be visually seen in the SEM images in Table 3.3. This is possibly due of the shearing of the lactose crystals causing them to become fragmented and thus becoming more elongated.

The C values illustrate how compact the particle is. The average C value reduced as the powder become more milled, and statistical analysis showed that, across all treatments, the C values of the samples were significantly different ($p < 0.05$). The reduction in C values due to milling weas unexpected as Gaiani *et al*. (2011) stated that the C values should decrease with increasing particle size, whereas the opposite was seen for this study where the DM powder had the smallest C value. No significant difference ($p > 0.05$) was shown between trials for this parameter. Statistical analysis demonstrated a significant difference ($p < 0.05$) between S values when comparing the CP sample with the AG, SM and DM sample. However, a significant difference ($p > 0.05$) was not demonstrated between the SM and DM samples. The average S value for CP, AG, SM and DM was 0.84 ± 0.00, 0.83 ± 0.00, 0.84 ± 0.00 and 0.84 ± 0.01, respectively. This suggests that the agglomerating and milling processes affect the smoothness

of the particle surface, but that the extent of milling does not cause a difference. In both milling and agglomerating processes, the particles were demonstrated to be smoother.

The difference in average R values were the most interesting of all descriptors analysed in this study. The average R value for the DM powder sample was 0.44 ± 0.02 which can be compared to the average R values for CP, AG and SM powders being 0.50 ± 0.01, 0.49 ± 0.03 and 0.50 ± 0.00. Statistical analysis using the paired multiple comparison test showed there was a significant difference ($p < 0.05$) between the DM sample when compared with the CP sample. However, there was no significant difference ($p > 0.05$) between the CP sample compared with the SM and AG sample. This indicates that the DM powder samples were significantly less round than the CP sample in this study which is possibly due to the breaking down of the lactose crystals as observed in Table 3.3.

From the particle shape analysis, it is evident that by milling the whey powder (SM and DM) the particles become more elongated and less spherical. Surprisingly, there was no major difference between the CP and AG powder samples according to particle shape analysis. This may be due to a very small sample being analysed which did not pick up a good portion of the agglomerated powder particles and thus the results are more similar than expected to the CP powder.

Table 3.4: Particle shape descriptor analysis of Control plant (CP), Agglomerated (AG), Single milled (SM) and Double milled (DM) whey powder. The table includes the average results for aspect ratio (AR), sphericity (S), convexity (C) and roundness (R).

		Aspect ratio	Convexity	Sphericity	Roundness
CP	Trial 1	0.76 ± 0.00	0.93 ± 0.00	0.83 ± 0.00	0.49 ± 0.00
	Trial 2	0.78 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.51 ± 0.01
	Trial 3	0.78 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.50 ± 0.00
	Average	**0.77 ± 0.01**	**0.93 ± 0.00**	**0.84 ± 0.00**	**0.50 ± 0.01**
AG	Trial 1	0.75 ± 0.01	0.93 ± 0.00	0.83 ± 0.00	0.46 ± 0.02
	Trial 2	0.78 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.52 ± 0.01
	Trial 3	0.77 ± 0.00	0.94 ± 0.00	0.83 ± 0.00	0.49 ± 0.00
	Average	**0.77 ± 0.02**	**0.93 ± 0.00**	**0.83 ± 0.00**	**0.49 ± 0.03**
SM	Trial 1	0.75 ± 0.00	0.93 ± 0.00	0.84 ± 0.00	0.50 ± 0.01
	Trial 2	0.77 ± 0.00	0.92 ± 0.00	0.84 ± 0.00	0.50 ± 0.02
	Trial 3	0.76 ± 0.00	0.92 ± 0.00	0.84 ± 0.00	0.50 ± 0.00
	Average	**0.76 ± 0.01**	**0.92 ± 0.00**	**0.84 ± 0.00**	**0.50 ± 0.00**
DM	Trial 1	0.73 ± 0.00	0.91 ± 0.00	0.84 ± 0.00	0.44 ± 0.00
	Trial 2	0.74 ± 0.00	0.91 ± 0.00	0.85 ± 0.01	0.46 ± 0.03
	Trial 3	0.73 ± 0.01	0.91 ± 0.00	0.84 ± 0.01	0.42 ± 0.03
	Average	**0.73 ± 0.01**	**0.91 ± 0.00**	**0.84 ± 0.01**	**0.44 ± 0.02**

3.3.1.4 Colour

The CP, AG, SM and DM powders produced in each trial were generated from the same base batch of whey powder, and thus changes in colour in each individual trial are not due to changes in processing conditions or composition, but are due to the effects of the agglomeration/ milling processes.

The L* value represents the brightness of the powder sample which ranges from 0-100, where 100 is the brightest. As expected, the average L* value for each powder followed the same

trend as particle size. The largest average L* value was obtained from the AG sample (93.20 ± 0.91) followed by the CP powder (94.00 ± 0.71), then the SM (94.50 ± 1.19) powder and the largest average L* value was obtained from the DM powder sample (95.00 ± 1.04). The higher L* values for the milled powders is due to the decrease in powder particle size which results in an increase in light scattering by the sample which can be reflected by a brighter powder sample (Haas *et al*., 2019).

The a* value is indicative of the redness (>0) to greenness (<0) of the powder sample. In this study, the smaller the particle size, the redder the sample is as the average a* value increased with increasing particle size. This indicates that the reddest sample was the DM sample while the least red sample was the AG sample. In terms of the b* value which represents the yellowness (>0) to blueness (<0), this decreased with decreasing particle size, as the average b* value for the CP, AG, SM and DM samples were 19.00 ± 0.48, 20.10 ± 0.65, 15.50 ± 1.06 and 13.20 ± 1.11. This indicates that the larger powder samples were more yellow. Statistical analysis of all L*, a* and b* values, showed that all samples were significantly different ($p < 0.05$). In addition, comparing the values for each trial, demonstrated there to be a significant difference ($p > 0.05$) between trials, which is most likely due to the effect of seasonality.

The ΔE value was used to understand the colour difference between the control (CP) powder and the treated (AG, SM, DM) powders. The ΔE values are displayed in Table 3.5; it is assumed that a colour difference of ΔE = >3 can be perceived by an average observer (Ghidouche *et al*., 2013). This indicates that there is not a noticeable colour difference between the AG powder sample and the CP powder sample as the ΔE = <3, whereas the SM and DM powder samples are seen to be noticeably distinguishable from the control, as the ΔE = >3.

3.3.1.5 Powder density

The average bulk density value for the CP powder was 0.60 ± 0.06 g/ml compared to 0.56 ± 0.06 and 0.52 ± 0.05 g/ml for SM and DM powders, respectively. This may be due to electrostatic interactions occurring between powder particles once they are milled. This agrees with the findings of Kaialy (2016), as, generally when milling occurs, particle charge increases with the level of energy introduced. The charges that are generated here are due to the interparticle and particle-wall collisions that occur during the milling process (Kaialy, 2016). This suggests that the milled powder particles in this study were thus occupying more space resulting in lower bulk density values due to the powder particles repelling each other. The more milling that occurred, the lower the bulk density levels were demonstrated; thus the DM powder had the lowest bulk density value. Statistical analysis demonstrated a significant difference ($p<0.05$) between the CP powder sample and the SM and DM samples, but little difference was shown between the bulk density of the CP powder and AG powder, with no statistical significance ($p>0.05$). This was unexpected as, when the particle size increases the density decreases as the larger particles occupy more space (Dhanalakshmi, Ghosal and Bhattacharya, 2011; Hazlett, Schmidmeier and O'Mahony, 2021a).

The values obtained for tapped density did not have the same large differences between them as demonstrated in the values obtained for bulk density. Statistical analysis of the tapped density values demonstrated a significant difference ($p <0.05$) between the CP sample and the AG and DM sample; however, there was no significant difference ($p <0.05$) between the CP and SM sample. The average tapped density values for CP, AG, SM and DM were, 0.73 ± 0.04, 0.74 ± 0.05, 0.74 ± 0.06 and 0.71 ± 0.06 g/ml, respectively. The tapped density is the measure of the density of a powder once it has been tapped with an external force in order to achieve a

high packing (Saw *et al*., 2013). The difference in bulk and tapped densities for the SM and DM powders were greater than that for the CP and AG powders. Saw *et al*. (2013) reported that finer powder particles had a greater change in density after the tapping occurred, most likely due to the tapping alleviating some of those electrostatic repulsion forces and thus compacting and increasing the density of the powder. These larger changes in bulk to tapped densities demonstrated for the milled powders can also be attributed to their poorer flowabilities (Saw *et al*., 2013). In the current study, samples for Trial 3 had lower densities when compared to the other two trials. Statistical analysis of both ρ_{bulk} and ρ_{tapped} demonstrated the values across all 3 trials were significantly different ($p < 0.05$). These differences may be due to differences in composition of the powders, as the three trials were conducted across the dairy season in June, September and November.

3.3.1.6 Flowability

Flowability is an important quality parameter for dairy powders as, if the flowability is poor, it can cause issues such as mechanical caking when transporting powder and during processing operations (Fitzpatrick *et al*., 2007; Ozel *et al*., 2022). In this study, the flowability of the powder was determined by finding the inverse of the flow function, known as the flow index (i); the higher the i value, the better the flowability the powder has (Fitzpatrick *et al*., 2004). The average *i* values for the CP, AG, SM and DM powders (Table 3.5) were 7.01 ± 0.16, 7.76 ± 1.50, 5.16 ± 0.51 and 4.04 ± 0.39 respectively. Statistical analysis demonstrated that there was a significant difference ($p < 0.05$) between the CP *i* value and all other samples. As expected, the AG powder had the highest flowability. The reason the agglomerated powder has better flowability when compared to the CP powder is due to less interactions occurring

between the powder particles, which in turn improved the flowability of the AG powder (Hazlett, Schmidmeier and O'Mahony, 2021b).

The increase in the amount of milling taking place, and thus the creation of smaller powder particles, resulted in the powders exhibiting poorer flowability characteristics. This agrees with the findings from a study conducted by Fu *et al*. (2012) which reported that finer lactose particles resulted in a more cohesive powder. The average *i* value for the DM powder was 4.04 ± 0.39, which is still classified as easy flowing in terms of the Jenike flow classification; however, an average i value of <4 would have classified the DM powder as cohesive.

Table 3.5: Commission Internationale d'Eclairage (CIE) colour space values, ΔE, bulk density (ρ_{bulk}), tapped density (ρ_{tapped}) flow index (i) and Jenike classification of Control plant (CP), Agglomerated (AG), Single mill (SM) and Double mill (DM) whey powder.

		Colour space values			ΔE	Density		Flow index (i)	Jenike
		L*	a*	b*		ρ_{bulk}	ρ_{tapped}		Classification
	Trial 1	93.50 ± 0.14	-5.14 ± 0.05	18.80 ± 0.13	-	0.66 ± 0.01	0.77 ± 0.01	6.93 ± 0.61	Easy flowing
CP	Trial 2	93.70 ± 0.17	-5.63 ± 0.03	19.60 ± 0.08	-	0.60 ± 0.01	0.73 ± 0.01	7.19 ± 0.94	Easy flowing
	Trial 3	94.80 ± 0.07	-5.65 ± 0.06	18.70 ± 0.08	-	0.54 ± 0.01	0.68 ± 0.01	6.90 ± 0.10	Easy flowing
	Average	**94.00 ± 0.71**	**-5.47 ± 0.29**	**19.00 ± 0.48**	**-**	**0.60 ± 0.06**	**0.73 ±0.04**	**7.01 ± 0.16**	Easy flowing
	Trial 1	92.50 ± 0.28	-5.33 ± 0.03	19.93 ± 0.05	1.52	0.65 ± 0.00	0.79 ± 0.01	9.43 ± 0.38	Easy flowing
AG	Trial 2	92.90 ± 0.31	-5.44 ± 0.03	19.60 ± 0.06	0.82	0.61 ± 0.01	0.75 ± 0.02	7.31 ± 1.23	Easy flowing
	Trial 3	94.20 ± 0.03	-6.35 ± 0.06	20.80 ± 0.09	2.29	0.54 ± 0.01	0.68 ± 0.01	6.53 ± 0.51	Easy flowing
	Average	**93.20 ± 0.91**	**-5.71 ± 0.56**	**20.10 ± 0.65**	**1.38**	**0.60 ± 0.06**	**0.74 ± 0.05**	**7.76 ± 1.50**	Easy flowing
	Trial 1	93.30 ± 0.11	-3.91 ± 0.04	14.30 ± 0.01	4.67	0.62 ± 0.00	0.79 ± 0.01	5.43 ± 0.44	Easy flowing
SM	Trial 2	94.60 ± 0.08	-4.68 ± 0.03	16.20 ± 0.07	3.64	0.57 ± 0.00	0.75 ± 0.01	5.48 ± 0.14	Easy flowing
	Trial 3	95.70 ± 0.10	-4.95 ± 0.03	16.10 ± 0.05	2.84	0.49 ± 0.00	0.67 ± 0.00	4.57 ± 0.35	Easy flowing
	Average	**94.50 ± 1.19**	**-4.51 ± 0.54**	**15.50 ± 1.06**	**3.66**	**0.56 ± 0.06**	**0.74 ± 0.06**	**5.16 ± 0.51**	Easy flowing
	Trial 1	93.80 ± 0.12	-3.24 ± 0.03	11.90 ± 0.05	7.16	0.56 ± 0.00	0.77 ± 0.01	3.65 ± 0.05	Cohesive
DM	Trial 2	95.40 ± 0.06	-4.18 ± 0.04	14.06 ± 0.05	5.97	0.53 ± 0.00	0.72 ± 0.02	4.44 ± 0.19	Easy flowing
	Trial 3	95.80 ± 0.16	-4.40 ± 0.02	13.50 ± 0.07	5.44	0.46 ± 0.01	0.65 ± 0.01	4.03 ± 0.32	Easy flowing
	Average	**95.00 ± 1.04**	**-3.94 ± 0.62**	**13.20 ± 1.11**	**6.08**	**0.52 ± 0.05**	**0.71 ± 0.06**	**4.04 ± 0.39**	Easy flowing

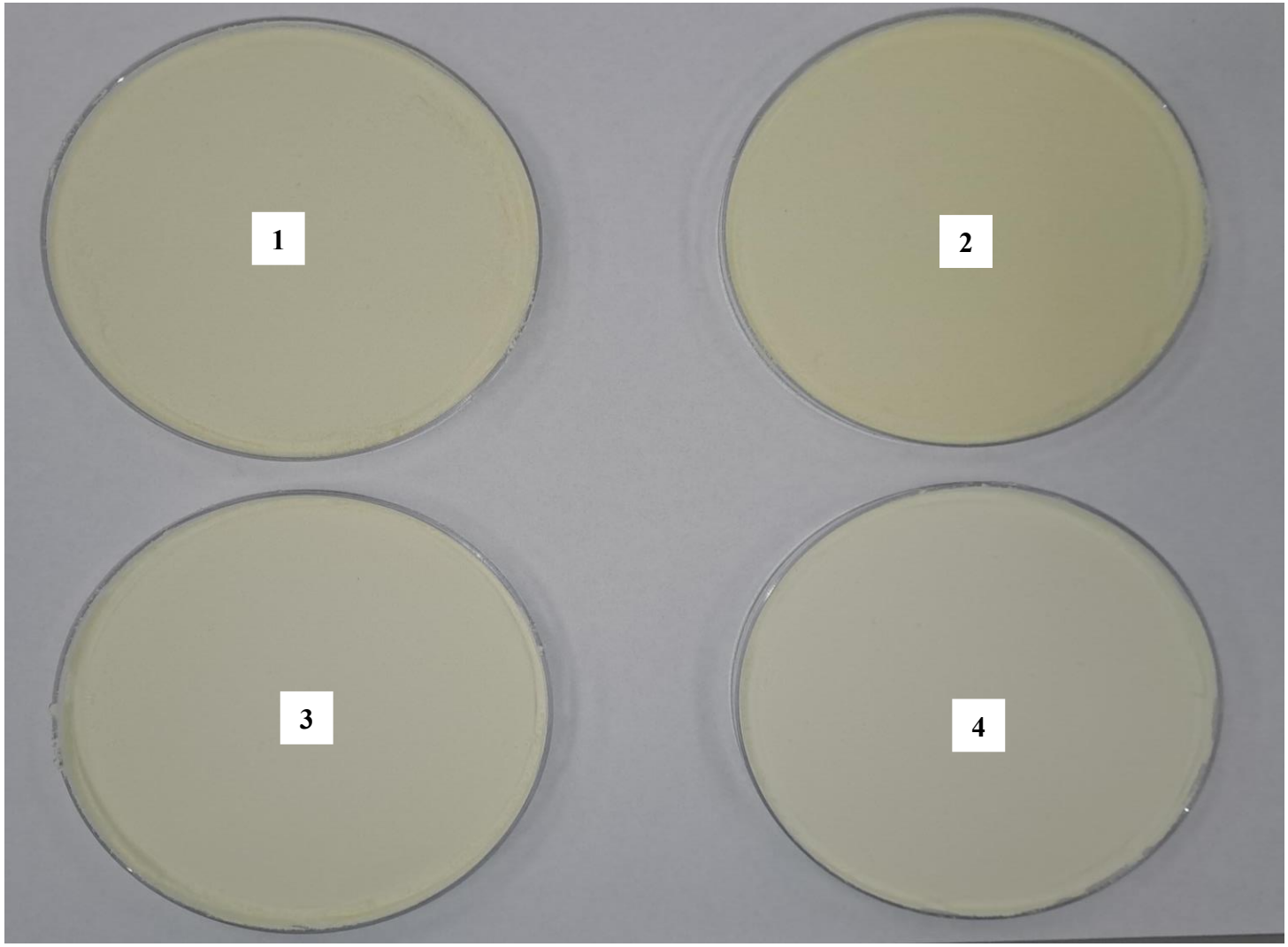


Figure 3.3 Whey powder samples (1) Control plant (CP), (2) Agglomerated (AG), (3) Single mill (SM) and (4) Double mill (DM) whey powder from Trial 1

3.3.2 Powder functional properties

3.3.2.1 Rehydration performance

Gas release from a powder in solution can be monitored using broadband acoustic resonance dissolution spectroscopy (BARDS). This can then be detected and quantified as a change in acoustic resonance which can explain the gas exchange occurring between a particular powder and a solution during the rehydration process (Wu *et al*., 2019). Figures 3.4, 3.5 and 3.6 provide rehydration behaviors of the CP, AG, SM and DM powders from the three different trials. The plots from Trial 1 and Trial 2 (Figure 3.4 and Figure 3.5) follow very similar patterns. As expected, the milled powders (SM and DM) had very different fundamental curves when compared to the control (CP) and agglomerated powder (AG). As seen in plot A in both Figures 3.4 and 3.5, the DM powder had the largest minimum frequency (f_{min}) of approximately 6 kHz. This can be reflected in the B plots of the same figures which showed very little gas release for the DM powders. The DM profile is similar to that of the SM profile but the f_{min} for the SM powder is slightly lower, at approximately 4.5 kHz, indicating slightly more gas release for the SM powder. Both SM and DM powders took approximately 600 s to return to steady state, longer than seen in profiles for the CP and AG powders. The difference in rehydration abilities of the SM and DM powders compared to the CP powder can be attributed to the milling process to which they were subjected.

The small particle size which gave the powders poor flowability characteristics (Table 3.5) may be the cause of the powders slower rehydration characteristics, because cohesive powders can have slow initial wetting due to a layer forming on initial contact with the solvent, which can slow down the remaining powder from dispersing in the solvent (Hazlett, Schmidmeier

and O'Mahony, 2021b). These results can be reflected in the Plot B from Figures 3.4 and 3.5, which display the gas volume profile; DM powders have the least amount of gas produced.

From Figures 3.4 and 3.5, the CP and AG powders follow similar rehydration profiles. The AG powder took a slightly shorter time to reach the minimum frequency of the curve (t_m) when compared to the CP powder sample. The faster the initial gas release/ faster t_m indicates more rapid and extensivewetting and dispersion ability of the powder (Wu *et al*., 2019). Agglomeration tends to improve the rehydration properties of a powder as the larger agglomerated particle size allows for water to penetrate the interstitial spaces between the particles (Wu *et al*., 2019). There was no notable difference between the fundamental curves for the CP and AG powders. This may be due to the rehydration properties of the CP powder already being very good due to the high lactose content.

Some of the rehydration curves displayed on plot A and B in Figure 3.6 for Trial 3 were unexpected. The rehydration profile for the DM and AG powder was similar to that seen in Trial 1 and Trial 2. However, the rehydration profiles for CP and SM powder samples were unusual. The SM powder took a lot longer to return to steady state than the SM powders in Trial 1 and Trial 2. This may again be linked to the flowability of the powders as the SM powder for Trial 3 had the poorest flowability value compared to the SM powders from Trial 1 and Trial 2 (Table 3.5). The unusual fundamental curve for the CP powder remains unexplained; this powder sample had very poor rehydration properties, where even after 800 s, it still did not return to steady state.

From the rehydration profiles of the different powders, it is evident that the milling treatments had negative effects on the rehydration properties of the whey powder. The agglomeration process (AG powder) had little effect on the rehydration properties when compared to the CP powder, as influences somewhat by the fact that the control powder already had very good rehydration properties.

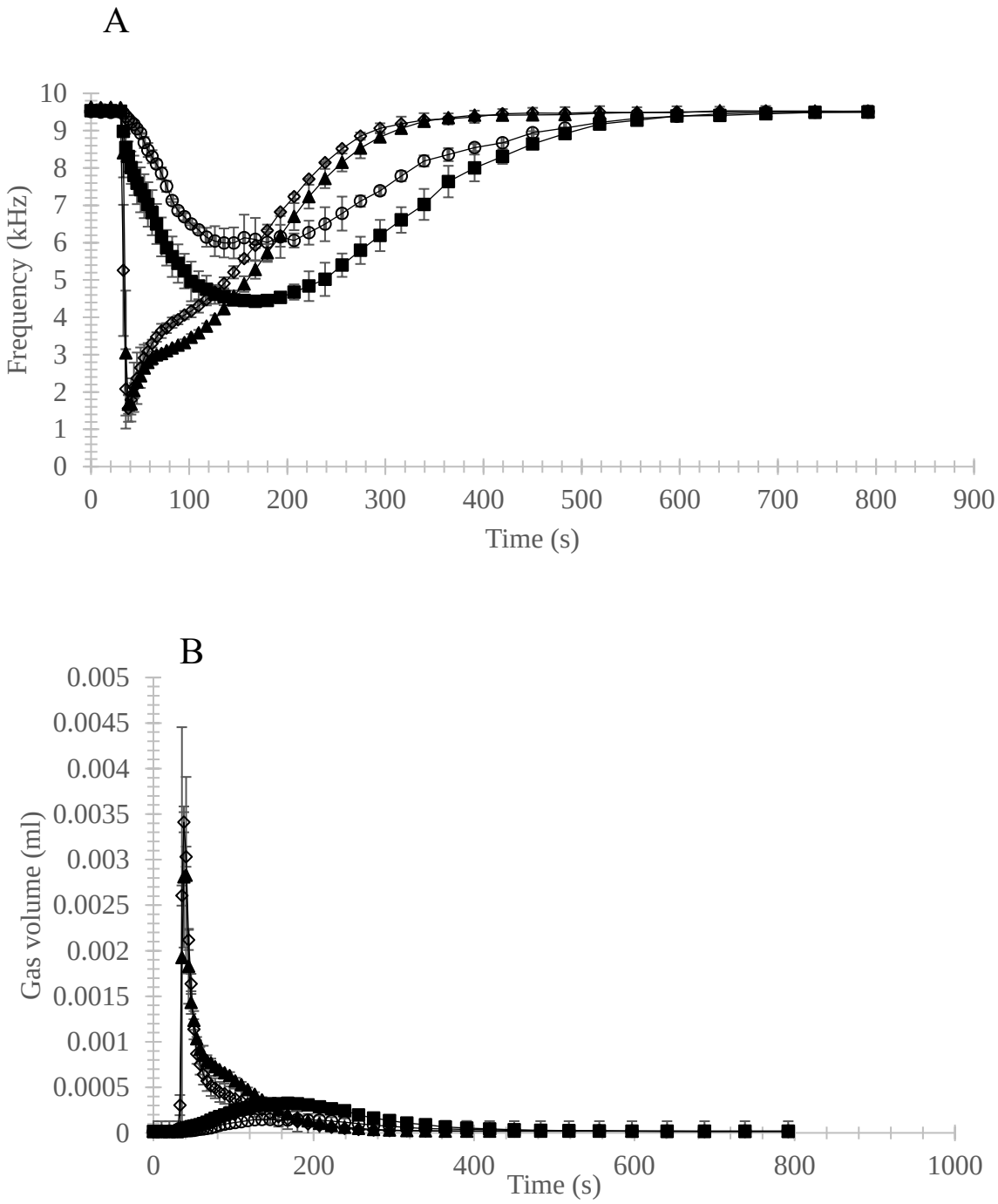


Figure 3.4 (A) BARDS frequency spectrum and (B) gas volume profile of Trial 3 Control plant (CP) —▲—, Agglomeration (AG) —◇—, Single mill (SM) —■— and Double mill (DM) —○— whey powder at 0.2% concentration

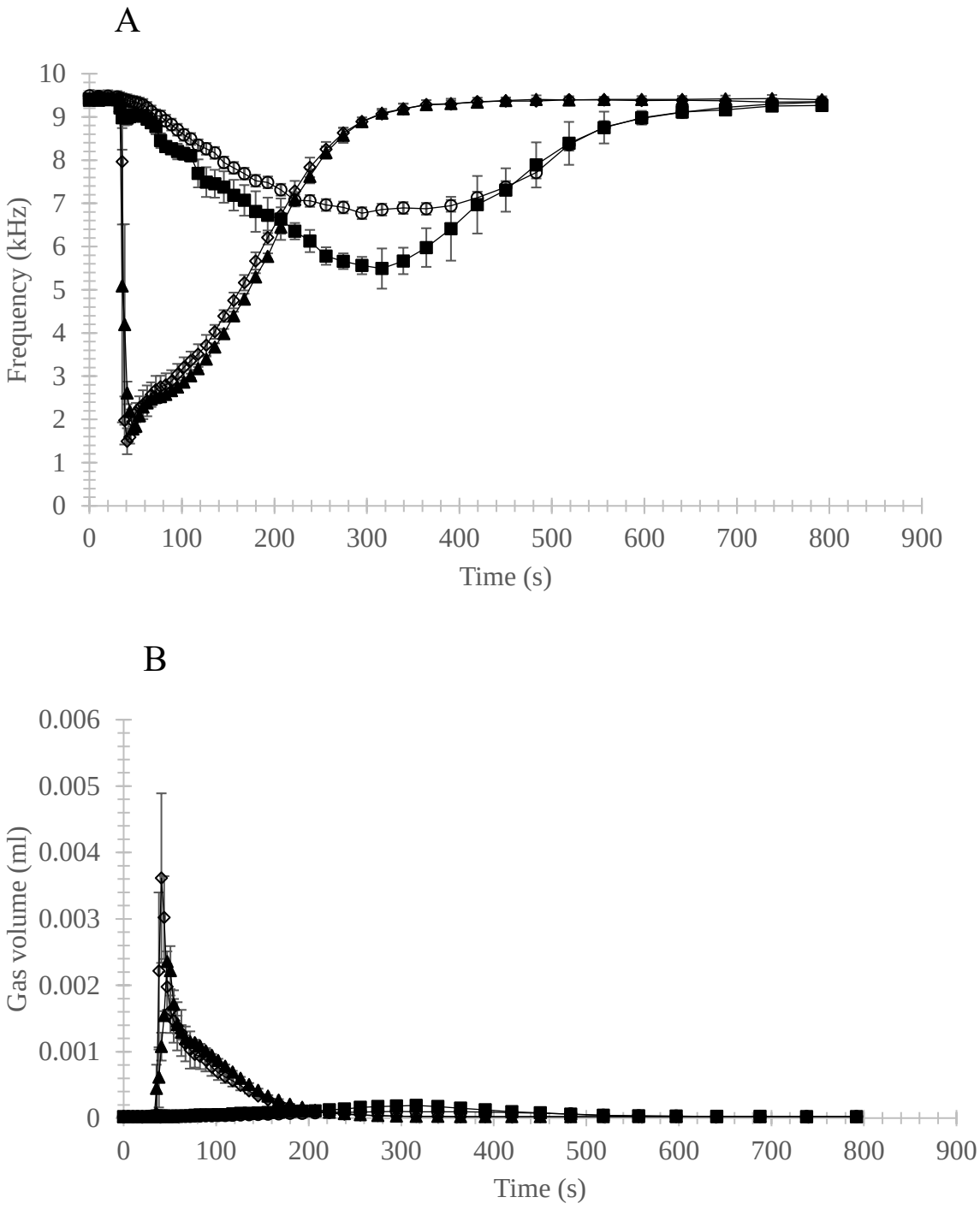


Figure 3.5 (A) BARDS frequency spectrum and (B) gas volume profile of Trial 3 Control plant (CP) —▲—, Agglomeration (AG) —◇—, Single mill (SM) —■— and Double mill (DM) —○— whey powder at 0.2% concentration

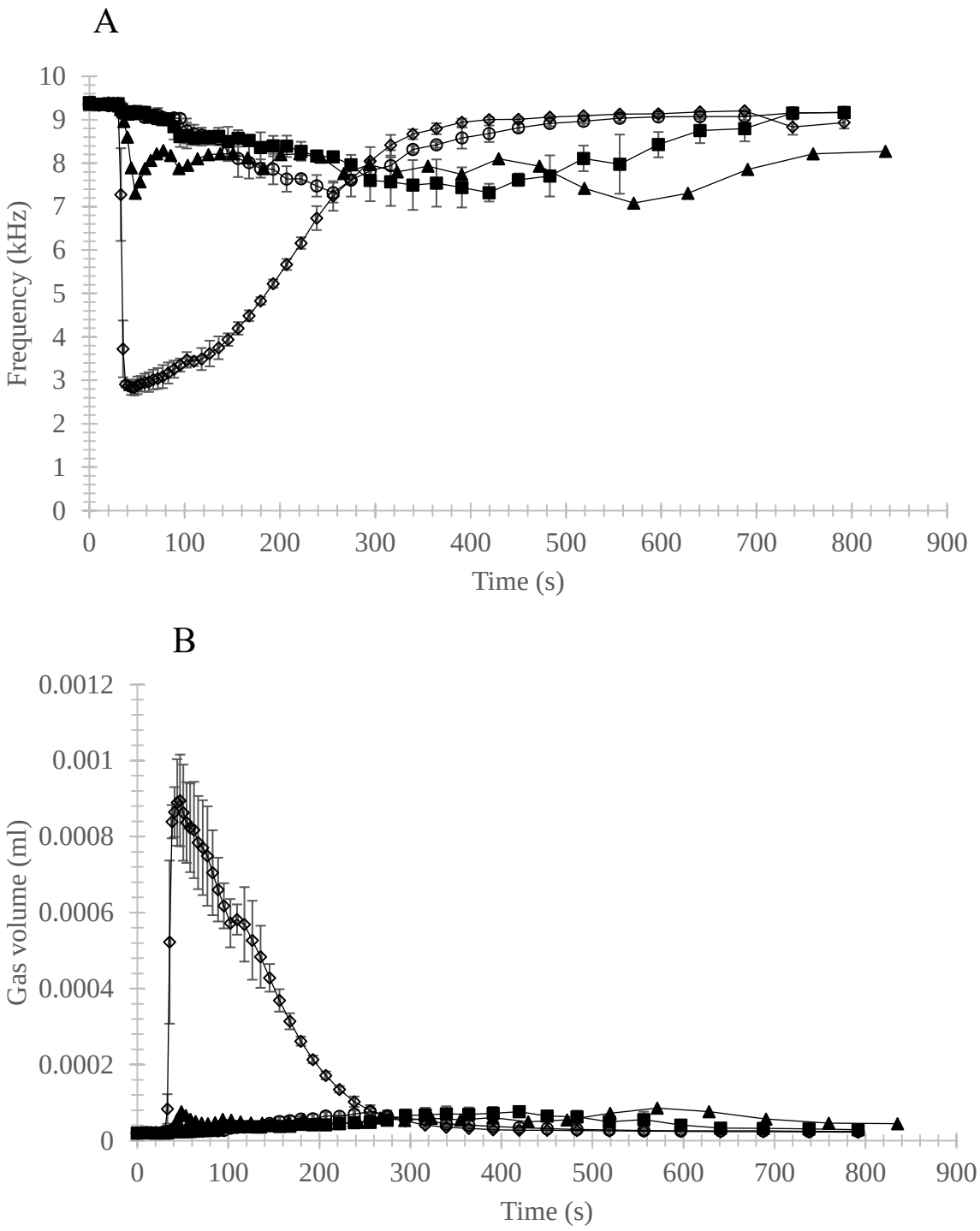


Figure 3.6 (A) BARDS frequency spectrum and (B) gas volume profile of Trial 3 Control plant (CP) —▲—, Agglomeration (AG) —◇—, Single mill (SM) —■— and Double mill (DM) —○— whey powder at 0.2% concentration

3.3.3. Protein profile, denaturation and aggregation

3.3.3.1 Protein profile

The protein profile of the whey powders from the three trials are displayed in the SDS-PAGE gel images in Figures 3.7 and 3.8. The main whey protein bands on the SDS gels from the three trials were identified as lactoferrin (LF), bovine serum albumin (BSA), IgG Heavy, β-lactoglobulin (β-lg) and α-lactalbumin (α-lac), as identified by comparison with the results of Gai *et al*. (2025) and their relative molecular weights.

3.3.3.1 Whey protein denaturation and aggregation

The CP powders from Trials 1, 2 and 3 are displayed on the SDS-PAGE gel image in Figure 3.7. There is little difference between the non-reducing bands (lanes 2-4) for the 3 trials. This indicates that across the dairy season, the change in whey protein profile isn't evident on the SDS gel image. The bands under reducing conditions, for the same powders are shown in lanes 5-7 on Figure 3.7. There are more protein bands evident in these lanes which indicates some whey protein denaturation had taken place during processing, in particular for the LF and BSA protein bands. Statistical analysis of the results of the denaturation of protein (Table 3.6) showed a significant difference ($p < 0.05$) between all trials, although this is not clear from the SDS gel in Figure 3.7 as the reducing lanes 5-7 look very similar. Typical SDS-PAGE gel images of the CP, AG, SM and DM rennet whey powders from the three trials are shown in Fig. 3.8. Fig. 3.8 A displays whey powders from Trial 1. There was little difference seen between the CP powder and the AG, SM and DM powders under non-reducing conditions (lanes 2-5) and the CP powder or the AG, SM and DM powders under reducing conditions (lanes 6-9). This suggests that the treatments applied to the whey powder (agglomeration and milling) had little effect on the protein profile of the whey powder and did not cause any

additional protein denaturation. Figure 3.8 C, which depicts Trial 3, also displayed a similar result.

Figure 3.8 B depicts the protein profiles for Trial 2. Under reducing conditions (lanes 6-9), it appeared the SM and DM powders had more extensive denaturation compared to the CP powder under the same conditions. Further denaturation is evident in the lactoferrin, bovine serum albumin (BSA) and IgG heavy chain bands in lanes 8 and 9 (SM and DM powder sample). Statistical analysis demonstrated that there was no significant difference ($p > 0.05$) between the CP sample and the AG and SM sample. However, there was a significant difference ($p < 0.05$) between the CP and DM sample, which may be due to the double milling process as mechanical denaturation may occur through milling, as reported by Mohammad *et al*. (2016). This was not clear from the SDS images for Trials 1 and 3 (Fig 3.8 A or C). However, these values can be reflected in Table 3.6, where there was a significant difference ($p < 0.05$) in the level of denaturation for DM powders when compared to their relevant CP powder.

Therefore, it can be concluded that the agglomeration process had little to no effect on whey protein denaturation. However, the more extensive milling process (DM) apparently caused some mechanical denaturation to the whey proteins in this study, like due to frictional heating during milling.

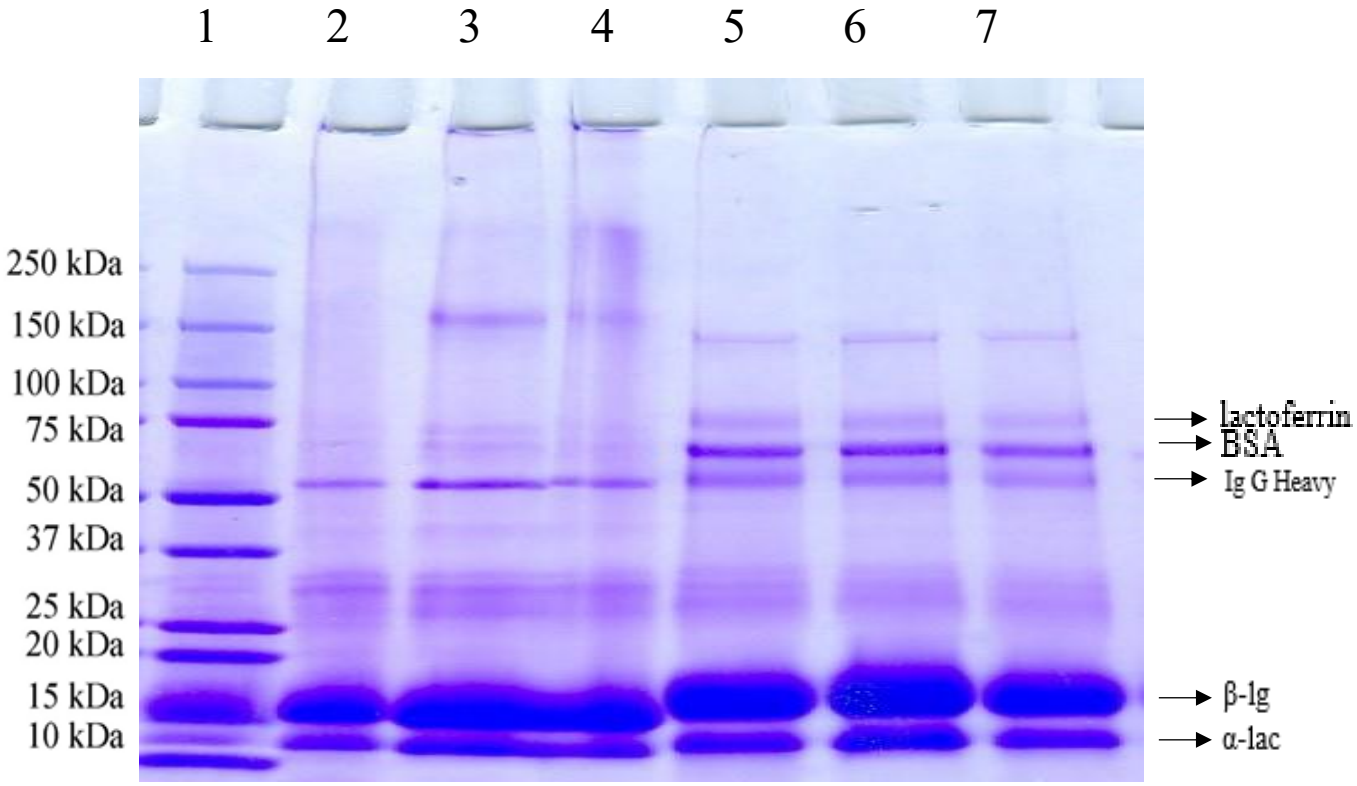


Fig 3.7: SDS-PAGE electrophoretograms of whey powder. Lane 1, protein standard; lane 2-4 Control plant (CP) powders Trial 1, Trial 2 and Trial 3 under non-reducing conditions; lane 5-7 Control plant (CP) whey powders Trial 1, Trial 2 and Trial 3 under reducing conditions.

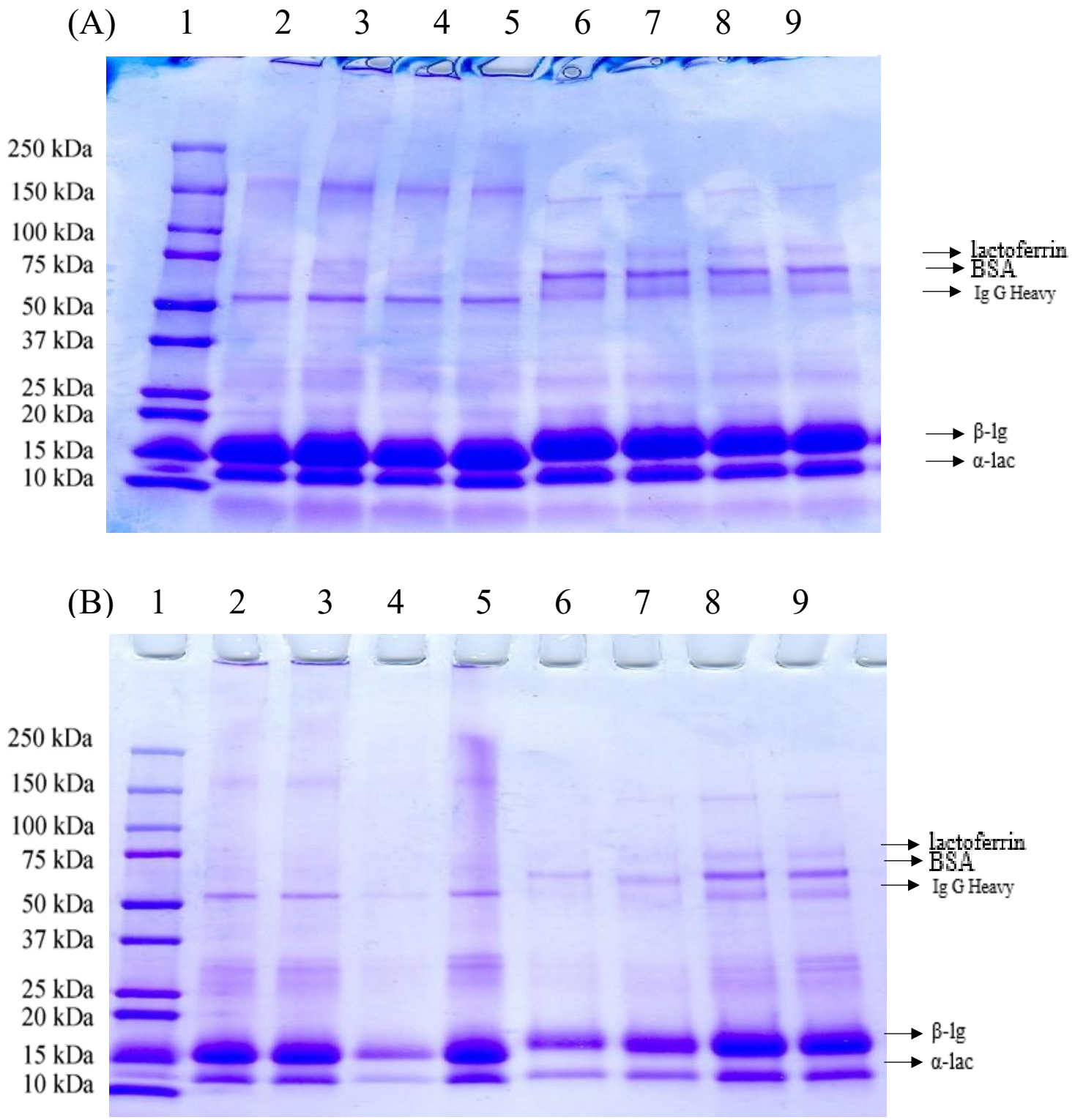


Fig 3.8: SDS-PAGE electrophoretograms of whey powder from Trial 1 (A), Trial 2 (B) and Trial 3 (C). Lane 1, protein standard; lanes 2-5, Control plant (CP), Agglomerated (AG), Single mill (SM) and Double mill (DM) powders under non-reducing conditions, lanes 6-9 Control plant (CP), Agglomerated (AG), Single mill (SM) and Double mill (DM) whey powders under reducing conditions.

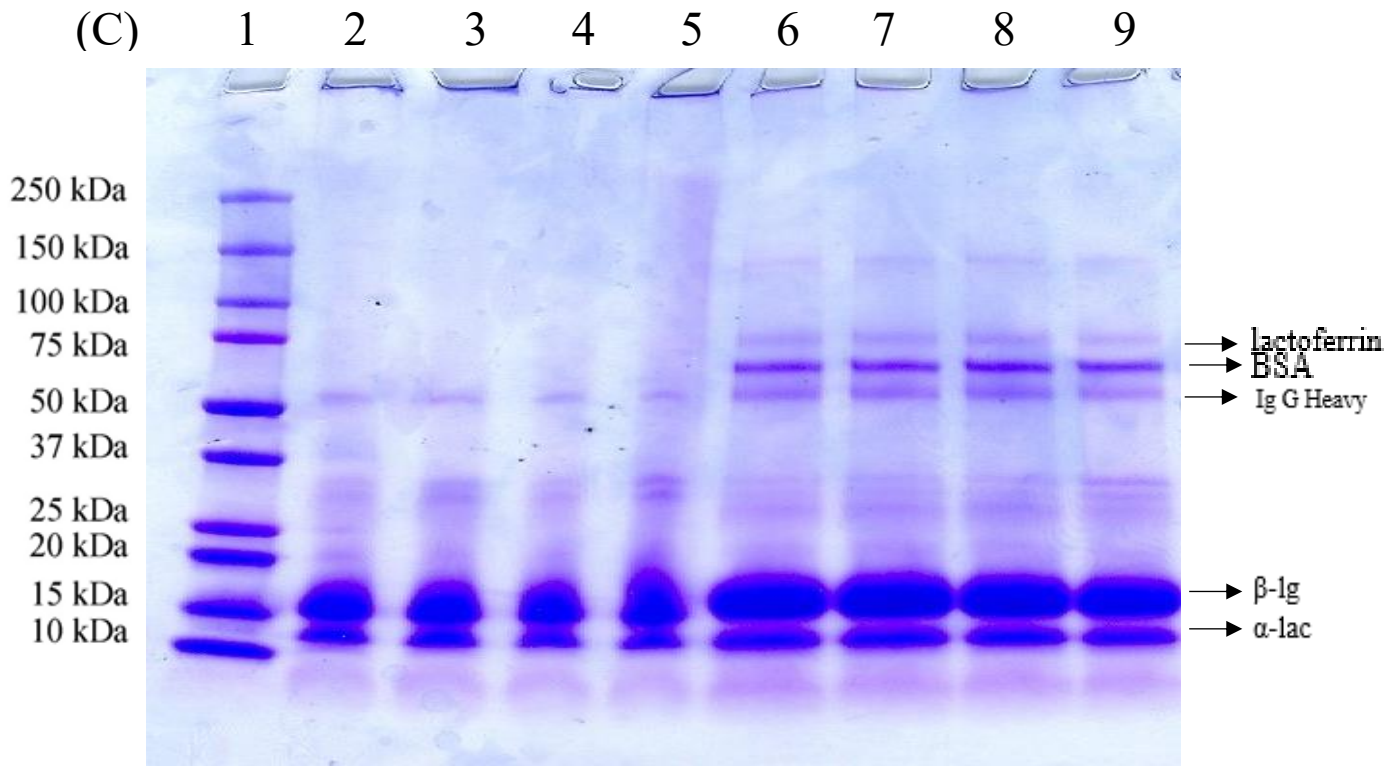


Fig 3.8: SDS-PAGE electrophoretogram cont.

3.3.3.2 Non-protein nitrogen contents

In general, apparently 25% of the total protein in whey powder consists of non-protein nitrogen (NPN) (Kelly and Kelly 1995). If the liquid whey is subjected to a membrane filtration concentration step, some of the NPN can be lost with the permeate. The non-protein nitrogen contents in the whey powder are displayed in Table 3.6, which is in agreement with what was reported by Kelly and Kelly (1995). Statistical analysis demonstrated no significant difference ($p > 0.05$) in NPN contents between the CP sample and AG, SM and DM samples, which indicates the processing treatments did not affect the content of NPN in the whey powder. However, a significant difference ($p < 0.05$) was demonstrated between trials. Ng-Kwai-Hang *et al*. (1985) reported higher NPN contents in late lactation milk; however, in this the highest NPN values were shown in Trial 2 (September) whey powder.

Table 3.6: The concentration (%) of total protein, denatured protein and non-protein nitrogen values of Control plant (CP), Agglomerated (AG), Single mill (SM) and Double mill (DM) whey powder. Denatured protein and Non-protein Nitrogen values are expressed as a % of total protein.

Treatment	Trial	Total Protein (%)	Denatured protein (% of total protein)	Non-protein Nitrogen (% of total protein)
	Trial 1	11.20 ± 0.02	13.80 ± 2.26	16.80 ± 0.59
CP	Trial 2	13.20 ± 0.11	20.40 ± 0.71	17.60 ± 0.58
	Tria1 3	16.30 ± 0.07	41.90 ± 0.33	14.80 ± 0.35
	Average	**13.50 ± 2.60**	**25.40 ± 14.70**	**16.40 ± 1.40**
	Trial 1	10.30 ± 0.08	13.00 ± 0.60	16.30 ± 0.62
AG	Trial 2	12.10 ± 0.06	21.10 ± 0.62	17.40 ± 0.02
	Tria1 3	13.00 ± 0.14	42.20 ± 0.70	14.50 ± 0.34
	Average	**11.80 ± 1.40**	**25.40 ± 15.10**	**16.10 ± 1.47**
	Trial 1	10.80 ± 0.08	14.70 ± 1.08	17.20 ± 0.11
SM	Trial 2	12.50 ± 0.04	19.80 ± 0.47	17.20 ± 0.44
	Tria1 3	16.80 ± 0.27	43.50 ± 0.94	14.10 ± 0.53
	Average	**13.40 ± 3.10**	**26.00 ± 15.40**	**16.10 ± 1.80**
	Trial 1	11.60 ± 0.06	18.10 ± 1.91	19.10 ± 0.01
DM	Trial 2	13.40 ± 0.13	22.40 ± 0.66	17.40 ± 0.70
	Tria1 3	15.80 ± 0.07	45.90 ± 1.53	14.40 ± 0.37
	Average	**13.60 ± 2.10**	**28.80 ± 15.00**	**17.00 ± 2.40**

3.3.4 Applications

3.3.4.1 Snack seasoning system

To fully understand the impact of a change in particle size of whey powder on selected applications, it was important to test the whey powder in a relevant application model. In this study, the whey powders were evaluated for performance in a topical snack seasoning system. The results showed that the AG powder seasoning sample had the largest amount of seasoning left in the bag after being shaken (residue in bag), and the DM powder seasoning sample had the least amount left. Statistical analysis of data for the quantities of residue in the bag demonstrated a significant difference ($p < 0.05$) between all samples. This trend can also be

reflected in the drop-off results, where the AG powder seasoning system gave a larger result of 0.92 ± 0.12 g compared to the CP powder seasoning system (0.74 ± 0.09 g). These results were aligned with what was demonstrated in a study by Ermis *et al*. (2009) which showed that a lower force was required to cause drop-off of larger particles from crisps and that seasoning with higher coarse particle fractions had higher levels of seasoning drop off from the snack, due to the larger particles being more loosely bound to the snack (Ermis *et al*., 2009). This study highlighted the impact of particle size in snack seasoning systems.

Table 3.7: The quantity of the snack seasoning system that did not adhere to the crisps (residue in bag) and dropped off the crisps (drop off) when screening whey powder of different particle size in a seasoning system. Control plant (CP), Agglomerated (AG) and Double mill (DM) whey powders were compared

Treatment	Residue in bag (g)	Drop off
CP	0.12 ± 0.02^{a}	0.74 ± 0.09^{ab}
AG	0.17 ± 0.02^{b}	0.92 ± 0.12^{a}
DM	0.00 ± 0.00^{c}	0.55 ± 0.14^{b}

Values in the same column not showing a common superscript are significantly different ($p<0.05$)

3.3.4.2 Beverage application

To screen the whey powders in another application, a beverage system was prepared using the CP, DM and AG whey powders as part of the system. The BARDS fundamental curve for these powders are displayed in Figure 3.9. Although the whey powder is used as an ingredient in the system, it is clear that there are no major differences in rehydration properties of different systems containing CP, AG and DM powder, as they all have a similar time at which the minimum frequency of the curve occurred (t_m) of approximately 50 s and also had similar

curves as they returned to steady state. It was interesting to note that as seen previously (Section 3.4.2.1), the DM powder had the largest minimum frequency *(f_{min}),* which could also be shown in Figure 3.9 as even within a beverage system, the DM powder system still had the largest f_{min} when compared to the beverage systems containing the CP and AG powders.

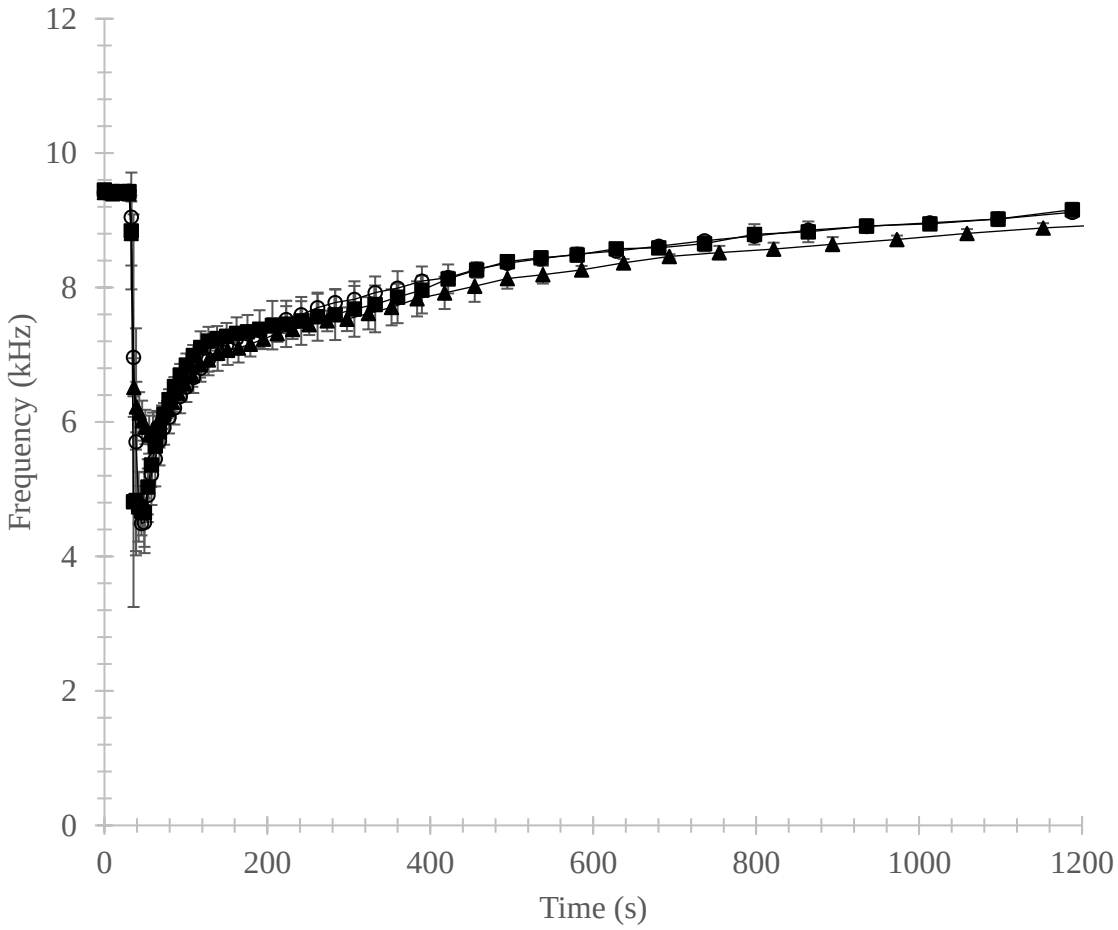


Fig 3.9: BARDS frequency spectrum of a beverage system containing Control plant (CP) —■— , Agglomerated (AG) —○— , and Double mill (DM) —▲— whey powder.

3.3.6 Shelf-life analysis

The CP whey powders from Trial 1 and Trial 2 was stored under three different conditions for a total of 12 weeks to evaluate how storage conditions and time affect colour and rehydration properties of the whey powder.

Table 3.8 shows the L*A*B* colour values and ΔE values for the whey powders throughout the twelve weeks of storage . As expected, in both Trial 1 and Trial 2, the ΔE values were largest for the CP powder held under the hot storage conditions for a total of twelve weeks with values of 9.04 and 4.56, respectively. When comparing the Trial 1 and Trial 2 powders stored under the hot conditions for 12 weeks with the respective powder's initial LAB values, the L* value decreased, which indicates that the powder got darker in colour, while the a* and b* values increased, implying that the powder is became more red/yellow in colour.

The combinations of these changes results in browning of the powder, which is a characteristic of aging of sweet whey powder (Patel *et al*., 2013; Norwood *et al*., 2017) and is most likely due to the Maillard reaction. The Maillard reaction is a three-stage chemical reaction that occurs between amino acids and reducing sugars (lactose) in dairy products (Zhou and Langrish, 2022). The result of this reaction is a change in colour due to the formation of high molecular weight nitrogenous brown pigments known as melanoidins (Zhou and Langrish, 2022). This change in colour can happen during processing when high temperatures are being used, but it can also happen in storage (Li *et al*., 2019), as seen in this study. It is clear that the "Hot" storage conditions accelerated the Maillard reaction as the largest ΔE values were demonstrated for those powders stored in this condition (Patel *et al*., 2013). Over the twelve

weeks, the colour of the powders stored in the "Ambient" and "Cold" conditions did not change greatly as the ΔE remained <3. However, statistical analysis demonstrated that, for all L*, a* and b* values, under all storage times and conditions, a significant difference ($p < 0.05$) was shown between all samples.

The rehydration properties of these powders were also analysed; Figures 3.10 and 3.11 display the BARDS fundamental curves for each powder after being stored under the respective conditions. It is clear from these plot, there is no major change in the rehydration properties of the powders with varying storage conditions over the twelve week period as they follow similar rehydration profiles. Conversely, it has been demonstrated that Maillard browning has been associated with a decrease in solubility of powders due to the formation of melanoidin molecules which are higher in molecular weight (Patel *et al*., 2013). This change in solubility was not demonstrated in this study; however; with increasing Maillard browning/ storage times, the change in solubility of the whey powder may be more evident.

Table 3.8: Commission Internationale d'Eclairage (CIE) colour space and ΔE values of the Control plant (CP) whey powder for Trial 1 and Trial 2 stored under three different environments over a period of 12 weeks.

Trial	Time	Ambient (10 ± 5°C)			ΔE	Cold (4°C)			ΔE	Hot (40°C/ 40%RH)			ΔE
	(weeks)	L*	a*	b*		L*	a*	b*		L*	a*	b*	
1	0	93.50 ± 0.14	-5.14 ± 0.05	18.80 ± 0.13	-	93.50 ± 0.14	-5.14 ± 0.05	18.80 ± 0.13	-	93.50 ± 0.14	-5.14 ± 0.05	18.80 ± 0.13	-
1	6	93.20 ± 0.01	-4.79 ± 0.02	18.50 ± 0.05	0.55	93.30 ± 0.05	-4.96 ± 0.04	18.70 ± 0.04	0.29	91.20 ± 0.06	-2.65 ± 0.02	19.50 ± 0.09	3.46
1	12	93.30 ± 0.05	-4.85 ± 0.00	18.90 ± 0.03	0.37	93.30 ± 0.14	-4.98 ± 0.04	18.90 ± 0.06	0.28	86.40 ± 0.17	0.09 ± 0.01	20.80 ± 0.10	9.04
2	0	93.70 ± 0.17	-5.63 ± 0.03	19.60 ± 0.08	-	93.70 ± 0.17	-5.63 ± 0.03	19.60 ± 0.08	-	93.70 ± 0.17	-5.63 ± 0.03	19.60 ± 0.08	-
2	6	92.70 ± 0.42	-5.16 ± 0.05	18.80 ± 0.12	1.36	93.60 ± 0.12	-5.25 ± 0.04	18.80 ± 0.05	0.89	92.10 ± 0.19	-4.34 ± 0.07	19.10 ± 0.08	2.11
2	12	93.80 ± 0.19	-5.32 ± 0.03	19.10 ± 0.03	0.60	93.90 ± 0.06	-5.52 ± 0.09	19.70 ± 0.21	0.55	90.80 ± 0.08	-2.18 ± 0.02	20.30 ± 0.06	4.56

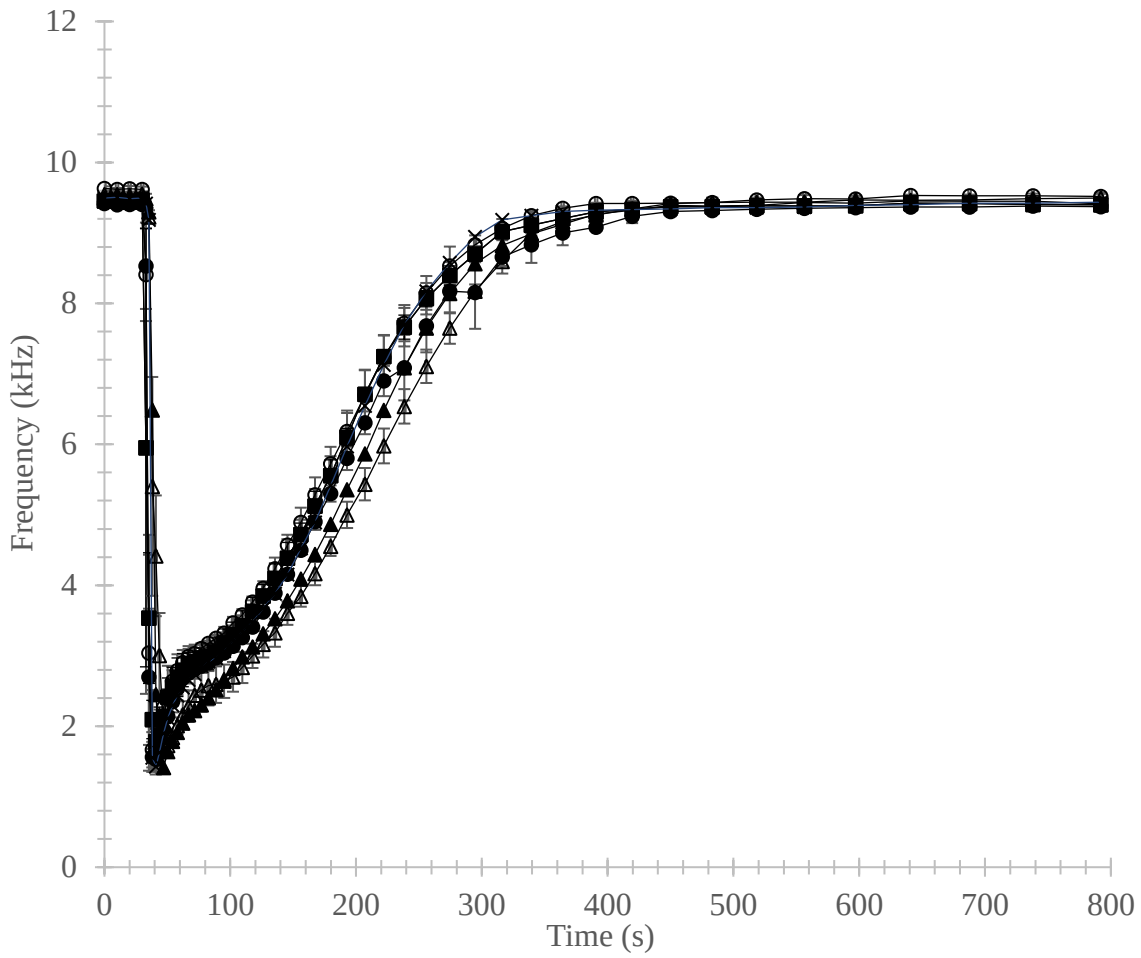


Figure 3.10 BARDS frequency spectrum of Trial 1 Control (CP) week 0, CP Ambient week 6, CP Cold week 6, CP Hot week 6, CP Ambient week 12, CP Hot week 12 and CP Cold whey powder week 12 at 0.2% concentration

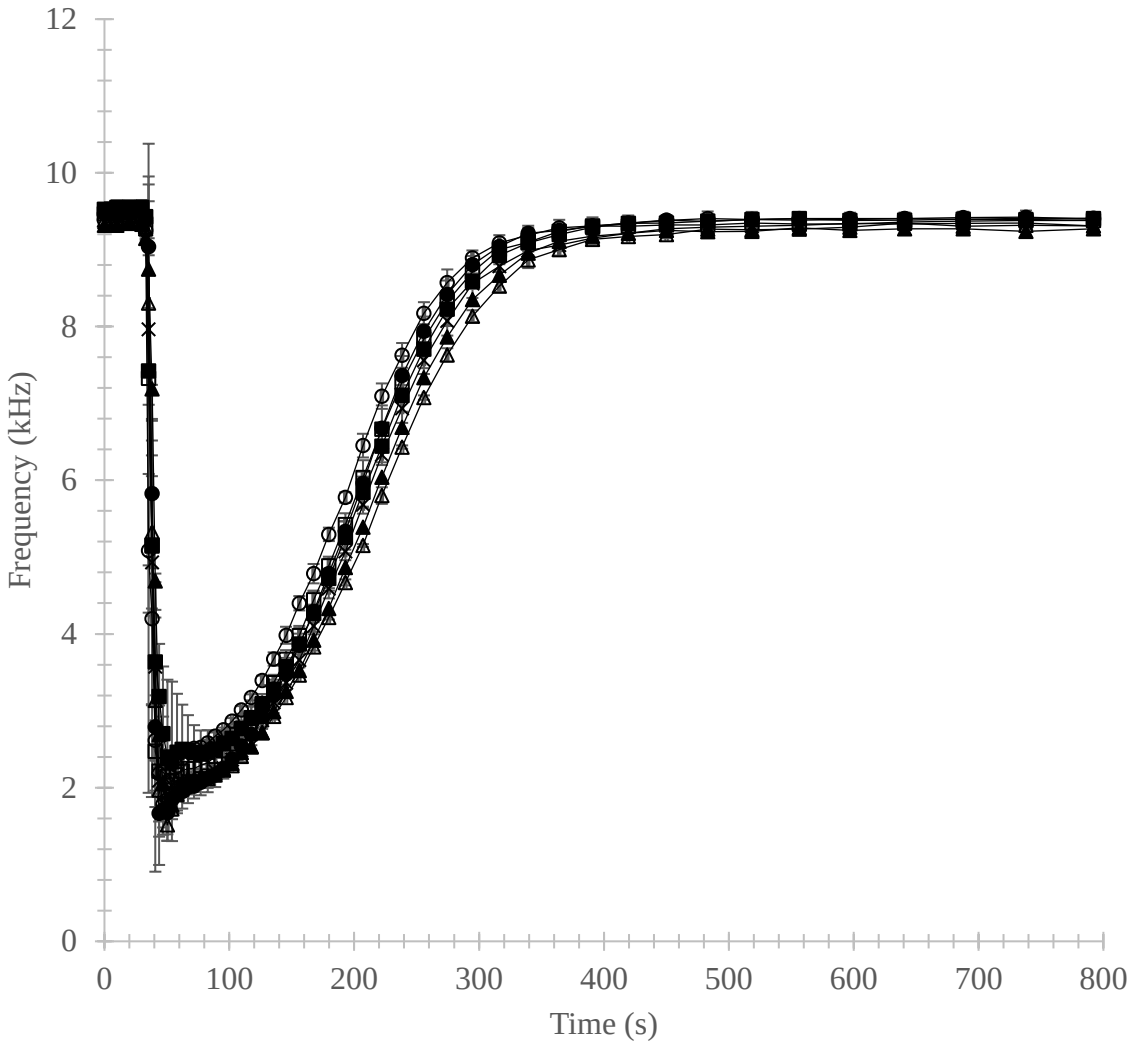


Figure 3.11 BARDS frequency spectrum of Trial 2 Control (CP) week 0, CP Ambient week 6, CP Cold week 6, CP Hot week 6, CP Ambient week 12, CP Hot week 12 and CP Cold whey powder week 12 at 0.2% concentration

3.4 Conclusions

The objective of this study was to investigate the impact of altering the particle size of whey powder post-drying on the physical and functional properties of the powder. The results from this study clearly show that the particle size of the whey powder can be altered through fluidized bed agglomeration (AG) and milling processes (SM and DM). Agglomeration resulted in an increase in particle size, while milling reduced the powder particle size. In addition to the particle size being affected, other physiochemical properties of the AG, SM and DM powders were also influenced including particle shape, morphology, flowability density and colour. Changes in the characteristics of whey powders can present challenges for both manufacturers and consumers. For instance, poor flowability (associated with the SM and DM powders) may lead to mechanical caking and difficulties in transporting the powder during processing operations (Ozel et al., 2022). In many whey powder applications, such as in RTM beverages, solubility is critical—if the powder does not rehydrate well (SM and DM powders), it can negatively affect key quality attributes of the final product for the consumer, such as appearance, texture, and flavour (Morr and Ha, 1993; Calton et al., 2019). This study provides dairy researchers and processors with deeper scientific insight into how modifying whey powder particle size after drying influences its physical and functional properties, and how these changes impact its suitability for specific applications.

3.5 References

Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Cappelli, F.P. and Calamari, L. (2015). Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science*, **98 (3)**, pp.1815-1827.

Bronsveld, E. (2015). Milk and Whey Powder In: Bylund, G. (2015). *Dairy processing Handbook.* 5th ed. available at: https://dairyprocessinghandbook.tetrapak.com/chapter/milk-and-whey-powder [Accessed on the 5th January 2025]

Crowley, S. V., Gazi, I., Kelly, A.L., Huppertz, T., and O'Mahony, J.A. (2014). Influence of protein concentration on the physical characteristics and flow properties of milk protein concentrate powders. *Journal of Food Engineering,* **135**, 31–38.

Dhanalakshmi, K., Ghosal, S. and Bhattacharya, S. (2011). Agglomeration of food powder and applications. *Critical Reviews in Food Science and Nutrition*, **51 (5)**, pp. 432-441.

Ermis, E., Farnish, R.J., Berry, R.J. and Bradley, M.S.A. (2009). Direct measurement of powder flavor adhesion onto crisp surface using a novel adhesion tester. *Particulate Science and Technology*, **27 (4)**, pp. 362-372.

Fitzpatrick, J.J., Barry, K., Cerqueira, P.S.M., Iqbal, T., O'Neill, J. and Roos, Y.H. (2007). Effect of composition and storage conditions on the flowability of dairy powders. *International Dairy Journal*, **17 (4)**, pp.383-392.

Fitzpatrick, J.J., Iqbal, T., Delaney, C., Twomey, T. and Keogh, M.K. (2004). Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. *Journal of food Engineering*, **64 (4)**, pp. 435-444.

Fu, X., Huck, D., Makein, L., Armstrong, B., Willen, U. and Freeman, T. (2012). Effect of particle shape and size on flow properties of lactose powders. *Particuology*, **10 (2)**, pp.203-208.

Gai, N., Uniacke-Lowe, T., O'Regan, J., Goulding, D.A. and Kelly, A.L. (2025). Influence of β-lactoglobulin and κ-casein genotype on whey protein denaturation in heated bovine milk and rennet whey. *International Dairy Journal*, **160**, p.106107.

Gaiani, C., Boyanova, P., Hussain, R., Pazos, I.M., Karam, M.C., Burgain, J. and Scher, J. (2011). Morphological descriptors and colour as a tool to better understand rehydration properties of dairy powders. *International Dairy Journal*, **21 (7)**, pp.462-469.

Gao, W., Chen, F., Wang, X. and Meng, Q. (2020). Recent advances in processing food powders by using superfine grinding techniques: A review. *Comprehensive Reviews in Food Science and Food Safety*, **19 (4)**, pp. 2222-2255.

Gausemel, Even & Schüller, Reidar & Rukke, Elling-Olav & Johansen, Anne-Grethe & Liland, Kristian & Jørgensen, Camilla. (2022). The Effect of Sampling Methods on Particle Size Distribution in Dry Whey Protein Concentrate (WPC). In: *Nordic Rheology Conference.* Reykjavik, Iceland, 8-10 August 2022. Norway: University of Stravanger.

Ghidouche, S., Rey, B., Michel, M. and Galaffu, N. (2013). A Rapid tool for the stability assessment of natural food colours. *Food chemistry*, **139 (1-4)**, pp.978-985.

Haas, K., Obernberger, J., Zehetner, E., Kiesslich, A., Volkert, M. and Jaeger, H. (2019). Impact of powder particle structure on the oxidation stability and color of encapsulated crystalline and emulsified carotenoids in carrot concentrate powders. *Journal of Food Engineering*, **263**, pp.398-408.

Hazlett, R., Schmidmeier, C. and O'Mahony, J.A., (2021a). Influence of mechanical integrity during pneumatic conveying on the bulk handling and rehydration properties of agglomerated dairy powders. *Journal of Food Engineering*, **288**, p.110103.

Hazlett, R., Schmidmeier, C. and O'Mahony, J.A. (2021b). Approaches for improving the flowability of high-protein dairy powders post spray drying– A review. *Powder Technology*, **388**, pp.26-40.

Kaialy, W. (2016). A review of factors affecting electrostatic charging of pharmaceuticals and adhesive mixtures for inhalation. *International Journal of Pharmaceutics*, **503 (1-2)**, pp.262-276.

Kelly, J. and Kelly, P. (1995). Nanofiltration of whey: quality, environmental and economic aspects. *International Journal of Dairy Technology*, **48 (1)**, pp.20-24.

Li, Y.H., Wang, W.J., Guo, L., Shao, Z.P. and Xu, X.J. (2019). Comparative study on the characteristics and oxidation stability of commercial milk powder during storage. *Journal of Dairy Science*, **102 (10)**, pp.8785-8797.

Mohammad, A.W., Teow, Y.H., Ang, W.L., Chung, Y.T., Oatley-Radcliffe, D.L. and Hilal, N. (2015). Nanofiltration membranes review: Recent advances and future prospects. *Desalination*, **356**, pp.226-254.

Mohammad, M.A., Grimsey, I.M., Forbes, R.T., Blagbrough, I.S. and Conway, B.R. (2016). Effect of mechanical denaturation on surface free energy of protein powders. *Colloids and Surfaces B: Biointerfaces*, **146**, pp.700-706.

Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley, J.E. and Monardes, H.G. (1985). Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. *Journal of Dairy Science*, **68 (5)**, pp.1257-1262.

Norwood, E.A., Pezennec, S., Burgain, J., Briard-Bion, V., Schuck, P., Croguennec, T., Jeantet, R. and Le Floch-Fouéré, C. (2017). Crucial role of remaining lactose in whey protein isolate powders during storage. *Journal of Food Engineering*, **195**, pp.206-216.

O'Donoghue, L.T., Haque, M.K., Kennedy, D., Laffir, F.R., Hogan, S.A., O'Mahony, J.A. and Murphy, E.G. (2019). Influence of particle size on the physicochemical properties and stickiness of dairy powders. *International Dairy Journal*, **98**, pp.54-63.

Ozel, B., McClements, D.J., Arikan, C., Kaner, O. and Oztop, M.H. (2022). Challenges in dried whey powder production: Quality problems. *Food Research International*, **160**, p.111682.

Pan, K., Song, Q., Wang, L. and Cao, B. (2011). A study of demineralization of whey by nanofiltration membrane. *Desalination*, **267 (2-3)**, pp.217-221.

Patel, K.N., Modi, R.B., Patel, H.G. and Aparnathi, K.D. (2013). Browning, its chemistry and implications in dairy products: A review. *Indo-American Journal of Agricultural and Veterinary Sciences*, **1**, pp.1-12.

Saito, Z. (1988). Lactose crystallization in commercial whey powders and in spray-dried lactose. *Food Structure*, **7 (1)**, p.9.

Salunke, P., Syamala, A. and Metzger, L.E. (2023). Microstructural Characterization of High-Protein Dairy Powders. *Dairy*, **4 (3)**, pp.462-481.

Saw, H.Y., Davies, C.E., Paterson, A.H. and Jones, J.R. (2013). The influence of particle size distribution and tapping on the bulk density of milled lactose powders. In: *Proceedings of CHEMECA 2013 Conference,* Brisbane, Australia 29th September- 2nd October 2013. Australia: Engineers Australia.

Sharma, A., Jana, A.H. and Chavan, R.S. (2012). Functionality of milk powders and milk-based powders for end use applications—a review. *Comprehensive Reviews in Food Science and Food Safety*, **11 (5)**, pp.518-528.

Wu, S., Fitzpatrick, J., Cronin, K., Ahmed, M.R., Fitzpatrick, D. and Miao, S. (2019). Application of broadband acoustic resonance dissolution spectroscopy (BARDS) to the gas release behaviour during rehydration of milk protein isolate agglomerates. *Journal of Food Engineering*, **253**, pp.14-20.

Wu, S., Cronin, K., Fitzpatrick, J. and Miao, S. (2022). Updating insights into the rehydration of dairy-based powder and the achievement of functionality. *Critical Reviews in Food Science and Nutrition*, **62 (24)**, pp.6664-6681.

Zhou, Z. and Langrish, T. (2022). Color formation and Maillard reactions during the spray drying process of skim milk and model systems. *Journal of Food Process Engineering*, **45 (1)**, p.13936.

Chapter 4

General Discussion and Conclusions

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Declaration

This chapter was written by author Emma J. Gordon (E.J.G.) and reviewed by all co-authors.

4.1 General Discussion and Conclusions

The demand for whey powder is continually increasing, due to its cost-effectiveness and versatility. Whey powder is used in a wide range of food products, including animal feed, soups, bakery goods, confectionery, powdered beverages, snack seasonings, and dairy products (Adamson, 2015). Dairy scientists and processors are constantly working to optimize the physicochemical properties and functionality of dairy powders to provide more tailored and targeted characteristics for end-users and, achieve optimal quality attributes of finished product qualities. In the two approaches explored in this thesis, whey powder can undergo pre- and post-drying treatments to modify these properties for specific end uses.

The first main aim of the work presented in this thesis was to investigate if in-line shearing of pre-crystallised whey concentrate was effective in reducing particle size and increasing homogeneity of resultant powders, to better control and/or predict the functionality of whey powder. Thus, Chapter 2 focused on applying established technologies, to shear the precrystallised whey concentrate to different extents, in producing three powders with different particle sizes ranges. Shearing the whey concentrate pre-drying reduced the particle size of the resultant powders and altered the morphology, particle shape, colour, density, and levels of protein denaturation in the whey powder.

Overall, the findings from this chapter identified an innovative approach to reduce the particle size of whey powder by targeting the concentrate pre-drying, by applying an in-line shearing treatment to the crystallised whey concentrate. The impact of this original research is the identification of an approach which can be used to control the particle size of whey powder, using an in-line process, without negatively affecting the physicochemical and functional

characteristics of regular whey powder. This process could be applied to produce whey powders that require a particular particle size distribution profile for a specific application, such as in a snack seasoning system. For example, it has been seen in previous studies, and also in Chapter 3, that smaller powder particles have better adhesion properties (Ermis *et al*., 2009). In the snack seasoning industry, it is essential that the seasoning adheres sufficiently as, if not, it can cause financial losses for the company and unsatisfied consumers due to poor flavour performance (Ermis *et al*., 2009).

Following on from the work completed in Chapter 2, Chapter 3 focused on the impact of altering the particle size post-drying on the physicochemical and functional properties of whey powder. The results of this study demonstrated that agglomeration (AG) using a pilot-scale fluid bed dryer was successful in achieving an increase in particle size, while milling (SM and DM) reduced the particle size. Agglomerating this powder resulted in differences in composition, particle size, morphology, colour, density and flowability. When the agglomerated powder was used as part of a seasoning blend in a snack application, it demonstrated higher levels of drop off of the seasoning from the crisps when compared with the control. This is likely due to weaker adhesion forces associated with the larger whey powder particles. The physicochemical and functional properties of SM and DM powder, i.e., particle size, particle shape, morphology, colour, density, flowability, rehydration profile and protein denaturation levels differed from those of the control. In particular, the flowability and rehydration properties of the DM powder were negatively affected as the powder became more cohesive and the rehydration process was slower. This change in physicochemical and functional properties of these two powders could cause issues for manufacturers during processing and for consumers. For example, poor flowability can contribute to issues with mechanical caking and problems with transporting powder during processing operations (Ozel

et al., 2022). In many applications of whey powder, such as sweetened condensed milk or a beverage system, the ability of whey powder to dissolve in solution is essential, and if rehydration ability of the powder is poor, this can have detrimental effects on the finished product quality attributes such as appearance, texture and flavour (Morr and Ha, 1993; Calton *et al*., 2019).

However, the DM powder performed best in a snack seasoning system, as there was the lowest amount of drop-off, which indicated that this powder had the best adhesion properties. The study conducted in Chapter 3 also highlighted the importance of appropriate storage conditions of whey powders. High temperatures and relative humidity promote Maillard browning in whey powder which can impact the colour of the product (Patel *et al*., 2013; Norwood *et al*., 2017); however, our analysis showed that this did not negatively affect the rehydration properties of the powder.

Overall, the results of this study indicate that the powder particle size can be altered post-drying, using agglomeration and milling technologies, and that these treatments subsequently affect the physicochemical and rehydration properties of the powder. The agglomeration process is the optimal way of altering the particle size distribution of the powder as it did not negatively affect physicochemical or rehydration properties. The findings from this chapter will help with selection of post-drying treatments for whey powders and adds to the understanding of how they will ultimately affect the physiochemical and rehydration properties of whey powder.

The results presented in this study thus show that the particle size of whey powder can not only be altered post-drying but can be reduced pre-drying by performing a shearing treatment on the crystallised whey concentrate.

When comparing findings of Chapter 2 and 3, more extensive modification to the physicochemical and functional properties were clearly achieved using the post-drying treatments (Chapter 3). However, in terms of feasibility for industry, the in-line shearing process may be the most practical. As it is an in-line process, there is less disruption to the whey powder production process and less concern regarding microbial contamination as integrating this technology would simply involve including an additional in-line pump/shear device into the process line. In comparison, the post-drying treatments lead to greater risks for microbial growth, as such treatments would be implemented after any of the typical thermal treatments. After the milling process, it was shown that the moisture levels were slightly higher compared to the control sample, which indicates that the milling process would most likely have to be completed in an environmentally controlled room which may not be feasible in a factory environment. As well as this, the milled powders could prove to be difficult to convey in the factory due to the reduced flowability. The agglomeration process may be achieved using established, traditional technology widely used today in spray dryers; however, most dryers are limited in the volume of fine powder that can be returned for the agglomeration process and expanding this would be a costly and complicated process. For these reasons, the in-line shearing pre-drying treatment would most likely be the more feasible for an industry process line.

Overall, these findings give dairy scientists and processors a greater insight into the effect of altering physicochemical properties of the whey powder and how in turn these altered properties can subsequently affect many other properties of the powder. It also emphasizes the importance of tailoring the powder's properties to the end-use application to obtain the optimal product.

4.2 Suggestions for future research

From the findings of the studies conducted in this thesis, it is evident that the area of pre- and post-drying alterations to powders requires further research to fully understand the implications of the treatments applied on the subsequent whey powders produced. Suggestions for future research topics to complement this body of work are as follows:

- Application work could be completed using the whey powder that was produced following the in-line treatments. As previously mentioned, whey powder has a wide range of applications from snack seasoning to soups. It would thus be useful to implement these whey powders into a variety of applications and identify for which application they perform best, compared with the control whey powder;
- Chapter 3 showed how agglomeration or milling may help reduce segregation in whey powders upon storage. This could be tested by sampling and testing the composition of these treated powders during storage under simulated industrial conditions to understand if these treatments aid in reducing the segregation of the particles and creating a more homogenous, uniform powder;
- It was also demonstrated that the milling process may cause some mechanical denaturation of the proteins. Further research could be completed to understand this mechanical denaturation and to investigate what implications, if any, this would have

on the functionally of the powder in applications. This milling process could be compared to a cryomilling process where the powders would be milled at low temperature which allows them to fracture more easily and may prevent the mechanical denaturation from occurring (Gao *et al*., 2020);

- A shelf life study could be carried out on the powder generated in each chapter (in Chapter 3 shelf life of the control powders only was studied). As the concentrate is being sheared prior to drying (Chapter 2), this could subsequently affect the stability of the lactose crystals upon storage which may result in having a powder that is less stable (i.e., in terms of susceptibility to caking) because of this impact on lactose crystals. This was also seen when milling the powder, whereby the shearing of lactose crystals may cause issues upon storage, a topic that was not addressed in this thesis. Shelf-life analysis of the physicochemical and rehydration properties of the powders could thus be conducted at intervals after the powder has been subjected to different storage conditions to fully understand this aspect of the potential use of the powder and technologies discussed;

4.3 References

Calton, A., Ma, H., Nordlund, E., Poutanen, K. and Sozer, N. (2019). Instant properties of ingredients used for point of consumption production of high-moisture food structures selectively fortified with protein and dietary fibre. *Journal of Food Engineering*, **263,** pp.204-212.

Ermis, E., Farnish, R.J., Berry, R.J. and Bradley, M.S.A. (2009). Direct measurement of powder flavor adhesion onto crisp surface using a novel adhesion tester. *Particulate Science and Technology*, **27 (4)**, pp.362-372.

Gao, W., Chen, F., Wang, X. and Meng, Q. (2020). Recent advances in processing food powders by using superfine grinding techniques: A review. *Comprehensive Reviews in Food Science and Food Safety*, **19 (4)**, pp. 2222-2255.

Morr, C.V. and Ha, E.Y.W. (1993). Whey protein concentrates and isolates: processing and functional properties. *Critical Reviews in Food Science & Nutrition*, **33 (6)**, pp.431-476.

Norwood, E.A., Pezennec, S., Burgain, J., Briard-Bion, V., Schuck, P., Croguennec, T., Jeantet, R. and Le Floch-Fouéré, C. (2017). Crucial role of remaining lactose in whey protein isolate powders during storage. *Journal of Food Engineering*, **195**, pp.206-216.

Ozel, B., McClements, D.J., Arikan, C., Kaner, O. and Oztop, M.H. (2022). Challenges in dried whey powder production: Quality problems. *Food Research International*, **160**, p.111682.

Patel, K.N., Modi, R.B., Patel, H.G. and Aparnathi, K.D. (2013). Browning, its chemistry and implications in dairy products: A review. *Indo-American Journal of Agricultural and Veterinary Sciences*, **1**, pp.1-12.