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INFLUENCE OF SPRAY DRYER AIR OUTLET TEMPERATURE AND NOZZLE ORIFICE DIAMETER ON CHARACTERISTICS OF HIGH-PROTEIN MILK PROTEIN CONCENTRATE (MPC)

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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1999

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ABSTRACT

The main goal of this research was to determine how spray drying conditions can affect the formation of aggregates in the manufacture of high protein (70%) Milk Protein Concentrates (MPCs). MPCs are produced by dehydration of retentates from ultrafiltering/diafiltering skim milk. These powders are sold for their functional properties as food ingredients.

Results are divided into two major parts: basic functionality of commercial MPC samples and characteristics of MPCs produced under controlled spray drying conditions. Analyses performed on basic functional characteristics of commercial samples showed a wide variation in all the variables studied, but no statistically significant correlation among them. This wide variability in functionality limits the marketability of MPCs.

Since the processing and storage histories of the commercial samples was unknown, MPCs were produced under controlled spray drying conditions. Two factors were varied: air outlet temperature and nozzle orifice diameter. An air inlet temperature of 200°C and a nozzle orifice of 0.8 mm were used for drying all the MPCs while air outlet temperature was set to 65°, 70°C, 75°, 85°C and 90°C. The samples dried at 75°C air outlet temperature were also dried with nozzles of 0.5 and 1.3 orifice diameter.
Evaluation of the spray dried MPCs show that increasing the air outlet temperature could induce the formation of large particles that had a much slower rate of hydration. This effect was evident between 75° and 85°C air outlet temperature. Both hydrophobic interactions and disulfide linkages seem to play a role in the formation of these large particles.

Varying the air outlet temperature also resulted in changes in moisture content and, therefore, in water activity of the powders. These differences were found to be important for the storage quality of the powders. MPCs dried at lower air outlet temperatures were more prone to non-enzymatic browning due to their higher water activity. Browning reactions resulted in molecular polymerization, as shown by SDS-PAGE.

Varying the nozzle diameter did not produce significant changes in the powder characteristics under the specific conditions used (75°C air outlet temperature and 200°C air inlet temperature).

Foaming of a 5% protein solution and Emulsion Volume Index (EVI) of a 3% protein, 10% o/w emulsion, did not show noticeable differences due to drying the powders at different temperatures. However, differences in their hydration characteristics suggest that the MPCs dried at low and high air outlet temperatures could perform differently when used as ingredients in foods.
ACKNOWLEDGMENTS

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Four companies provided most of the commercial samples of milk protein concentrates that were analyzed in the first phase of this research project. These were: New Zealand Milk Products; Main Street Foods, DMV and Avonmore.

I would like to acknowledge the valuable help from Main Street Foods, for supplying raw materials and allowing me to use their spray drying facilities to produce milk protein concentrates under controlled conditions. Special thanks to Phil Rakes for his continuous cooperation and insights.

All the members from my committee provided very helpful advice and suggestions. Dr. Michael E. Mangino’s help with milk protein chemistry was most valuable.
My labmates, especially Kwok Man Lee and C. Josephine Kuo, generously helped me at some point with some of the laboratory techniques. I also wish to express my gratitude to Charles (Chip) Pretzman, from the Animal Science Department, for his valuable guidance with electrophoresis techniques.

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CHAPTER 1

INTRODUCTION

The consumers' acceptance of foods is based largely on their sensory characteristics, namely color, flavor (including taste, smell and trigeminal sensations) and texture. Texture constitutes one of the most important aspects within the sensory profile of a food. Certain ingredients are included in a food product solely or mostly because they enhance the sensory acceptance of the food through a favorable effect upon the food's textural attributes. These ingredients are known collectively as 'functional' ingredients.

Proteins are used in foods not only for their contribution to the nutritional quality of the food, but also, and perhaps more importantly in many instances, for their functional role. Certain proteins are well known for a single functional attribute. For example, gelatin is used mainly for its thermo-reversible gelling properties and egg proteins provide excellent foaming and heat setting. Some proteins like those in milk can provide multiple functionality in various food systems (Kinsella, 1984). Their versatility and the fact that they are label-friendly make milk proteins a preferred choice as a functional ingredient for many foods.
Much research has been devoted to the study of functional performance of fairly pure milk protein products, such as caseinates and more recently whey proteins. Caseinates have been used in the food industry for many years as a surfactant and ‘whitening’ agent. Whey, the by-product from cheese or casein manufacture, has constituted a major waste problem in the dairy industry. Hence, considerable resources have been invested in developing technologies that can add value to whey, turning it into a valuable food ingredient. Membrane filtration has been used to concentrate whey, washing away a significant portion of lactose and minerals. The concentrated fluid is dehydrated later on, resulting in protein-rich whey protein concentrates (WPCs) which have found many uses as functional food ingredients.

Recently, the same technology applied in the manufacture of whey protein concentrates has started to be used for the production of milk protein concentrates (MPCs). In this case, the raw material is skim milk instead of whey and the finished product is a high-protein powder that comprises both the whey proteins and the caseins along with other minor milk proteins. The composition of MPCs makes them attractive versatile functional ingredients with the potential of replacing the more expensive caseinates in some food applications. However, the heterogeneous composition of MPCs as compared to WPCs or caseinates also makes it more difficult to control and predict their functional performance.
As evidenced by the research work done at the J.T. Parker Chair laboratory in Ohio State University in 1996 and 1997, commercial MPCs from different suppliers available at this moment exhibit very different functionalities\(^1\). The lack of standardization among MPCs limits their current use as functional food ingredients by the food industry. Differences in processing conditions may affect the way caseins and whey proteins interact, therefore affecting the functionality of MPCs. Some of these products, for example, may be tailor-made to the specifications of a customer. However, no information is available about the exact manufacturing procedure followed by the manufacturers.

The aim of this research is to determine whether varying the process conditions during spray drying can induce different modes of aggregation in MPCs, which could, in turn, affect their functionality as food ingredients. To provide a theoretical background for this project, first a definition of protein functionality as food ingredients is provided. Viscosity is briefly discussed. Second, the approaches to assess protein functionality are briefly described. A discussion of the relationship between protein structure and function (in the context of their use in food systems) follows. The manufacturing procedure of milk protein concentrates is summarized, giving emphasis to spray drying. Since spray drying is both a mass and heat transfer process, heat-induced changes in milk proteins are highlighted.

\(^1\) The results from this research are summarized in Chapter 4 of this document.
CHAPTER 2

BACKGROUND AND OBJECTIVES

In this section, a few highlights of the preliminary results obtained in MPC functionality research conducted during 1996 and 1997 in the J. T. Parker Chair laboratory at the Ohio State University are given. These results motivated the direction of the research presented in this dissertation.

Basic functionality tests showed that various MPC samples of similar protein content (>70%) from different countries performed differently as functional ingredients. Among the functional attributes studied, viscosity showed the widest variation. Differences among samples were more noticeable in solutions with a high protein concentration (10%). In this case, viscosity of MPC solutions varied from very low (~10-20 mPa-s), Newtonian to very viscous (>10,000 mPa-s), thixotropic and pseudoplastic. Such wide range in viscosity values suggested that the form of aggregation of the milk proteins differed for the various powders analyzed, presumably as a result of different processing conditions.
Samples that showed extreme differences in viscosity and also came from different countries were selected for particle size analysis, as determined with laser diffraction (Malvern Mastersizer Microplus). Both the particle size distributions and the disaggregation patterns after treating with various denaturing agents provided further evidence that the mode of aggregation of different MPCs could be linked to differences in functionality. The treatments used to dissociate the MPCs included ultrasonication, sodium dodecyl sulfate (SDS), and \( \beta \)-mercaptoethanol.

MPCs of ca. 70% protein were custom-made by varying the air inlet temperature in the spray dryer (200°, 250° and 300°C). Particle size analysis showed that not only was the mean particle size increased with increasing air inlet temperature but also that powders produced by using higher temperature were more difficult to dissociate by ultrasonication and chemical dissociating agents.

The main objectives of this research were:

1. To determine the spray drying conditions that effect the formation and characteristics of aggregates in high-protein MPC.

2. To determine the nature of protein-protein interactions that occur due to spray drying under different conditions.
CHAPTER 3

LITERATURE REVIEW

3.1 Functionality Defined

"Functionality" in the context of food ingredients is seen as any way in which the ingredient affects the characteristics of the food. Functionality of food proteins typically includes all properties except their nutritional value (Pomeranz, 1985). Some authors also exclude flavor effects and enzymatic activity from the scope of food protein functionality. Patel and Fry (1987) state that from a manufacturer’s standpoint, "functional proteins are used to produce or stabilize certain structures in foods which are attractive to consumers”.

Kinsella (1982) listed the typical functional properties that proteins can perform in food systems as: solubility, water absorption and binding, viscosity, gelation, cohesion-adhesion, elasticity, emulsification, fat adsorption, flavor binding and foaming. Solubility (or the lack of it) is not viewed as a functional property per se, but rather as a prerequisite for other functions to take place (Damodaran, 1996; Patel and Fry, 1987).
Functionality in food products will ultimately determine the value of a protein ingredient in formulated foods (Morr, 1985). The desired functional property to be performed by a protein will depend on the type of food system. Table 3.1 summarizes functional properties in food systems.

<table>
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<tr>
<th>General Property</th>
<th>Functional Criteria</th>
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<tr>
<td>Organoleptic</td>
<td>Color, flavor, odor</td>
</tr>
<tr>
<td>Kinaesthetic</td>
<td>Mouthfeel, texture, smoothness, grittiness</td>
</tr>
<tr>
<td>Hydration</td>
<td>Solubility, wettability, water sorption, swelling, thickening, gelling, syneresis, viscosity, gelation</td>
</tr>
<tr>
<td>Surface</td>
<td>Emulsification, foaming, film formation</td>
</tr>
<tr>
<td>Rheological/textural</td>
<td>Elasticity, cohesiveness, chewiness, adhesiveness, network formation, aggregation, dough formation, texturizability, extrudability</td>
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</table>

From: Kinsella et al., 1994.

Table 3.1: Functional Properties in Food Systems

A protein must be able to interact with other components of the food system in order to exhibit functionality (Mangino, 1984; Mangino and Harper, 1996). As the protein is exposed to a different environment, it will change its conformation in order to attain the lowest kinetically attainable free energy. Some of the mechanisms that a protein may use in order to minimize its free energy are the removal of hydrophobic groups from the aqueous environment, and hydrophobic interactions. (Mangino, 1984).
3.1.1 Viscosity

Intuitively, viscosity is defined as a fluid's resistance to flow (Toledo, 1991). Viscosity plays a major role in the texture of many foods such as soups, sauces, salad dressings, gravies, and beverages. For most foods, the concept of viscosity is dynamic, as a single value may not reflect the viscosity behavior of the fluid. Viscosity depends on a number of factors, such as shear rate, length of exposure to shear, and temperature. Very few food products have a single viscosity value that is independent of shear rate. Such products are said to exhibit Newtonian flow behavior. Typically, viscosities of different products are compared by using the same conditions of shear rate and time.

In aqueous solutions, there are a number of mathematical models that allow to estimate a protein's intrinsic viscosity based on the shape and size of the molecules (Rha & Pradipasena, 1986). Protein hydration, extent of denaturation, and electrostatic charge are variables used in some of these models. Rha & Pradipasena (1986) note that there exists a critical concentration above which protein solutions will exhibit non-Newtonian behavior. The critical concentration is typically much larger for globular proteins than for fibrous proteins (10-35% for the former vs. less than 1% for the latter). Some mathematical models have been devised to determine this critical concentration and to model the rheological behavior of concentrated protein solutions.
The effect of proteins in increasing the viscosity of fluid foods is difficult to predict, since the interaction between proteins and other food ingredients may significantly alter the rheological properties of the fluid and thus the protein's effect on viscosity may be different than that which would be predicted from the rheology of pure protein-water mixtures. In addition, the effect of heat, pH, shear and other processing parameters may affect significantly the proteins' contribution to viscosity.

3.2 Approaches to Assessing Protein Functionality

Since foods are complex systems from a physicochemical standpoint, the functional performance of a protein in a particular food system is difficult to predict. Two basic approaches have been used in assessing protein functionality. One is to evaluate individual functional properties in aqueous systems. The other approach consists in measuring functionality in model food systems.

A limitation to basic functionality testing in aqueous systems is the lack of standardized methods (Patel and Fry, 1987). Some collaborative studies have been conducted in order to avoid inter-laboratory variations due to differing procedures. Morr et al. (1985) developed a method to evaluate protein solubility; Patel and Fry (1987) suggested a number of methods for evaluating various functional properties, and Phillips et al. (1990) published a standardized method to evaluate foaming of three different protein products.
However, there are yet some functional properties that lack standard procedures to be evaluated. Examples of these are the enzymatic gelation of milk proteins, and the meltability and sliceability of process cheese foods.

While basic functionality tests in aqueous systems can provide a means to differentiate various protein ingredients, these tests may not be useful in predicting the functional performance of the protein in a food (Harper, 1984). Foods are very complex and ingredient-ingredient and ingredient-process interactions can play a major role in determining the protein's functionality. In an attempt to simulate the complexity of foods and evaluate protein functionality under more realistic conditions, a number of model food systems have been developed. Model food systems use a simplified formula and can be prepared in a benchtop or pilot scale. Ingredients that are not an important fraction of the total or that do not play an important functional role are excluded. For instance, the formulation of a model food system typically does not include any added flavor compounds. Model food systems are designed in a way that allows to detect differences among various protein ingredients (Harper, 1984).

Despite the fact that model food systems try to account for a food's complexity, as Harper (1984) warns, they may not be useful to optimize the use of a protein ingredient in the actual food product. A possible reason for this has to do with process-ingredient interactions, which can yield very different results after scaling up. Damodaran (1996) points out that protein denaturation during food manufacture is probably one of the major
reasons why a protein's functional behavior in a model food system is often different than that in real foods. Model food systems, however, are considered a better approach in understanding protein functionality in foods.

3.3 Functionality and Protein Structure

Structure-function relationships are more easily envisioned in the context of a biological function, such as those performed by enzymes or muscle proteins. However, from the point of view of functions as food ingredients, the relationship between structure and function is not so well understood. As noted by Damodaran (1994), the lack of a good understanding of the molecular bases for protein functionality in foods constitutes the major constraint to increasing the use of proteins from inexpensive sources (such as plant proteins) in formulated foods.

There are several levels of protein structure, namely primary, secondary, tertiary and quaternary. Primary structure refers to the amino acid sequence in the polypeptide chain, and is conceptualized as one-dimensional. Secondary structure has to do with repeating local three-dimensional patterns or motifs, such as α-helices and β-sheets. Tertiary structure is given by the spatial or three-dimensional conformation of the whole protein. Finally, quaternary structure has to do with the association of subunits (Campbell, 1995). For example, monomers may associate to form dimers, tetramers or polymeric units. All
of these levels of structure exert some influence in the functional performance of proteins.

The importance of primary structure in milk protein functionality can be illustrated by two examples. One is the presence of cysteine residues in β-lactoglobulin, κ-casein and αs1-casein. The availability of sulfhydryl groups in these proteins makes possible the formation of disulfide bonds that are vital for heat gelation (Creamer and MacGibbon, 1996). Another example that relates primary structure and protein functionality is the large number of serine and threonine residues in the α- and β-caseins. Through post-translational modifications, these residues are phosphorylated, which makes these caseins Calcium-sensitive, an essential factor in gel formation during cheese-making.

Swaisgood et al. (1994) explain the ability of some proteins to exhibit various functional attributes in food systems to the existence of several well-defined domains in the protein structure. These domains allow the protein to interact with other molecules in the food. The authors present multidomain evidence for β-casein and αs1-casein. They suggest the use of limited proteolysis in immobilized enzyme bioreactors and subsequent cross-linking of specific mixtures of domains as a means of modifying protein functionality.

The relatively large amount of proline residues in the caseins precludes the formation of a large amount of α-helices or β-sheets. Data from Raman and Fourier-transformed infrared spectroscopy analyses has determined that caseins have around 6-14% helix and
20-40% β-structures (Farrell et al., 1993). Caseins' secondary structure, therefore, can be best characterized as mainly "random coil". This makes them very flexible, which allows caseins to work as good emulsifiers (Mangino and Harper, 1996). The lack of secondary structure also provides heat stability to the caseins.

The loss of the original (native) tertiary structure of the protein is referred to as denaturation. In actuality, some functional properties required denaturation of the protein. For example, heat gelation of whey proteins. Partial denaturation increases hydrophobicity and this, in turn, decreases the energy barrier to adsorption at air-liquid interfaces. Based on this, foaming properties of whey proteins can be enhanced by partial denaturation as the result of controlled heat treatment (Fligner and Mangino, 1991).

Some of the milk proteins occur as dimers in the milk environment. For example, αs2-casein is found as both a monomer and a dimer (Creamer, 1991), while β-lactoglobulin is found as a dimer at low temperatures and at the normal milk pH (Gallagher and Mulvihill, 1997). Native κ-casein is known to exist as polymers of varying size (Creamer, 1991; Creamer and McGibbon, 1996). But by far, the most widely studied quaternary structure in milk proteins is the association of the different caseins in micelles. The formation of micelles allow the caseins to remain in a colloidal suspension in an aqueous medium despite their relatively large number of hydrophobic residues. Scientists recognize the role of κ-casein in stabilizing the micelle, but the exact mechanism is still being debated (Creamer, 1991). Micelle destabilization by specific
cleavage of κ-casein by the enzyme chymosin (rennin) is essential for cheese making, as this allows the aggregation of Calcium-sensitive caseins.

Various kinds of forces are responsible for stabilizing protein structure. In increasing order of interaction energy, these are: van der Waals forces, electrostatic interactions, hydrogen bonds, hydrophobic forces and covalent bonds—most notably disulfide bridges—(Phillips et al., 1994). Heat processing of food products will induce changes in protein conformation. Depending on the severity of heat (temperature and time) applied to the food, some of the structure-stabilizing forces will be disrupted and interactions different than the ones found in the native protein will occur. The kind of change and how it will affect the functional performance of the protein will depend, among other factors, on the extent of heat loaded on the product, protein composition, environmental conditions (e.g., pH, ionic strength, solutes, etc.).

There are four major ways of modifying protein structure: chemically, enzymatically, physically or by genetic engineering (Dickinson and McClements, 1996). These modifications have provided insight on structure-function relationships. However, the prediction of functional performance based on the molecular properties of a protein has not been successful (Damodaran, 1996). Some of the reasons for the lack of success lie in our incomplete knowledge of the protein structures themselves. For example, the three-dimensional structure of caseins have not been completely elucidated yet with the analytical methods available today. There have been tremendous advances in this area
with the use of energy-minimization models, but no complete agreement has been reached (Farrell et al., 1993).

On the other hand, most studies on milk protein functionality have been conducted using relatively pure proteins in simple model systems (Singh, 1993). MPC, however, is a mixed, heterogeneous product in which all the different proteins found in milk are present in the original proportions. Rather than determining structural changes in individual proteins of the MPC, it becomes important to understand how processing can affect the characteristics of the powder particles. These changes in particle characteristics can arise not only from the structural changes of each protein, but also from protein-protein interactions, as will be discussed later on. Features such as particle size and the tenacity of MPC particles to resist dissociation by various agents are conceivably related to the way MPC can interact with other ingredients in the food, which is what ultimately will determine its functionality.

3.4 Milk Protein Concentrate (MPC) Processing Technology

The term MPC is currently applied to powders with a protein content varying from 50 to 85 percent (Rosenberg, 1995). The scope of this research is limited to high-protein MPCs, which were considered to be those with at least 70% protein.
A general manufacturing process for MPCs is summarized in the flow chart provided in Figure 3.1. The raw material for MPC production is skimmed milk. Total solids are increased typically up to 22-25% by membrane concentration. This step is discussed in more detail in section 3.4.1. Diafiltration is necessary for the production of high-protein MPC, since the lactose and minerals content can be reduced significantly, thereby increasing the relative protein concentration in the retentate and thus in the end product.

The retentate is typically concentrated by evaporation to up to 40-50% total solids. Evaporation is a much more energy efficient and therefore a more economical process than spray drying, but it cannot achieve complete dehydration of the product. Since viscosity increases significantly above a solids concentration of 45% in milk products and this will reduce the droplet size during spray drying, 40-50% solids becomes a practical limit for evaporation (Singh and Newstead, 1992). Finally the UF retentate is dehydrated by spray drying. The dehydration process is outlined in section 3.4.2.

Novák (1991) outlines a manufacturing process for MPC in which he states that raw milk is pasteurized before ultrafiltration and the retentate obtained after ultra/diafiltration is also heat-treated prior to evaporation.
Figure 3.1: MPC Manufacturing Process Flowchart
3.4.1 Ultrafiltration and Diafiltration

Membrane filtration technologies started being used in the industry in the late sixties (Ostergaard, 1986). Among various other applications, membrane filtration is widely used for fractionation and concentration purposes. The various types of membrane filtration processes are roughly classified on the basis of pore size as: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). In general, microfiltration is used alone or in combination with ultrafiltration when the objective is to fractionate the milk proteins. When UF is used alone, the original casein to whey ratio is maintained (Novák, 1991). The driving force behind the hydrodynamic flow across the membrane is the existence of a hydrostatic pressure gradient. Other factors, such as membrane type, process conditions and equipment configuration are also important in determining the mechanisms of mass transport through the membrane (Rosenberg, 1995).

In the dairy industry, the major application is for the production of whey protein concentrates. Typically, ultrafiltration membranes are used, since the molecular weight cutoff of these membranes ranges around 1 to 200 kDa (Rosenberg, 1995). These membranes are therefore suited for the concentration of milk proteins. If skim milk (and not whey) is the starting material, retentates that can be further evaporated and dried into milk protein concentrates.
With ultrafiltration, total solids can be typically increased up to around twenty percent. The actual concentration achieved by UF depends on processing conditions. For example, "cold" UF, performed at 10°C, can produce retentates of about 20% solids when using spiral wound membranes, while "hot" UF (50°C) can concentrate the dry matter up to 28% for the same kind of membranes (Getler et al., 1997). Higher solids concentrations (i.e., 24% for "cold" UF and 33% for "hot" UF) can be achieved by using a combination of spiral wound and plate and frame membranes. As Getler et al. (1997) point out, "cold" UF is preferred since it reduces the thermal load on the product. However, "hot" UF may be used if the evaporation step prior to spray drying is to be skipped.

The total solids composition also changes as small molecules are eluted with the permeate. The pore size of UF membranes is large enough to allow for the passage of molecules smaller than proteins, e.g. lactose and mineral ions and salts.

Diafiltration is necessary to obtain MPC with a protein content or 65% or more (Getler et al., 1997). This process consists in diluting the retentate with water after it has reached the highest practical solids concentration that can be obtained with a particular membrane and then ultrafiltering again. This allows for a greater extent of removal of small watersoluble compounds.
3.4.2 Spray Drying

3.4.2.1 Basic Concepts

Spray drying is probably the most widely used dehydration method in foods (Potter and Hotchkiss, 1995). This process is limited to fluid foods of relatively low viscosity that can be pumped and atomized into very small droplets. Through atomizing, surface area of the liquid can be increased substantially. The exposure of this extremely large surface area to a stream of hot air makes it possible for water to evaporate very rapidly (Farrall, 1980). Spray drying constitutes simultaneously a mass and heat transfer process, since a large amount of water needs to be removed from the product and this is achieved by evaporation, so heating is necessary.

Although there exists a wide variety of designs, the main components of a spray dryer, as outlined by Pisecky (1987) are: atomizer with feed supply system, air dispenser with hot air supply system, drying chamber, powder collector, and powder after-treatment system.

Two distinct phases occur during dehydration of foods with a moisture content of 50 percent or more: the “constant rate” and the “falling rate” periods (Sokhansanj and Jayas, 1987). In the first phase, moisture is being removed from the food particle at a fairly constant rate. Water removal is controlled by the heat transfer between the hot air and the water on the surface of the droplet (Pérez-Correa and Farias, 1995). At this stage, water
can migrate easily from the interior to the surface of the droplets (Pisecky, 1987). The change of state of water from liquid to gas exerts a cooling effect on the drying particle, due to the removal of the latent heat of evaporation. For this reason, spray drying is used for foods that are fairly heat sensitive, such as milk, coffee, juices, etc. (Potter and Hotchkiss, 1995).

As drying proceeds, the droplet reaches a point when it loses its fluid character and becomes a wet solid (Pisecky, 1987). In the process of drying skim milk, a smooth skin is formed on the surface of the droplet and later a crust is formed underneath the skin. The formed skin constitutes a physical barrier that seals off the path of water diffusion to the surface of the particle (Hassan and Mumford, 1996). The drying material enters what is known as the “falling rate” stage in dehydration processes. In this stage, internal water diffusion, rather than temperature difference, becomes the driving force (Pérez-Correa and Farias, 1995). The moisture content of the food material at which the drying rate changes from a constant to a falling one is called the critical moisture content. Pisecky (1987) states that the critical moisture content for milk products lies between 15 and 30%.

Spray drying in the milk industry has traditionally been used to produce powdered milk. Other milk products, such as whey, whey protein concentrates or isolates, caseinates, and milk protein concentrates are also dehydrated by means of spray drying. The typical operating conditions for spray drying of milk products are outlined in Table 3.2. The
liquid-air layout most widely used is cocurrent flow, since the final temperature attained by the dried particles are lower than when using a countercurrent liquid-air flow (Filkóva and Mujumdar, 1987).

No information is available about actual spray drying conditions for MPC in the industry. El-Samgray et al. (1993a) produced MPC by spray drying a skim milk UF retentate (20% solids) at 160°C air inlet temperature and 70°C air outlet temperature. The retentate was fed at 25°C.

Table 4.2: Typical Spray Dryer Operating Conditions for Milk Products

<table>
<thead>
<tr>
<th>Material</th>
<th>Moisture Inlet (%)</th>
<th>Moisture Outlet (%)</th>
<th>Atomizing Device</th>
<th>Air Temperature Inlet (°C)</th>
<th>Air Temperature Outlet (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk</td>
<td>48-55</td>
<td>4</td>
<td>Wheel, Pressure nozzle (170-200) bar wheel</td>
<td>&lt;250</td>
<td>95-100</td>
</tr>
<tr>
<td>Whey</td>
<td>50</td>
<td>4</td>
<td>Wheel</td>
<td>150-180</td>
<td>70-80</td>
</tr>
<tr>
<td>Milk</td>
<td>50-60</td>
<td>2.5</td>
<td>Wheel, Pressure nozzle (100-140 bar)</td>
<td>170-200</td>
<td>90-100</td>
</tr>
</tbody>
</table>

Source: Adapted from Filkova and Mujumdar (1987), p. 245.
Control factors in spray drying

Control of the following processing parameters determines ultimately the heat effect on the particles:

1. **Air inlet temperature.** This is the temperature of the dry hot air to which the fluid is atomized.

2. **Air outlet temperature.** Is the temperature of the air/powder mixture as it leaves the drier. Since the temperature of the drying particles approaches that of the air outlet temperature towards the completion of the drying process, this can have a significant effect on heat damage to dry milk products (Singh and Newstead, 1992).

De Wilder *et al.* (1976), as cited by Knipschildt (1986), found that increasing the air outlet temperature decreased the solubility of dried homogenized whole milk concentrate more significantly than increasing the air inlet temperature.

3. **Air flow:** Turbulence makes moisture removal more rapid, particularly during the constant rate period. Typically measured by the Reynolds number (Re), the degree of turbulence in air flow depends on the spray pattern and chamber design, among other things. Although this is recognized as being an important factor in determining the
drying rate and thus the end product characteristics, once a spray drying equipment has been installed, changes in the air flow pattern are not practicable.

4. **Droplet size.** The increase in surface area that results from reducing the droplet size will increase the drying speed (Filková and Mujumdar, 1987; Furuta *et al.*, 1994). Droplet size can be influenced by the type of nozzle used, orifice diameter, feed pressure, and feed viscosity. A rotary atomizer, for example, is better suited for atomizing highly viscous liquids (Knipschildt, 1986). The selection of a particular type of atomizer is intimately related to the dryer design. For example, spray angles produced by rotary atomizers are larger, and this requires a drying chamber of larger diameter (Furuta *et al.*, 1994). Higher orifices in the atomizer, lower pressure and smaller spray angle generally produce larger particles (Farrall, 1980).

The uniformity in droplet sizes of the atomized liquid is also important in determining the powder characteristics. Small particles may overheat while large particles will require a longer heating time to reduce the moisture content to the desired level (Singh and Newstead, 1992).

Viscosity of the fluid fed to the atomizer also influences the atomized droplet size. Knipschildt (1986) reports how by preheating a skim milk concentrate immediately before spray drying, its viscosity could be reduced significantly, producing a smaller average droplet size. This made possible a reduction in the air outlet temperature while
still obtaining the desired moisture content in the powder. Milder drying conditions resulted in improved solubility. However, the author warns that if the heated concentrate is held at temperatures above 60°C, albeit for a very short time, its viscosity can actually increase before atomization.

3.4.2.3 Heat-Induced Reactions

3.4.2.3.1 Denaturation

Many of the bonds that stabilize the secondary and tertiary protein structures are sensitive to temperature. For example, an increase in temperature will weaken hydrogen bonds. Hydrophobic bonds, on the other hand, can become stronger with increasing temperatures up to around 60°C, but weaken at higher temperatures. Thus, protein structure will be affected by heat. Usually, denaturation that occurs at temperature below 60°C is reversible. At temperatures of 80-90°C, inter- and intra-molecular interactions of denatured proteins can result in the formation of aggregates or precipitates. Heating to temperatures in excess of 100°C could lead to the loss of serine and lysine residues as the result of the formation of isopeptide bonds (Singh, 1993).

Heat effects on milk protein structure and functionality are implied when characterizing non-fat dried milk with common working terms such as “low heat” “medium heat” and “high heat”. These are industry classifications, rather than legal standards, and they are
based on the amount of undenatured whey proteins (Kinsella, 1984; Anonymous, 1971). These different types of powders are obtained by adjusting the heat treatment of the fluid prior to evaporation and dehydration rather than by manipulating spray drying conditions (Singh and Newstead, 1992). According to Pisecky (1986), spray drying has a negligible effect on the Whey Protein Nitrogen Index. It is known that low-heat milk powders are more soluble and less prone to Maillard reactions and therefore are preferred ingredients for products such as recombined milks. High-heat powders contain a higher amount of denatured whey proteins and are therefore preferred as ingredients for products such as yogurt, in which partial denaturation of whey proteins is desirable since it increases water holding capacity and in turn reduces syneresis.

3.4.2.3.2 Aggregation

Probably the best characterized heat-induced protein-protein interaction in milk is the association of β-lactoglobulin and κ-casein. The formation of this complex has been studied by a number of researchers. Some of them have proposed hydrophobic interactions as the force that holds these aggregates together at the initial stages of aggregation, but there exists some agreement on the fact that disulfide linkages cause the formation of the final aggregate (Dalgleish, 1990a). Denaturation of β-lactoglobulin precedes the formation of aggregates, either with other molecules of β-lactoglobulin or with κ-casein. Kinetic studies of β-lactoglobulin denaturation have found non-linear
Arrhenius plots. Activation energies decrease significantly at temperatures above 90°C. Dalgleish (1990a) determined a pseudo-first order reaction for β-lactoglobulin denaturation, which seemed to be in good agreement with the results obtained previously by other researchers.

The formation of the β-lactoglobulin-κ-casein complex is regarded as the cause of poor rennetability of high heat treated milks (Dalgleish, 1990b). Gallagher and Mulvihill (1997) used a synthetic milk system to assess the importance of the sulfhydryl-disulfide interchange between β-lactoglobulin and κ-casein in the heat-stability profile and rennet coagulation time. They compared synthetic milk systems containing either porcine or bovine β-lactoglobulin. The increase in rennet coagulation time after heating was much larger when using bovine β-lactoglobulin than when porcine β-lactoglobulin was present. Since the latter lacks a free sulfhydryl group, which is present in bovine β-lactoglobulin, the decrease in rennetability was attributed to the formation of a disulfide-induced complex between bovine β-lactoglobulin and κ-casein.

Although the complex formed between β-lactoglobulin and κ-casein is recognized as the main one produced as the result of heat treatment, other interactions are possible as well. For example, complexes between α-lactalbumin and β-lactoglobulin may form, especially at temperatures below 90°C. Since αs2-casein also contains a cysteine residue, it might play a role in complex formation with β-lactoglobulin (Corredig and Dalgleish, 1996).
Protein-carbohydrate interactions, especially those that result from the reactions between amino acids and reducing sugars, may also play an important role in protein aggregation. The Lobrey de Bruyn-Alberda van Ekenstein transformation of lactose results in a number of isomers. One of these isomers is lactulose (4-\(O\)-\(\beta\)-D-galactopyranosyl-D-Fructose). Even though lactulose is roughly ten times less reactive than lactose, it is believed to strongly induce protein crosslinking and polymerization (O'Brien, 1995; Matsuda et al., 1991). The aldo-keto rearrangement from lactose to lactulose occurs at high temperatures or in alkaline solution (Matsuda et al., 1991).

Maillard reactions can result in the formation of brown pigments and loss of solubility and reduced nutritional quality due to loss of lysine, the most reactive amino acid in these reactions. Protein polymers are formed as the result of Maillard reactions (Kato et al., 1988; Kato et al., 1989). Intermediate products from the Maillard reaction, such as \(\alpha\)-dicarbonyl compounds generated through the reaction of glucose and lysine can induce the formation of protein polymers (Kato et al., 1988). 3-deoxyglucosone was identified by Kato et al. (1988) as the major factor responsible for protein polymerization as a result of Maillard reactions.
3.4.2.3.3 Assessment of Heat Damage during Spray Drying

The changes undergone by milk proteins as the result of the heat treatment have been studied more extensively on fluid processes such as pre-heating, pasteurization, UHT, retorting, etc. A number of studies have assessed the effect of pre-heating before concentration and/or dehydration of skim milk and ultrafiltered skim milk. El-Samragy et al. (1993a) varied the heat treatments of UF skim milk retentate prior to spray drying. Their study showed a loss of solubility with higher heat treatment (85°C/28sec), which was attributed to protein unfolding and aggregation. In a later study, it was shown that the heat treatment affected significantly gel water holding capacity, acid gel strength and emulsifying capacity, but not foaming capacity (El-Samragy et al., 1993b). An interaction term between pH and heat treatment was significant for all the functional properties analyzed.

The effect of heat during spray drying is more difficult to assess due to the dynamic nature of this process. Solids concentration is increasing throughout the process, evaporation rate goes from a constant to a falling period as diffusivity of the material changes. Complex mathematical models have been developed to describe the energy flow and product changes during the spray drying process. As any other model, these are based on a number of assumptions that limit their application.
The heat "damage" as a result of spray drying has been traditionally assessed by either microbial or enzyme inactivation studies. In microbial studies, usually a pseudo-z value is determined, since microbial inactivation can be partially due to dehydration. Therefore, loss of microbial viability cannot be solely attributed to heat, as in a true z-value. Teixera et al. (1995) investigated the death kinetics of *Lactobacillus bulgaricus* during spray drying of inoculated skim milk at various outlet air temperatures (62° to 105°C). They found a smaller energy of activation for the destruction of *L. bulgaricus* when air outlet temperatures exceeded 70°C. The break in the Arrhenius plot suggested a different mechanism for bacterial death at higher temperatures.

Daemen and van der Stege (1982) studied inactivation of the enzymes alkaline phosphatase, chymosin and α-amylase and of the bacteria *Serratia marcescens* and *Staphylococcus* sp. They found that the effect of the air outlet temperature was much more significant than that of the air inlet temperature.

Etzel et al. (1995) studied the inactivation of alkaline phosphatase by spray drying of condensed skim milk. A linear decrease in alkaline phosphatase activity was found with increasing air outlet temperature (80°C to 120°C). Mathematical models which included bubble formation were used for simulation. Predictions based on the model simulations indicated a significant inactivation of alkaline phosphatase in the critical region of 20-30% moisture.
Matzinos and Hall (1993) varied the air outlet temperature in the spray dryer and studied the effect on denaturation of lactate dehydrogenase in bovine plasma. When the enzyme's residual activity was plotted against air outlet temperature, two linear segments were found. Above 89°C, the reduction in residual activity as the result of increasing the air temperature was much steeper.

An analogy can be drawn between protein denaturation and microbial inactivation during spray drying. The effect can be due to both heating and dehydration. The concentration effect is important, as it increases the chances of inter-molecular associations (i.e., polymerization, complexing).

Babella and Novák (1982), as cited by Novák (1991), studied the effect of different spray drying conditions on the emulsifying capacity of MPC. They found that the emulsifying capacity of powders spray dried from both UF retentate or UF/EV concentrate at a peripheral speed of 145 m/s in the disc atomizer decreased with increasing drying air temperature. The effect was greater on powder produced from UF/EV concentrate. However, the temperature of the drying air did not seem to have such an effect when the peripheral speed of the spraying disc was 165 m/s. This suggests that droplet size is particularly important in determining the final heat load on the product during dehydration.
To the best of our knowledge, the heat effect of spray drying on milk proteins of MPC practically has not been studied. Results from skim milk drying experiments cannot be extrapolated to drying of skim milk UF retentate for the production of high-protein MPC. Since proteins are more closely packed after membrane concentration, the chance for protein-protein interactions is higher (Damodaran, 1996). Heat-induced aggregation, for example, is said to be largely dependent on protein concentration (Kato et al., 1995).

Also, the removal of lactose during ultrafiltration will affect the heat-sensitivity of proteins during spray drying. For example, Mistry (1989) determined that alkaline phosphatase could be inactivated much more rapidly in 5-fold concentrated UF skim milk than in retentates of lower concentration and than non-UF milk. This was attributed to the removal of lactose. Other researches have stated that the removal of lactose accelerates the heat denaturation of whey proteins. On the other hand, variations in the salt balance and pH affect the heat stability of caseins (Pisecky, 1986). Both of these factors are changed with membrane concentration.

Finally, the differences in protein content may decrease the drying rate of skim milk UF retentate (as compared to skim milk). Greater viscosity may account for a longer drying time. A more rapid skin formation may also result in smaller drying rate. Hassan and Mumford (1996) determined that an increase in the initial solids concentration of skim milk decreased markedly the drying rate, possibly due to a more rapid appearance of a skin in the surface of the droplet. It is reasonable to think that a higher protein fraction in
the droplet solids may also influence skin formation and thus the rate of drying. If the rate of drying diminishes, the residence time in the drying chamber will be longer, increasing the heat damage on the proteins.
CHAPTER 4

BASIC FUNCTIONALITY IN COMMERCIAL MPC SAMPLES

This chapter summarizes the results obtained during my first two years of research in the J. T. (Stubby) Parker Chair Laboratory at the Ohio State University. As it will be shown later, the wide variation in the basic functionality tests of commercial samples motivated the spray drying experiments that constitute the core of this study.

4.1 Methods

4.1.1 Samples

Commercial MPC samples were obtained from ten different countries (Australia, France, Hungary, Ireland, the Netherlands, New Zealand, Sweden, the United Kingdom and U.S.A.). The geographical origin of some of the samples was unknown. The samples were analyzed for protein content and solubility, viscosity, foaming and particle size. Some samples were selected on the basis of their differences in viscosity to study their behavior in model food systems. A whipped topping system was chosen because it would be a potential application in which MPCs could substitute sodium caseinates.
4.1.2. Protein Content

Protein content was based on micro-Kjeldahl Nitrogen determination, using a conversion factor of 6.38. A Kjeltec Tecator 1025 was used for automatic distillation and titration of the samples.

4.1.3 Protein Solubility

The method described by Morr et al. (1985) was used to determine protein solubility at pH 7.0. Based on this method, 500 mg of dry MPC were dispersed in about 40 ml of 0.1 M NaCl solution and hydrated for 1 hour. pH is adjusted to 7.0 and the dispersion is diluted to 50 ml with 0.1 M NaCl solution. Aliquots are centrifuged at 20,000 x g for 30 minutes and the supernatant was filtered using Whatman #1 filter paper. Protein content of the filtrate was determined as described in section 4.1.2 above. Protein solubility was calculated as:

\[
\text{Protein solubility (\%) = \frac{\text{Supernatant protein concentr. (mg/mL) x 50 mL} - \text{Sample protein content (\%) \times 100}}{\text{Sample wt (mg) \times 100}}}
\]
4.1.4 Viscosity

Viscosity was determined on 5% and 10% protein solutions with a Brookfield RV rotational viscometer. Only the results on the 10% solutions will be shown, since these are the ones where differences among the samples are evident.

4.1.5 Foaming

Foaming was determined using the procedure described by Phillips et al. (1990), modified to use 150 ml of solution instead of 75 ml. A 5% protein solution was hydrated for one hour and whipped in a Sunbeam® Mixmaster model 0193 using the smaller bowl (1.9 L). Overrun was determined by weighing a tared 125 ml plastic boat filled with foam. The following formula was used to calculate overrun:

\[
\% \text{ Overrun} = \frac{(125 - \text{Foam weight}) \times 100}{\text{Foam weight}}
\]

4.1.6 Emulsion Volume Index (EVI)

The emulsion volume index (EVI) is an accelerated centrifugal method that measures the propensity to creaming during storage. The method described by McDermott et al. (1981) was used to determine EVI in a model whipped topping containing 20% fat. 1 ml
of sample was mixed thoroughly in a porcelain plate with 10 μl of methylene blue dye and 10 μl of Oil Red "O" dye. Microhematocrit centrifugal tubes were filled with the stained mix and sealed on one end with Critoseal capillary tube sealant (Fisher Scientific). The tubes were centrifuged at 13,500 g for 30 minutes in a Fisher Marathon 13K micro-centrifuge and the EVI was calculated as:

\[
EVI = \frac{\text{Emulsion Length}}{\text{Total Length}} \times 100
\]

Relative fat percent in formulation / 0.9

4.1.7 Particle Size

The particle size distribution was determined by laser diffraction, using a Malvern Mastersizer Microplus (Malvern Instruments Ltd.). MPC solutions standardized to 10% protein were hydrated for at least one hour. Deionized water was used as a dispersant. Three to five ml of sample solution were dispersed in the water until obtaining a 10-30% obscuration of the laser beam. Particle size distributions were generated by the instrument's software (Mastersizer μ+, 2.15) based on Mie's theory. The effective range of this particular model is 0.5-550μm. The standard presentation (5OHD) was chosen for calculation of the particle size distributions. This presentation uses a relative refractive index of 1.15 and an absorptive index of 0.1. No assumption was made on the type of particle size distribution, and therefore the data obtained are from polydisperse distributions.
The "initial" (after one hour hydration) particle size distribution was compared to that obtained after treating the sample with the following dissociating treatments:

- Sodium Dodecyl Sulfate (SDS)
- β-Mercaptoethanol
- Ultrasonication (built-in device)

Since the actual amount of milk protein that needs to be dispersed in water to obtain 10-30% obscuration is very small, it is assumed that dissociating agents are added in excess. Changes in particle sizes can be assessed by comparing the volume or the surface area (Sauter) mean diameters or by the shift of the major peak from the size range of aggregates to that of micelles. The susceptibility of particles to be disaggregated by each of the above agents can provide an indication of the nature of the forces that hold the aggregates together. For example, hydrophobic interactions can be weakened by the use of SDS while mercaptoethanol can reduce disulfide bridges. The dissociating effect of ultrasonication may be attributed to the simultaneous disruption of several types of forces, which may include hydrogen bonding and hydrophobic interactions.
4.2 Results – Commercial MPC Samples

4.2.1 Protein Content

The commercial samples analyzed encompassed a wide range of protein contents. On a wet basis, protein ranged from a minimum of 41.5% to a maximum of 90.2%. In general, the suppliers of MPC have three categories based on the protein content. The first would be of around 55% protein or less; the second one is around 70% and the third one is 80% protein or above. At this moment there are no standards of identity for this kind of product, so the rough classification based on protein content is merely used by the industry.

4.2.2 Protein Solubility

Protein solubility was determined at pH 7.0. The variation in the data is very large, ranging from below 20% to around 90%.

In order to increase the protein content of MPCs, the retentate needs to be diafiltered (i.e., diluted with water and ultrafiltered again). This process removes additional minerals and lactose. The removal of salts, minerals and sugars by ultrafiltration could render the milk
proteins (especially the whey proteins) more susceptible to heat denaturation (Kinsella, 1984) and hence reduce the solubility of the finished product. However, no correlation with protein content is apparent, as can be seen in Figure 4.1. This could be largely explained by differences in the actual heat treatment received by the samples, but those data were not available.

Figure 4.1: Scatterplot of protein solubility vs. protein content in commercial samples of MPCs.
4.2.3 Viscosity

Viscosity of 10% protein solutions showed great differences, from very low and Newtonian values to very high viscosities that were both time- and shear-dependent.

Figure 4.2. is a scatterplot showing the wide ranges of viscosity as compared to the samples' protein content. If all processing factors had been equal, a greater viscosity would have been expected from MPCs with higher protein content, since protein-protein interactions would be much more significant than for the MPCs with lower protein content. However, a clear relationship cannot be ascertained from Figure 4.2. Again, actual processing differences may account for some of the variations in viscosity.

![Figure 4.2: Scatterplot of viscosity after 5 min. at 5 rpm vs. protein content for commercial MPC samples.](image-url)

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4.2.4 Foaming

Five percent protein solutions showed great variations in foaming. Overrun after mixing for 5 minutes ranged from 0% to 940%. There was no significant correlation between overrun and any of the other variables.

Since maximum protein foaming is usually obtained near the isoelectric point (Mangino, 1984), overrun might be related to protein solubility. However, as seen in Figure 4.3, there is so much variation in the commercial samples studied that the apparent positive relationship is not statistically significant ($R^2=20\%$).

![Figure 4.3: Overrun vs. Protein Solubility at pH 7.0](image)
In summary, the commercial samples analyzed showed extreme differences in basic functional characteristics. Statistical correlations among protein content, protein solubility, viscosity, foaming and pH are shown in Table 4.1. A few pairs of variables had statistically significant correlations (p<0.05) but even in these cases the correlation coefficients were not too different than 0.5.

<table>
<thead>
<tr>
<th>Prot. Solubility</th>
<th>Protein Content</th>
<th>Protein Solubility</th>
<th>pH</th>
<th>Viscosity at 5 rpm</th>
<th>Viscosity at 20 rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.375</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(0.022)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.066</td>
<td>-0.651</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.717)</td>
<td>(0.000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscosity at 5 rpm</td>
<td>0.284</td>
<td>-0.426</td>
<td>0.395</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.094)</td>
<td>(0.010)</td>
<td></td>
<td>(0.025)</td>
<td></td>
</tr>
<tr>
<td>Viscosity at 20 rpm</td>
<td>0.340</td>
<td>-0.473</td>
<td>0.408</td>
<td>0.967</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.043)</td>
<td>(0.004)</td>
<td></td>
<td>(0.020)</td>
<td>(0.000)</td>
</tr>
<tr>
<td>Overrun after 5 min.</td>
<td>0.084</td>
<td>0.456</td>
<td>-0.400</td>
<td>-0.551</td>
<td>-0.531</td>
</tr>
<tr>
<td></td>
<td>(0.705)</td>
<td>(0.029)</td>
<td></td>
<td>(0.059)</td>
<td>(0.008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.011)</td>
</tr>
</tbody>
</table>

Note: Cell contents: Correlation (p value)

Table 4.1: Correlation Matrix for Basic Functionality Tests, Commercial MPC samples
4.3 Results – Selected High Protein Samples

Given the extreme differences in basic functionality, four high protein samples were selected for further analysis in model food systems. These samples were from different geographical origins and had shown very different viscosities. A summary of their characteristics is given in Table 4.2.
### Protein Content

#### Prot. Solubility at pH 7

<table>
<thead>
<tr>
<th>pH (5% solution)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.82%</td>
<td>85.74%</td>
<td>82.04%</td>
<td>80.68%</td>
<td></td>
</tr>
<tr>
<td>35.73%</td>
<td>45.75%</td>
<td>31.95%</td>
<td>47.66%</td>
<td></td>
</tr>
<tr>
<td>7.14</td>
<td>7.14</td>
<td>7.29</td>
<td>7.19</td>
<td></td>
</tr>
</tbody>
</table>

### Viscosity

<table>
<thead>
<tr>
<th>Viscosity at 20 rpm</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>378</td>
<td>1442</td>
<td>5180</td>
<td>576</td>
<td></td>
</tr>
<tr>
<td>Viscosity at 5 rpm</td>
<td>768</td>
<td>3968</td>
<td>17900</td>
<td>576</td>
</tr>
<tr>
<td>Δ Viscosity</td>
<td>-50.78%</td>
<td>-63.66%</td>
<td>-71.06%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

*After Heating at 60°C for 30 min.*

<table>
<thead>
<tr>
<th>Viscosity at 20 rpm</th>
<th>182</th>
<th>36</th>
<th>104</th>
<th>12.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity at 5 rpm</td>
<td>312</td>
<td>40</td>
<td>168</td>
<td>10</td>
</tr>
<tr>
<td>Δ Viscosity</td>
<td>-41.67%</td>
<td>-10.00%</td>
<td>-38.10%</td>
<td>25.00%</td>
</tr>
</tbody>
</table>

### Particle Size

| D(v,0.1) | A | 18.1 | 12.61 | 11.62 | 0.52 |
| D(v,0.5) | 63.5 | 75.53 | 35.04 | 30.77 |
| D(v,0.9) | 136.78 | 237.83 | 82.71 | 89.09 |
| D[4,3]   | 71.38 | 103.23 | 42.26 | 39.75 |
| D[3,2]   | 33.22 | 28.84 | 23.58 | 2.22 |
| Sp. Surface Area | 0.1806 | 0.208 | 0.2545 | 2.6976 |

*After Heating at 60°C for 30 min.*

| D(v,0.1) | 10.62 | 0.41 | 5.01 | 0.23 |
| D(v,0.5) | 36.61 | 12.15 | 21.78 | 7.66 |
| D(v,0.9) | 101.25 | 44.07 | 73.88 | 62.09 |
| D[4,3]   | 49.2 | 18.75 | 32.42 | 22.01 |
| D[3,2]   | 14.55 | 1.63 | 4.96 | 0.63 |
| Sp. Surface Area | 0.4123 | 3.6698 | 1.2088 | 9.5291 |

Units: Viscosity: mPa.s; Particle size diameters: μ; Specific surface Area: m²/g.

**Table 4.2: Characteristics of Selected Commercial MPC Samples**
4.3.1 Whipped Topping Model

In order to assess functionality under conditions that would approach those under industrial settings, a whipped topping was used as a model food system for the selected high protein MPC commercial samples.

The whipped topping was formulated as shown in Table 4.3. MPC solids were calculated based on a standard 2% protein in the formulation. Water was added to 100%.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenated Coconut Oil</td>
<td>20.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15.00</td>
</tr>
<tr>
<td>Na₂PO₄</td>
<td>0.10</td>
</tr>
<tr>
<td>Xantham Gum</td>
<td>0.05</td>
</tr>
<tr>
<td>Polysorbate 60</td>
<td>0.10</td>
</tr>
<tr>
<td>Protein</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 4.3: Whipped Topping Formulation

The water soluble components were hydrated for 1 hour at 60°C. Melted oil was added while stirring. The mixes were homogenized twice using a bench top homogenizer (A/S Foss Electronic, Denmark) and immediately placed in an ice bath until cooled down to 4°C. The mixes were ripened overnight at 4°C.
The emulsion volume index (EVI) was determined for the whipping mix. EVI results are shown in Figure 4.4. As seen here, three of the MPC samples analyzed had significantly higher emulsion volume index than the sodium caseinate used as a control.

Figure 4.4: Emulsion Volume Index (EVI) of Whipped Topping Mix, Selected Commercial MPC Samples.

Note: Different subscripts indicate statistically significant difference at α=0.05.
For the foaming test, 300 g were placed on the bowl of a Kitchen Aid® Heavy Duty mixer and beat at speed 6 with the wire whip attachment for a total of 15 minutes. Overrun was determined at five minutes intervals.

Foaming results are shown in Figure 4.5. After whipping for 5 minutes, there was no statistically significant difference in the overrun of the different samples. Differences become evident after 10 and 15 minutes whipping time. Samples C and D achieve maximum overrun after 10 minutes, as compared to samples A, B, and sodium caseinate, whose maximum overrun was obtained after just five minutes whipping. These differences did not appear to be related to protein solubility or viscosity or to EVI of the whipping mix. However, samples C and D had smaller particle size than samples A and B, as shown previously in Table 4.2. Sample B behaves similarly to sodium caseinate in this model food system.

Foam stability was determined by gravitational drainage by placing the whipped topping in a plastic funnel (upper diameter: 145mm; lower diameter: 25 mm). A piece of cheese cloth was tied up to the lower part of the funnel using a rubber band and the time for the first drop to fall down was measured. All of the MPC samples produced less stable foams than sodium caseinate. Among the whipped toppings prepared with MPCs, sample B had the most stable foam. This reinforces the fact that this particular sample was the one that could most closely perform like sodium caseinate in this system.
Figure 4.5: Whipped Topping Foaming for Selected Commercial MPC Samples

Note: Different letter subscripts indicate significantly different samples.
4.4 Results – Custom-Made Samples

In the previous section it was shown that high protein commercial MPC samples from different manufacturers exhibited different functionalities. However, no information was available about the processing conditions of those samples. In trying to find out the basis for the differences, production of MPC samples under known conditions was consigned to two processing plants in two different countries. Table 4.6. shows the processing conditions used to manufacture the MPCs. Samples labeled “P”, “Q” and “R” were produced in a commercial plant and had a very high protein content (80%+). Samples “X”, “Y” and “Z” were produced in a pilot plant and had a protein content around 66%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Heat Treatment prior to Spray Drying</th>
<th>Spray Drying Temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air Inlet</td>
</tr>
<tr>
<td>P</td>
<td>72°C/15 sec</td>
<td>160°C</td>
</tr>
<tr>
<td>Q</td>
<td>76°C/15 sec</td>
<td>200°C</td>
</tr>
<tr>
<td>R</td>
<td>80°C/15 sec</td>
<td>250°C</td>
</tr>
<tr>
<td>X</td>
<td>76°C/15 sec</td>
<td>300°C</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4: Processing Conditions for Custom-Made MPC Samples

Protein content and viscosity of the samples are given in Table 4.5. It can be noticed that the high protein samples (P, Q, R) had much higher viscosity than the lower protein samples (X, Y, Z). Viscosity was higher for the samples treated at higher temperatures.
prior to spray drying (R>Q>R). There was a small increase in viscosity for the samples dried at higher air inlet temperatures, but the magnitudes of the differences are not significant (Z>Y>Z).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein Content</th>
<th>Viscosity 10% Solution* (mPa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>81.6%</td>
<td>855</td>
</tr>
<tr>
<td>Q</td>
<td>80.7%</td>
<td>1067</td>
</tr>
<tr>
<td>R</td>
<td>82.5%</td>
<td>1359</td>
</tr>
<tr>
<td>X</td>
<td>66.3%</td>
<td>18</td>
</tr>
<tr>
<td>Y</td>
<td>66.0%</td>
<td>33</td>
</tr>
<tr>
<td>Z</td>
<td>66.2%</td>
<td>34</td>
</tr>
</tbody>
</table>

*After 5 min. at 5 rpm.

Table 4.5: Protein Content and Viscosity of Custom-Made MPC Samples

4.4.1 Particle Size

Particle size distributions were determined for the custom-made MPC samples after hydration for one hour and after treating with ultrasonication and β-mercaptoethanol as dissociating agents. The size distributions for samples P, Q, and R are shown in Figures 4.6 through 4.8. Figures 4.9 to 4.11 show the particle size distributions for samples X, Y and Z.
Figure 4.6: Particle Size Distribution after 1 hour hydration, custom-made MPC samples P, Q & R.

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>Sample</th>
<th>Sample</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>P</td>
<td>Q</td>
<td>R</td>
</tr>
<tr>
<td>68.95 µ</td>
<td>83.06 µ</td>
<td>66.54 µ</td>
<td></td>
</tr>
<tr>
<td>D[3,2]</td>
<td>P</td>
<td>Q</td>
<td>R</td>
</tr>
<tr>
<td>5.91 µ</td>
<td>10.71 µ</td>
<td>11.81 µ</td>
<td></td>
</tr>
<tr>
<td>D(v,0.5)</td>
<td>P</td>
<td>Q</td>
<td>R</td>
</tr>
<tr>
<td>46.98 µ</td>
<td>50.41 µ</td>
<td>45.99 µ</td>
<td></td>
</tr>
<tr>
<td>Sp. Surface Area</td>
<td>P</td>
<td>Q</td>
<td>R</td>
</tr>
<tr>
<td>1.0148 m²/g</td>
<td>0.5603 m²/g</td>
<td>0.5082 m²/g</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6: Particle Size Diameters for samples P,Q,R after 1 hour hydration
Figure 4.7: Particle Size Distribution after 5 min. ultrasonication, custom-made MPC samples P, Q & R.

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>Sample</th>
<th>Sample</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>44.97 μ</td>
<td>43.27 μ</td>
<td>43.59 μ</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>0.95 μ</td>
<td>1.23 μ</td>
<td>1.23 μ</td>
</tr>
<tr>
<td>D(v,0.5)</td>
<td>26.01 μ</td>
<td>25.21 μ</td>
<td>25.21 μ</td>
</tr>
<tr>
<td>Sp. Surface Area</td>
<td>6.3347 m²/g</td>
<td>4.8809 m²/g</td>
<td>4.8809 m²/g</td>
</tr>
</tbody>
</table>

Table 4.7: Particle Size Diameters for samples P, Q, R after 5 min. ultrasonication.
Figure 4.8: Particle Size Distribution after treating with β-mercaptoethanol, custom-made MPC samples P, Q & R.

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>Sample P</th>
<th>Sample Q</th>
<th>Sample R</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>33.31 μ</td>
<td>33.86 μ</td>
<td>35.91 μ</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>1.14 μ</td>
<td>0.66 μ</td>
<td>0.96 μ</td>
</tr>
<tr>
<td>D(ν,0.5)</td>
<td>22.95 μ</td>
<td>17.54 μ</td>
<td>24.74 μ</td>
</tr>
<tr>
<td>Sp. Surface Area</td>
<td>5.2713 m²/g</td>
<td>9.0609 m²/g</td>
<td>6.2333 m²/g</td>
</tr>
</tbody>
</table>

Table 1: Particle Size Diameters for samples P, Q, R after treating with β-mercaptoethanol

54
Figure 4.9: Particle Size Distribution after 1 hour hydration, custom-made MPC samples X, Y & Z.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Particle Size</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D[4,3]</td>
<td>41.76 μ</td>
<td>65.49 μ</td>
<td>79.99 μ</td>
</tr>
<tr>
<td></td>
<td>D[3,2]</td>
<td>3.09 μ</td>
<td>11.72 μ</td>
<td>11.27 μ</td>
</tr>
<tr>
<td></td>
<td>D(v,0.5)</td>
<td>35.77 μ</td>
<td>57.46 μ</td>
<td>58.41 μ</td>
</tr>
<tr>
<td></td>
<td>Sp. Surface Area</td>
<td>1.9408 m²/g</td>
<td>0.5120 m²/g</td>
<td>0.5323 m²/g</td>
</tr>
</tbody>
</table>

Table 4.9: Particle Size Diameters for samples X, Y, Z after 1 hour hydration
Figure 4.10: Particle Size Distribution after 5 min. ultrasonication, custom-made MPC samples X, Y & Z.

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>Sample</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td></td>
<td>25.27 µ</td>
<td>47.84 µ</td>
<td>72.66 µ</td>
</tr>
<tr>
<td>D[3,2]</td>
<td></td>
<td>0.70 µ</td>
<td>1.31 µ</td>
<td>2.57 µ</td>
</tr>
<tr>
<td>D(v,0.5)</td>
<td></td>
<td>11.48 µ</td>
<td>31.66 µ</td>
<td>46.04 µ</td>
</tr>
<tr>
<td>Sp. Surface Area</td>
<td></td>
<td>8.5679 m²/g</td>
<td>4.5636 m²/g</td>
<td>2.3307 m²/g</td>
</tr>
</tbody>
</table>

Table 4.10: Particle Size Diameters for samples X, Y, Z after ultrasonication
Figure 4.11: Particle Size Distribution after treating with β-mercaptoethanol, custom-made MPC samples X, Y & Z.

<table>
<thead>
<tr>
<th>Sample</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>18.28 μm</td>
<td>41.47 μm</td>
<td>50.90 μm</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>0.58 μm</td>
<td>1.88 μm</td>
<td>4.77 μm</td>
</tr>
<tr>
<td>D(v,0.5)</td>
<td>0.52 μm</td>
<td>32.09 μm</td>
<td>37.89 μm</td>
</tr>
<tr>
<td>Sp. Surface Area</td>
<td>10.4276 m²/g</td>
<td>3.1985 m²/g</td>
<td>1.2583 m²/g</td>
</tr>
</tbody>
</table>

Table 4.11: Particle Size Diameters for samples X,Y,Z after treating with β-mercaptoethanol
Samples P, Q, and R had much larger particles than X, Y and Z. The former were also more resistant to dissociation by ultrasonication and β-mercaptoethanol than the latter. However the set of samples P, Q, R was produced under very different processing conditions than X, Y, and Z. P, Q and R had a much larger protein content and were dried under commercial conditions, which allows to use milder drying temperatures while removing the desired amount of moisture. X, Y and Z, on the other hand, had been produced in a pilot plant, using higher temperatures and only one-stage drying. They had smaller protein content, so the chances for protein-protein interactions were smaller than for samples P, Q, R.

For samples P, Q, and R, even though there was a small increase in viscosity of a 10% solution, but there was no significant change in the particle size characteristics due to the heat treatment of milk prior to spray drying. This could be explained by the fact that the different heat treatments used did not cover a large range of temperatures (72°, 76°C and 80°C for 15 seconds). Also, the spray drying conditions were very mild in terms of air inlet and outlet temperatures.

Samples X, Y, Z showed some differences in particle size. These differences were more noticeable when comparing the samples produced at 200°C and 250°C air inlet temperature (X and Y, respectively). Increasing the inlet temperature from 250°C to 300°C did not produce significant changes in particle size and dissociation.
These observations led us to believe that spray drying conditions can indeed play a role in the formation of particles with different sizes and dissociation behaviors. Dissociability of particles might be related to the ability of MPCs to interact with other ingredients in the food, and therefore it might be related to its functionality.

In the next part of this document, results from a spray drying experiment where the air outlet temperature was varied will be shown.
CHAPTER 5

SPRAY DRYING EXPERIMENTS

In the previous chapter, results from basic functionality tests in commercial MPC samples of varying protein contents were provided. It was shown that commercial products exhibit a wide range of functional properties that were not correlated to protein content or solubility. Selected MPC samples showed that there were also differences in functionality in model food systems. Some custom-made MPC samples were analyzed for their particle characteristics, indicating the possibility that varying the spray drying conditions could result in the formation of particles with different characteristics. This could have an incidence in product functionality as an ingredient.

This chapter covers the spray drying experiments. MPCs were produced by varying the air outlet temperature and the nozzle diameter.
5.1 Hypotheses

1. Higher heat treatments during spray drying, which can be applied by increasing the air outlet temperature or by increasing the atomized droplet size, can result in the formation of larger MPC aggregates.

2. The formation of aggregates in MPC manufacture is the result of protein polymerization, either by denaturation of whey protein and complexation with the caseins or as induced by intermediate products from the Maillard reaction.

3. Differences in the dissociability of MPC aggregates reflect different bonding mechanisms at lower and higher drying temperatures.

5.2 MPC Preparation

Skim milk had been previously pasteurized and ultrafiltered (UF) at 50°C in commercial dairy plant in Canton, Ohio. The retentate was chilled to 4°C and shipped overnight to another plant in LaCrosse, Wisconsin, where it was spray-dried. All of the UF skim milk retentates came from a single production lot, so confounding effect due to possible differences in the raw material could be avoided. The retentate from UF skim milk was kept refrigerated throughout the production period. At the time of spray drying, the
needed amount of retentate was warmed up to 50°C in a water bath in order to reduce viscosity and facilitate pumping and spray drying.

The UF skim milk retentate was dehydrated in a Niro portable spray dryer equipped with a two-fluid nozzle. The spray dryer had a conical-based drying chamber with a cylindrical height of 600 mm and a diameter of 800 mm. An air flow rate of 75% was used, providing an air pressure of ca. 2.55 bar. The air inlet temperature was set at 200°C, while the outlet temperature was varied.

5.3 Experimental Design

Time and raw material constraints limited the number of batches that could be run to produce MPC. Two factors were varied: air outlet temperature and atomizer orifice diameter. With respect to the air outlet temperature, 65°C turned out to be the lowest possible temperature within practical limits for the particular spray dryer used. The orifice diameters used were dictated by availability of nozzles.

The treatments used are summarized in Table 5.1. The figures in the cells indicate the number of replicates for each treatment.
As can be seen from Table 5.1, all treatments, except for the one where the outlet temperature was set at 70°C, were run in duplicates. The order in which the treatments were ran was randomized.

### 5.4 Analyses

#### 5.4.1 Moisture Content

Moisture content was calculated as the weight loss after drying ca. 3 g on an atmospheric pressure oven at 100°C for 24 hours and expressed as a percentage of the initial weight of the sample.

<table>
<thead>
<tr>
<th>Spray Dryer Outlet Temp.</th>
<th>Nozzle Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>65°C</td>
<td>-</td>
</tr>
<tr>
<td>70°C</td>
<td>-</td>
</tr>
<tr>
<td>75°C</td>
<td>2</td>
</tr>
<tr>
<td>85°C</td>
<td>-</td>
</tr>
<tr>
<td>90°C</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.1: Drying conditions used for producing MPCs
5.4.2 Water Activity

Water activity (Aw) was determined instrumentally using a CX-2 AquaLab water activity analyzer (Decagon Devices, Inc.). The instrument uses a chilled-mirror dewpoint to determine Aw.

5.4.3 Protein Content and Solubility

These were determined as described in Chapter 4. Solubility was determined both at pH 7.0 and 4.5.

5.4.4 Bulk Density

Apparent (bulk) density was measured by filling a tared 25 ml graduated cylinder with powder and tapping the cylinder 20 times. The weight of the filled cylinder was divided by the volume of the powder. Bulk density is typically expressed in g/ml.

5.4.5 Particle Size Analysis

Particle size distributions were determined as described in Chapter 4.
5.4.6 Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis is a separation technique based on the differences in the net charge of macromolecules, which allow them to migrate in an electric field. Since proteins contain basic and acidic amino acids, they will be able to move in a solvent as a result of the electric field, provided the pH is different than the isoelectric one (i.e., that in which the protein’s net charge is zero).

Polyacrylamide gels are the preferred matrix used in electrophoretic analysis of milk proteins. These gels typically use a denaturing agent, sodium dodecyl sulfate (SDS), which can break down hydrophobic interactions. The technique is typically referred to as SDS-PAGE (Sodium Dodecyl Sulfate - PolyAcrylamide Gel Electrophoresis). A pore size close to the macromolecule’s actual size needs to be used in order to obtain a good separation. Pore size is controlled by the polyacrylamide concentration and the degree of cross-linking in the gel.

The size to charge ratio is important in determining electrophoretic mobility. When SDS is used, this molecule binds to the proteins through hydrophobic interactions, resulting in a high negative charge in the molecule (Grappin and Ribadeu-Dumas, 1992). The size to charge ratio being roughly equal in the proteins, size of the molecule determines its mobility. The distance of migration is proportional to the logarithm of the molecular
weight, so by using standards of known molecular weight, it is possible to estimate the molecular weight of the protein bands.

In this research, milk protein concentrates were analyzed by SDS-PAGE (Mini-Protean II System, Bio-Rad Laboratories) under reducing and non-reducing conditions. Samples were prepared by making a 1% protein solution and diluting it 1:4 with sample buffer. The sample buffer was prepared as shown in Table 5.2. After diluting the milk protein solutions with sample buffer, the vials were immersed in boiling water for 4 minutes.

<table>
<thead>
<tr>
<th>Reducing (%v/v)</th>
<th>Non-Reducing (%v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>47.5</td>
</tr>
<tr>
<td>0.5 M Tris-HCl buffer (pH 6.8)</td>
<td>12.5</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10.0</td>
</tr>
<tr>
<td>10% (w/v) SDS</td>
<td>20.0</td>
</tr>
<tr>
<td>β-Mercaptoethanol</td>
<td>5.0</td>
</tr>
<tr>
<td>0.5% Bromophenol Blue</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Table 5.2: Sample Buffer Recipes for SDS-PAGE**

Polyacrylamide gels were prepared by mixing 30:0.8 Acrylamide:Bis with water, and Tris-SDS-HCl buffer (pH 8.8 for resolving gel and 6.8 for stacking gel). Polymerization was induced by adding 10% Ammonium Persulfate as a source of free radicals and TEMED (N, N, N', N'-tetramethylethlenediamine) as a catalyst. A monomer concentration of 15% was used for the resolving gel while the stacking gel had a 4%
concentration. A Tris-Glycine-SDS buffer, pH 8.3, (Bio-Rad Laboratories) was used for filling the running chamber.

The gels were casted by hand on 10 x 7 cm mini-gel glass plates using 1 mm spacers. 8 μl of sample were loaded in each well. A broad range molecular weight standard (14.4 to 200 kDa) was purchased from Bio-Rad Laboratories and used as a reference. Pure bovine serum albumin (BSA), β-casein, β-lactoglobulin and α-lactalbumin (Bio-Rad Laboratories) were used to prepare a milk protein standard, which was also used as a reference in some of the gels.

Electrophoresis was performed under a constant current of 50 mAmps. The gels were stained overnight on Coomassie® brilliant blue R-250. The gels were destained by immersing them in a solution containing 40% methanol and 10% acetic acid for half an hour. After this, the gels were destained in by 10% acetic acid for about eight hours, changing the destaining solution several times throughout the destaining period. This destaining method was found to provide a clear background without leaching of the stained protein bands.

Densitometric scanning (GS-700 Densitometer, Bio-Rad) of the gels was used to acquire the electrophoregrams and analyze the profiles on each lane to determine the relative mobility (Rf) of individual peaks.
5.5 **Theoretical Framework**

Before proceeding with a more in-depth discussion of the results, it is necessary to define some of the terms used throughout this chapter. Specifically, the concepts of particle, aggregation and polymerization will be discussed in this section.

5.5.1 **Particle**

The notion of particle is one of great interest at an industrial level. Particle size analysis is used in fields like geology and soil science, metallurgy, pharmaceutics, industrial hygiene, environmental science, and food science, among many others. Particle size standards are usually specified for food powders. Standards have been traditionally set by sieving, and expressed as a mesh number.

Much confusion exists in the way the term “particle” has been defined by various scientists. In some instances, the words “particle” and “particulate” are used interchangeably. Kaye (1981) proposed the use of the term “fineparticle” to refer to finely divided substances (e.g., powders, smokes, aerosols, etc.). His argument was that the word “particle” had already been well defined and established by physicists as a structural component of the atom. However, most authors do use the term particle in a macromolecular context.
Groves (1992) provides the following definition for particle:

*A particle is a discrete entity of solid or liquid matter existing in a dispersed state with a diameter at or below approximately 50 μm.*

The author notes, however, that there is no complete agreement on what is the maximum size of a particle. According to Groves (1992), other researchers place this upper limit at 1000 μm diameter. Allen (1968) defined particle simply as “an aggregation of molecules without limit to the upper size”. Cadle (1965) limited his definition of particle to those with diameters between 0.5 and 10,000 μm. Kaye (1981) stated that fineparticles have dimensions of 0.1-2,000 μm diameter. However, there exists some agreement about the fact that there is a well defined boundary between the dispersed phase and the dispersant, irrespective of the size of the particle (Cadle, 1965; Groves, 1992).

Particle size is usually expressed as the diameter of an equivalent sphere with the same volume or surface area as the particle (Groves, 1984). This simplifies enormously the particles’ characterization, since only one dimension, i.e., the diameter, is needed to describe their size.

Most methods of particle size analysis do not take into consideration the shape of the particles. Morphology, or overall shape, and more specifically the topography (profile outline), topology (basic structure with respect to included holes) and texture (detailed structure of elements in the surface of the profile) are all very important particle characteristics (Kaye, 1981). Particle shape, however, is difficult to assess.
The limits that should be used to characterize particles within a certain size range are imprecise. Groves (1992) arbitrarily defines a colloidal range to be between 100 nm-10 μm diameter and states that particles smaller than 100 nm can be considered molecular aggregates. In an earlier publication (Groves, 1984), the author defines the size of a colloid to be 1-100nm. The emphasis was, however, placed on submicroscopic particles that would be below the detection level of the microscope but still detectable by the Tindall beam effect.

Particle size is an important consideration when choosing the method used for size determination. The size of particles can be determined by either direct or indirect methods. Direct methods include microscopy, sieving and sedimentation, while indirect methods are based on a physical principle like drag in turbulent flow, adsorption of dyes, or light scattering. As Groves (1992) points out, the only absolute direct method is light microscopy, but sizes under 1-2 μm cannot be accurately viewed. This method is not only limited in terms of the size that the microscope can discriminate, but sampling errors can be great (Rawle, 1994). Sieving is limited to very large particles, typically greater than 37 μm or 400# mesh (Allen, 1992). Sedimentation is limited to particles of 2-50 μm and it is not suitable for emulsions (Rawle, 1994).

Light scattering methods use mathematical models to estimate particle diameter based on the way in which they interact with light. The choice of mathematical model depends on
the size of the particle relative to the wavelength of the light source and on optical properties of both the particles and the medium in which they are dispersed (e.g., refractive index, and absorbance). Rayleigh scattering theory is used when the particles are much smaller than the light’s wavelength, while Mie scattering theory is used for particles very close or larger in size than the wavelength of the light source (Cadle, 1965; Plantz, 1984). Fraunhofer models can be used when the particles are very large respective to the light’s wavelength (Plantz, 1984).

Since the instrument used in this research for the determination of particle size distributions encompasses a size range of 0.05-550 μm, the discussion of particles will be limited to this size.

5.5.2 Aggregation and Polymerization

The meaning of the term “aggregate” needs to be placed in the appropriate context. When referring to particles, Kaye (1981) distinguishes between “aggregate” and “agglomerate” depending on how strongly the particles are associated. An aggregate would be a loose association of particles, likely to disintegrate during its operational life, while an agglomerate would tend to maintain its identity throughout the operational system. Irani and Callis (1963) defined these two terms in an opposite way. An aggregate, according to these authors, is “a particle or assembly of particles held together
by strong inter- or intramolecular or atomic cohesive forces”. In contrast, when particles are held together by weak cohesive forces, then they would form an agglomerate. Jelinek (1970) uses only the term agglomerate, without distinguishing whether the process by which this was formed was reversible or irreversible. Although there is contradictory use of the terms aggregate and agglomerate, the different authors coincide in the fact that there are “primary” particles that can associate—either weakly or strongly—into larger or “secondary” particles, called either aggregates or agglomerates.

In dairy processing, ‘agglomeration’ is specific used to describe a process by which the particle size is increased on purpose to improve the powder’s dispersibility, wettability and sinkability (Hall and Hedrick, 1971; Campbell and Marshall, 1975). For this reason, in the particle size discussion, we will favor the use of the term ‘aggregate’ if there is reason to suspect that it has resulted from the association of smaller or primary particles.

Aggregation could occur as a result of physical phenomena at the macromolecular level. For example, moisture sorption in a food powder can result in the formation of inter-particle liquid bridges that can increase powder cohesiveness. Humidity caking can result in particles of larger size (Peleg, 1985).

When referring to proteins at the molecular level, the terms aggregation acquires a different meaning. Protein “aggregation” and “polymerization” are used interchangeably in the literature. For example, Kato et al. (1989), use both terms. Matzinos and Hall
(1993) say that "heat-induced aggregation and denaturation of proteins were revealed by the appearance or disappearance, and the dye intensity reduction, of some protein bands in the electrophoretic patterns". Monahan et al. (1995) use a very similar definition for polymerization, when they define it as "the appearance of high molecular weight protein bands in the stacking gel and at the top of the resolving gel with a concomitant decrease in the intensity of the monomeric protein bands".

Due to the lack of convention with respect to these terms, the definition by Monahan et al. (1995) will be used in this research.

The classical definition of a polymer implies homogeneity (repeating units). In food chemistry, polymers are more easily understood when studying polysaccharides. For example, starch is a polymer of glucose, pectin is a polymer of galacturonic acid, and so on. When there exists a heterogeneous mixture of proteins, as it is the case for milk protein concentrates, then polymerization, even if using the definition provided by Monahan et al. (1995), needs to be seen in a broader sense. The disappearance of monomeric bands does not necessarily imply that polymers of the same sub-unit have been formed. It is possible that complexes can be formed with different proteins (For example: $\alpha_{s1}$-casein and $\beta$-lactoglobulin; vs. dimers or polymers of $\beta$-lactoglobulin).

Many of the studies of protein polymerization have been conducted using simple models. For example: ovalbumin-glucose; $\beta$-lactoglobulin-glucose or -lactose; etc. While these
are useful to elucidate the mechanisms of a reaction, they may not necessarily reflect what happens when the system is much more complex.

5.6 Results and Discussion

5.6.1 Ultrafiltered Skim Milk Retentate Characteristics

The characteristics of the ultrafiltered skim milk retentate are summarized in Table 5.3:

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>19.06%</td>
<td>0.215%</td>
</tr>
<tr>
<td>Protein (wet basis)</td>
<td>13.54%</td>
<td>0.004%</td>
</tr>
<tr>
<td>Protein (dry basis)</td>
<td>71.01%</td>
<td>—</td>
</tr>
<tr>
<td>Protein solubility at pH 7.0</td>
<td>45.18%</td>
<td>0.015%</td>
</tr>
<tr>
<td>Protein solubility at pH 4.6</td>
<td>18.13%</td>
<td>0.002%</td>
</tr>
</tbody>
</table>

Table 5.3: UF Skim Milk Retentate Characteristics

Since the UF skim milk retentate was kept refrigerated until the spray drying, when it was warmed up to 50°C in a water bath, viscosity was determined at various temperatures to verify that heating the retentate resulted in a reasonable reduction of viscosity. Results are shown in Figure 5.1.
Holding the UF skim milk retentate at 50°C for the length of drying (2-3 hours for each batch) did not appear to have a significant effect on protein denaturation. This was verified by comparing the protein solubility of the refrigerated retentate versus that for retentate heated and held at 50°C for 2.5 hours. There was no statistically significant difference for the protein solubility at pH 4.6.
5.6.2 Milk Protein Concentrates

5.6.2.1 Temperature Effect

5.6.2.1.1 Moisture Content and Water Activity

The moisture content varied from 2.95% to 9.11% for the samples dried at high and low outlet temperatures respectively. The reason for such a large variation is that the spray dryer air outlet temperature can only be controlled by varying the speed at which the retentate is being pumped into the atomizer. For lower air outlet temperatures a high pumping speed is needed. Conversely, if the flow into the atomizer is slow, this will result in a higher air outlet temperature.

Although no standards of identity have been set yet for MPCs, commercial dried milks typically specify a maximum moisture level of 4 percent (21 CFR). Hence, the MPCs dried at the lower temperatures in this experiment (75°C and below) would not be commercially acceptable products.

A boxplot of moisture content for the various air outlet temperatures used is shown in Figure 5.2. The center line on each box indicates the median value, while the edges of each box mark the 25% and the 75% quartiles. The mean is indicated by a solid circle.
Same subscripts next to each bar indicate that differences are not statistically significant at $\alpha = 0.05$.

Figure 5.2: Moisture Content vs. Spray Dryer Outlet Temperature

A similar pattern was seen for water activity. Water activity ($A_w$) was determined instrumentally based on the chilled-mirror dewpoint by using a CX-2 AquaLab water activity analyzer (Decagon Devices, Inc.). Water activity values are depicted in Figure 5.3.
Although the term water activity (\(A_w\)) is used throughout this document, it should be noted that many authors deem as more appropriate the term "relative vapor pressure", since this is what is really determined using laboratory instruments while true \(A_w\) is a term derived from thermodynamics, rarely measured for foods (Fennema, 1995).

Figure 5.3: Water Activity vs. Spray Dryer Outlet Temperature

5.6.2.1.2 Protein Content

The MPCs had an average crude protein content of 69.5\% (±0.637\%) on a dry basis, as determined by micro-Kjeldahl (\%N × 6.38).
5.6.2.1.3 Protein Solubility at Neutral pH (7.0)

As can be seen from Figure 5.4, there was no statistically significant difference for the protein solubility at neutral pH of the MPCs dried at the different air outlet temperatures.

![Box plot showing protein solubility at pH 7.0](image)

**Figure 5.4:** Protein Solubility at pH 7.0 vs. Spray Dryer Outlet Temperature.

5.6.2.1.4 Protein Solubility at the Isoelectric pH (4.6)

Protein solubility at the isoelectric pH of milk is a crude measure of the solubility of whey proteins, since the caseins precipitate at this pH. Because the whey proteins are

79
heat labile, the loss of solubility at pH 4.6 can provide an indication of whey protein denaturation (Kinsella, 1984). Solubility at pH 4.6 for the MPCs dried at different temperatures is shown in Figure 5.5.

![Box plot showing protein solubility at pH 4.6 vs. spray dryer outlet temperature.](image)

**Figure 5.5: Protein Solubility at pH 4.6 vs. Spray Dryer Outlet Temperature**

Although protein solubility at pH 4.6 for the sample dried at 70°C outlet temperature seems to be lower than the rest in the graph, only two replicates were available for statistical analysis, so the difference can be considered irrelevant. The differences were not significantly different at $\alpha = 0.05$, as indicated by like subscripts next to each bar.
Therefore, for the temperature range used, the higher outlet temperature (90°C) did not result in detectable protein denaturation of the whey proteins.

It should be noted that the relative content of whey and caseins in MPCs should be the same as in milk, since all the proteins are retained in the ultrafiltrate. Milk proteins are comprised of around 80% protein (Swaisgood, 1996). Since the caseins are precipitated at pH 4.6, the remaining 20% of the total proteins present in the product, might be too low to provide an accurate indicator of heat damage in MPCs by determining its solubility.

5.6.2.1.5 Bulk Density

An indirect relationship between packed bulk density and the spray dryer outlet temperature was found, as shown in Figure 5.6. The MPCs dried at lower temperatures had considerably higher bulk density than those dried at higher temperatures. Typical values for spray dried milk range between 0.5-0.6 g/ml (Hall and Hedrick, 1971). The fact that all of the MPCs had a lower bulk density than the typical values for spray dried milk could be attributed to the fact that the total solids in the feed were much smaller than the solids concentration used in commercial operations (Hall and Hedrick, 1971; Mistry and Pulgar, 1996).
Figure 5.6: Bulk Density of MPCs dried at various spray dryer air outlet temperatures

Bulk density has a direct incidence on packaging costs (Hall and Hedrick, 1971). The fact that the MPCs spray dried at lower air outlet temperatures had higher bulk density means that the powder can pack more closely, reducing the amount of air voids between adjacent particles. True density, or the density of the powder devoid of entrapped air, either within the particles or between them, is larger in the MPCs dried at higher air outlet temperatures because they had a significantly lower moisture content.
5.6.2.1.6 Particle Size

A comparison of the different particle sizes is difficult because with the instrument used, a particle size distribution is obtained. The size distributions are usually polydispersed, i.e., they might be bi-modal or multi-modal and they might deviate considerably from a normal distribution. Typically, some of the following mean diameters are used:

\[ D[4,3] = \text{Equivalent volume mean diameter} = \frac{\sum d^4}{\sum d^3} \]

\[ D[3,2] = \text{Equivalent surface area mean diameter} = \frac{\sum d^3}{\sum d^2} \]

\[ D(v,0.5) = \text{Volume Median Diameter} \]

In a normal distribution, all the different ways to calculate a mean diameter should yield the same result. However, for polydispersed distributions, each of the above mean diameters can produce very different results (Rawle, 1994). The equivalent surface area mean diameter \( D[3,2] \), also known as the Sauter mean diameter, is probably the one that can best relate to ingredient functionality, since chemical reactivity will be influenced by surface area.

Figure 5.6 shows the particle size distributions for the MPCs produced at the different temperatures. The distributions were obtained after making a 10% protein solution which was hydrated for 1 hour and dispersing it in 500ml of water until a laser beam
obscuration of 10-30% was obtained. The mean diameters are tabulated below each chart. Volume percent distributions are shown because this is the type of distribution calculated by the instrument’s software using the mathematical equations and algorithms based on Mie’s diffraction theory. Volume distributions can be transformed into Surface or Number distributions, but the manufacturer warns against this kind of transformation, as they greatly increase the probability of calculation errors (Mastersizer μ+, version 2.15 Software Manual).

The volume distributions of all of the MPCs show two distinct peaks after one hour hydration. The first peak is around 0.3μm, while the second peak is between 50 and 80 μm particle diameter. The samples produced at higher spray dryer air outlet temperatures (85°C and 90°C) show a noticeable higher volume percent of particles of large diameter.
Figure 5.7: Particle size distributions after 1 hour hydration, MPCs produced at various spray dryer air outlet temperatures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>65°C</th>
<th>70°C</th>
<th>75°C</th>
<th>85°C</th>
<th>90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>35.18</td>
<td>32.28</td>
<td>49.83</td>
<td>58.79</td>
<td>58.37</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>μm</td>
<td>1.06</td>
<td>0.88</td>
<td>1.52</td>
<td>6.68</td>
<td>11.44</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>23.49</td>
<td>19.25</td>
<td>38.39</td>
<td>53.45</td>
<td>53.63</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>5.6592</td>
<td>6.7826</td>
<td>3.9534</td>
<td>0.8981</td>
<td>0.5243</td>
</tr>
</tbody>
</table>

Table 5.4: Mean particle diameters after 1 hour hydration.
The particles at around 0.3 μm diameter can be considered primary particles while the large size ones will be referred as aggregates in this discussion.

In order to test the molecular forces involved in holding the aggregates, various treatments were given to the samples. These were the addition of sodium dodecyl sulfate (SDS, an anionic detergent), β-mercaptoethanol (a reducing agent) and ultrasonic energy.

MPCs produced at 85°C and 90°C outlet temperatures were more resistant to dissociation using ultrasonic energy than the ones produced at lower air outlet temperatures. This is depicted in Figure 5.8. Ultrasonication probably have multiple effects in terms of the molecular forces it can disrupt, so the only inference that can be made based on the particle size distributions after ultrasonication is that the aggregates present in higher temperature MPCs were more difficult to break down.
Figure 5.8: Particle Size Distributions after Ultrasonication, MPCs produced at various spray dryer air outlet temperatures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>65°C</th>
<th>70°C</th>
<th>75°C</th>
<th>85°C</th>
<th>90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>4.73</td>
<td>1.34</td>
<td>1.71</td>
<td>15.55</td>
<td>37.71</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>μm</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.46</td>
<td>0.92</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
<td>0.44</td>
<td>26.21</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>16.0709</td>
<td>16.3802</td>
<td>16.3054</td>
<td>12.4417</td>
<td>6.4919</td>
</tr>
</tbody>
</table>

Table 5.5: Mean Particle Diameters after Ultrasonication
The particle size distributions after treating with SDS are shown in Figure 5.9. Most polypeptides bind SDS in a constant ratio of 1.4g of SDS per gram of protein (Hames, 1990). However, this binding ratio is sought for electrophoretic separations, where a complete dissociation of the proteins into their monomeric units is needed for molecular weight determinations. In this case, the total concentration of SDS in the dispersant was 0.05% and the protein concentration was typically 0.06% or less. The binding ratio might be less than 1.4, but it was assumed that SDS concentration was large enough to break existing hydrophobic associations in the MPC particles. A reduction in particle size after 5 minutes exposure to SDS at room temperature could reflect the importance of hydrophobic interactions for particle aggregation.

It can be seen from Figure 5.9 that most of the MPCs were solubilized by SDS, reducing the particles to the primary particle size range. Although it seems graphically that the sample dried at 90°C remained in the aggregate state, a comparison of the values given in Table 5.6 with those in Table 5.4 reveals that the specific surface area experienced a more than tenfold increase in the samples dried at high air outlet temperatures (85°C and 90°C), while the specific surface area was doubled or tripled in the samples dried at low air outlet temperatures (65°C, 70°C and 75°C). These changes indicate that hydrophobic interactions are an important factor in aggregate formation for the higher temperature MPCs.
Figure 5.9: Particle Size Distributions after treating with SDS, MPCs produced at various spray dryer air outlet temperatures.

### Table 5.6: Mean Particle Diameters after treating with SDS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>65°C</th>
<th>70°C</th>
<th>75°C</th>
<th>85°C</th>
<th>90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>12.01</td>
<td>6.28</td>
<td>7.44</td>
<td>28.00</td>
<td>41.27</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>μm</td>
<td>0.46</td>
<td>0.46</td>
<td>0.48</td>
<td>0.43</td>
<td>1.04</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>0.44</td>
<td>0.44</td>
<td>0.44</td>
<td>0.48</td>
<td>42.40</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>12.9435</td>
<td>13.1692</td>
<td>12.5271</td>
<td>13.7934</td>
<td>5.7591</td>
</tr>
</tbody>
</table>
The treatment with a reducing agent may show the importance of disulfide bridges in the formation of aggregates. The particle size distributions after treating with β-mercaptoethanol are shown in Figure 5.10. These results also show significant reductions in particle size as compared to the initial ones.

β-mercaptoethanol was added to the dispersant in a concentration of 0.1% v/v. Sample buffers used for electrophoresis under reducing conditions contain a much larger concentration of with β-mercaptoethanol, around 5%. In electrophoresis, however, a complete dissociation of the proteins and reduction of disulfide linkages is needed in order to obtain a good resolution of the protein bands in the gels. For electrophoresis separations, reducing agents are always added in conjunction to detergents and boiled for a few minutes. Detergents and high temperatures can partially unfold the proteins, making the internal disulfide linkages accessible to the reducing agent.

The purpose of adding with β-mercaptoethanol to the dispersing medium during particle size determinations was not to achieve a complete reduction of all the disulfide linkages in the protein, but rather to reduce disulfide bonds that could constitute a cohesive force in the formation of large aggregates. Conceivably, these disulfide bonds would be relatively accessible to the reducing agent. Therefore, the exposure of the MPC particles to the reducing agent was conducted under very mild conditions (i.e., room temperature, relatively low concentration of β-mercaptoethanol and lack of detergent).
Figure 5.10: Particle size distributions after treating with β-mercaptoethanol, MPCs produced at various spray dryer air outlet temperatures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>65°C</th>
<th>70°C</th>
<th>75°C</th>
<th>85°C</th>
<th>90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>6.56</td>
<td>1.73</td>
<td>3.36</td>
<td>28.86</td>
<td>14.42</td>
</tr>
<tr>
<td>D[3.2]</td>
<td>μm</td>
<td>0.36</td>
<td>0.36</td>
<td>0.39</td>
<td>0.92</td>
<td>0.44</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>0.36</td>
<td>0.35</td>
<td>0.37</td>
<td>20.85</td>
<td>0.46</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>16.5818</td>
<td>16.4967</td>
<td>15.2834</td>
<td>6.5230</td>
<td>13.5752</td>
</tr>
</tbody>
</table>

Table 5.7: Mean Particle Diameters after treating with β-mercaptoethanol.
5.6.2.1.6.1 Hydration Characteristics

Solubility is recognized as a prerequisite for an ingredient to be functional in food systems (Kinsella, 1984; Damodaran, 1996). At an industrial level, the ingredient's ability to interact with water is evaluated using looser terms, such as dispersibility. Tests to assess dispersibility, though, are subject to a certain degree of subjectivity. In this research, it was found that hydration time has an important effect in the mean particle size distribution for some of the powders. Changes in particle size distribution were monitored during a five hour period. In general, it was found that the size of the particle decreased for all MPCs with longer hydration times. However, the MPCs produced at higher air outlet temperatures (85°C and 90°C) showed only a slight reduction in particle size, while those produced at lower temperatures (65°C and 75°C) hydrated relatively faster. Figures 5.11 through 5.14 show the change in particle size distributions with respect to hydration time.
Figure 5.11: Particle Size Distribution with different hydration times, 70% MPC dried at 65°C Spray Dryer Air Outlet Temperature.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>43.11</td>
<td>34.93</td>
<td>14.28</td>
<td>10.42</td>
<td>9.56</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>μm</td>
<td>1.18</td>
<td>0.77</td>
<td>0.53</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>28.49</td>
<td>17.12</td>
<td>0.46</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>5.1070</td>
<td>7.8678</td>
<td>11.6361</td>
<td>13.0954</td>
<td>13.1835</td>
</tr>
</tbody>
</table>

Note: Values are the average of two observations.

Table 5.8: Mean particle diameters for different hydration times, MPC dried at 65°C air outlet temperature.
Figure 5.12: Particle Size Distribution with different hydration times, 70% MPC dried at 75°C Spray Dryer Air Outlet Temperature.

Table 5.9: Mean particle diameters for different hydration times, MPC dried at 75°C air outlet temperature.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>33.74</td>
<td>17.11</td>
<td>8.69</td>
<td>3.73</td>
<td>5.46</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>μm</td>
<td>0.78</td>
<td>0.50</td>
<td>0.43</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>19.59</td>
<td>0.47</td>
<td>0.39</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>7.3916</td>
<td>12.1449</td>
<td>14.2279</td>
<td>15.8505</td>
<td>15.4658</td>
</tr>
</tbody>
</table>

Note: Values are the average of two observations.
Figure 5.13: Particle Size Distribution vs. Hydration Time, 70% MPC dried at 85°C Spray Dryer Air Outlet Temperature.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{4,3}$</td>
<td>μm</td>
<td>64.79</td>
<td>63.94</td>
<td>58.98</td>
<td>43.73</td>
<td>29.71</td>
</tr>
<tr>
<td>$D_{3,2}$</td>
<td>μm</td>
<td>7.05</td>
<td>3.05</td>
<td>1.70</td>
<td>0.86</td>
<td>0.58</td>
</tr>
<tr>
<td>$D_{v,0.5}$</td>
<td>μm</td>
<td>57.41</td>
<td>53.88</td>
<td>47.13</td>
<td>27.11</td>
<td>3.74</td>
</tr>
<tr>
<td>SSA</td>
<td>m$^2$/g</td>
<td>0.8531</td>
<td>1.9669</td>
<td>3.6448</td>
<td>7.0414</td>
<td>10.3563</td>
</tr>
</tbody>
</table>

Note: Values are the average of two observations.

Table 5.10: Mean particle diameters for different hydration times, MPC dried at 85°C air outlet temperature.
Figure 5.14: Particle Size Distribution with different hydration times, 70% MFC dried at 90°C Spray Dryer Air Outlet Temperature.

Table S.II: Mean particle diameters for different hydration times, MFC dried at 90°C air outlet temperature.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>D[4,3]</td>
<td>μm</td>
<td>64.01</td>
<td>68.29</td>
<td>68.06</td>
<td>62.51</td>
<td>55.26</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>μm</td>
<td>11.38</td>
<td>6.88</td>
<td>3.78</td>
<td>2.02</td>
<td>1.38</td>
</tr>
<tr>
<td>D[v,0.5]</td>
<td>μm</td>
<td>59.04</td>
<td>61.01</td>
<td>58.42</td>
<td>50.76</td>
<td>42.35</td>
</tr>
<tr>
<td>SSA</td>
<td>m²/g</td>
<td>0.5272</td>
<td>0.8729</td>
<td>1.5753</td>
<td>2.9796</td>
<td>4.38</td>
</tr>
</tbody>
</table>

Note: Values are the average of two observations.
Masters (1972) describes four powder properties that are important in the reconstitution process: wettability, sinkability, dispersibility and solubility. Wettability is defined as "the ability of a milk powder to adsorb water on its surface". Sinkability is the ability of the powder particles to sink down into the water after being wetted. Dispersibility has to do with the ability of the powder to be distributed throughout the water. This is related to the easiness with which lumps or aggregates can fall apart. Finally, Masters states that solubility can be defined both in relative (i.e., rate of dissolving) or absolute terms.

Various processes have been designed to “instantize” dried milk powders. The objective of these processes is to produce porous agglomerates that have very fast rates of wetting, sinking and dispersing (Masters, 1972).

Within this context, the relative changes in particle size with respect to hydration time for the MPCs produced at different temperatures could be related to their wettability and dispersibility. MPCs dried at higher air outlet temperatures are relatively less wettable and dispersible than those produced at the lower air outlet temperatures.

Considering the fact that the differences in moisture content and water activity of the samples was fairly large, it was thought that the lower particle sizes for the samples produced at lower air outlet temperature could just be due to the fact that the powder had hydrated during storage. To rule out this possibility, a small sample of the MPCs dried at 90°C air outlet temperature was placed in a desiccator with a saturated solution of
potassium carbonate (Aw = 0.433). After 36 hours, the MPCs had equilibrated to Aw = 0.396, a value similar to the water activity of the MPCs dried at low air outlet temperature. Particle size was determined for these samples, showing that the high temperature MPCs still had a significant portion of large particles that hydrated very slowly. These results were consistent with the ones obtained before, suggesting that the differences in particle characteristics could be the result of the drying process.

It is possible that a higher air temperature, coupled with a more extensive removal of moisture, can produce a strong form of aggregation in the MPC powders. This can be conceptualized as the formation of a case-hardened shell that is fairly hydrophobic and contains individual or primary MPC particles inside. The “effective” particle, as detected by light scattering, would be a very large one. When drying at lower temperatures, the outer skin might be thinner and porous, so that water can penetrate to the core of the particles more easily, thereby showing a much smaller effective particle size with shorter hydration times. The hypothesized aggregation mechanisms for high and low air outlet temperatures are schematized in Figure 5.15.

The particle size distributions observed after 5 hours hydration are similar to those obtained after ultrasonic vibration was applied to solutions that have been hydrating for just one hour. Ultrasonication of the samples could, therefore, be used as a means to simulate long hydration time, providing a quick comparison of the hydration ability of different samples.
Figure 5.15: Hypothesized MPC Aggregate Formation at High and Low Spray Dryer Air Outlet Temperatures
Given the extremely large size of some of the particles, it was hypothesized that these were too large to remain in a true solution. To verify this, 10% solutions were prepared for the MPCs dried at low (65°C) and high (90°C) air outlet temperatures. After 1 hour hydration, this solutions were centrifuged at 10,000 G for five minutes. Particle size and protein content of the supernatant were determined and compare to those of the original solution. The results from protein analysis are shown in Table 5.12. As seen in this Table, 6.4% protein was precipitated by centrifugation for the MPCs dried at 65°C while 11.2% of the protein in the MPCs dried at 90°C was removed after centrifugation. The particle size distributions of the supernatants were practically identical for the supernatants of both samples, showing a single mode at size ~0.35µm. This indicates that the large size particles constitute a significant portion of the volume of the dispersed protein.

<table>
<thead>
<tr>
<th>MPC Air Outlet Temperature</th>
<th>65°C</th>
<th>90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Content of Solution*</td>
<td>9.48%</td>
<td>9.40%</td>
</tr>
<tr>
<td>Protein Content in Supernatant**</td>
<td>8.88%</td>
<td>8.35%</td>
</tr>
<tr>
<td>Δ% Protein (Precipitated)</td>
<td>-6.38%</td>
<td>-11.12%</td>
</tr>
</tbody>
</table>

*Average of two observations.
**After centrifugation at 10,000 G for 5 min.

Table 5.12: Protein Content of Solutions Used for Particle Size Analysis, Before and After Centrifugation.
5.6.2.1.6.2 Final Remarks about Particle Size Distributions

The particle size distributions, as determined with laser diffraction using the Malvern Mastersizer Microplus, are calculated under a number of assumptions. One of these is that the particles' shape is approximately spherical. Other assumptions have to do with the refractive index of the sample relative to the medium.

The changes in particle size after the exposure to detergent (SDS) may produce changes in both the shape of the particles and their optical properties. Since SDS can partially unfold the protein molecules, it is possible that the shape of the particles becomes less spherical after binding detergent molecules. On the other hand, SDS caused a rapid drop in obscuration of the laser beam, from around 30% to about 10% in three to five minutes. This suggests that a large number of the particles may have been reduced to particles that are very small and possibly outside the range of detection of the instrument. The dispersion also lost opacity, becoming almost transparent after a couple of minutes. This is an obvious indication of changes in the refractive index of the particles.

Hydration might have a similar effect. As noted by Groves (1982), a protein molecule that is heavily hydrated behaves as if it were in a sphere of some material with a density intermediate between the density of the dispersing medium and the particle. Hydration of the surface of the molecules would result in an interface that lacks a sharp definition.
(Groves, 1982). This could affect the optical properties of the particles and therefore limit the accurate size determination by laser diffraction.

The changes in optical properties of the particles due to the changes in environmental conditions (i.e., addition of detergent to the dispersant, long hydration time, etc.) should be evaluated in order to make the necessary changes to the optical model used by the instrument’s software and allow a more accurate determination of particle sizes. This was outside the scope of the present study but needs to be recognized as one of the limitations of particle size determination as conducted in this research.

5.6.2.1.7 Electrophoretic Patterns

SDS-PAGE showed no major differences among the MPCs dried at different spray dryer air outlet temperatures. Electrophoregrams for SDS-PAGE carried out under non-reducing and reducing conditions are provided in Figures 5.16 and 5.17. As expected, electrophoresis of non-reduced samples showed some bands in the stacking gel region, indicating the presence of large aggregates. However, the densitometric profiles were practically identical and therefore the relative concentrations of the aggregates and the different protein bands were basically equal for the samples produced at high and low spray dryer air outlet temperatures.
Figure 5.16: Electrophoregram of non-reduced SDS-PAGE for MPCs dried at various spray dryer outlet temperatures.
Figure 5.17: Electrophoregram of reduced SDS-PAGE for MPCs dried at various spray dryer outlet temperatures.

*Note: For exact molecular weights, please refer to Fig. 5.16
5.6.2.1.8 Accelerated Storage Tests

5.6.2.1.8.1 Effect on Color

Since the different temperatures used did not seem to have a significant effect on particle aggregation at the molecular level, as shown by SDS-PAGE, it was decided to explore the possibility that some of the changes could occur during storage of the MPCs. In this case, since the MPCs produced at 65°C had a significantly higher water activity than those produced at higher temperatures, it was hypothesized that protein polymerization could occur as a result of post-process chemical reactions. In particular, Maillard reactions have been postulated by various authors (Kato et al., 1989, Matsuda et al., 1991, Cho et al., 1986) as a cause of protein polymerization.

Long term storage was simulated by taking a sub-sample of the MPCs and placing them on an incubator at 55°C for 14 days. Color changes were monitored during this period by using a Hunter Lab UltraScan XE dual beam xenon flash spectrophotometer (Hunter Associates Laboratory, Inc.). This instrument uses the L*a*b scale recommended by the International Commission on Illumination (Commission Internationale de l'Eclairage, CIE).
The whiteness index per the CIE colorimetry committee was chosen for a general color comparison. This index is calculated using tristimulus values\(^2\). Based on this, perfect white would have a whiteness index of 100%. As the sample color deviates from white, this index decreases.

![Diagram](image)

**Figure 5.18**: Brown color development for MFCs dried at various spray dryer air outlet temperatures.

\(^2\) Whiteness Index = \(Y + 800(x_n - x) + 1700(y_n - y)\), where \(Y = Y\) tristimulus value of the sample and \(x, y =\) chromacity coordinates of the perfect diffuser for the CIE 1921 2º Standard Observer. (Anonymous, 1994).
It is obvious from Figure 5.18 that those samples dried at lower spray dryer air outlet temperatures underwent more intense browning during high temperature storage. This was expected because the samples dried at lower temperatures had significantly higher $A_w$. At low moisture content, non-enzymatic browning reactions (i.e., Maillard reactions) proceed at a much faster rate as $A_w$ increases, reaching a maximum at $A_w$ 0.4-0.8. Above a certain water activity value, the rate of browning reactions actually decreases due to the diluting effect of water (Labuza and Saltmarch, 1981). The $A_w$ of the MPCs produced in this research ranged from around 0.15 to 0.35, so they fall in the range where increasing water activity can impart mobility to the substrates.

5.6.2.1.8.2 Effect on Apparent Molecular Weight

The aged MPCs were analyzed using SDS-PAGE with and without the reducing agent β-mercaptoethanol to assess the extent of possible polymerization. Figures 5.19 and 5.20 show the electrophoreograms of the aged MPCs. These results show that the samples produced at a lower spray dryer outlet temperature, and therefore with a higher $A_w$, had reduced electrophoretic mobility in most of the proteins. The effect is more evident for the whey proteins, which not only have reduced mobility but also show significant smearing.
Figure 5.19: Electrophoregram of non-Reduced SDS-PAGE for aged MPCs dried at various spray dryer outlet temperatures.

*Note: For exact molecular weights, please refer to Figure 5.16.
Figure 5.20: Electrophoregram of Reduced SDS-PAGE for aged MPCs dried at various spray dryer outlet temperatures.

*Note: For exact molecular weights and milk protein standard identification, please refer to Figures 5.16 and 5.17.
The changes in electrophoretic mobility experienced by the different samples can be seen also in Figure 5.21. The relative mobilities (Rf) of the main bands of the gel are plotted vs. the spray dryer air outlet temperature. The proteins are presumed to be the ones indicated in the legend, but the identification of the proteins might be masked by confounding factors such as the formation of dimers from β-lactoglobulin that could be mistaken for some of the caseins. Notwithstanding these problems, it is obvious that the major whey proteins (β-lactoglobulin and α-lactalbumin) were the ones that experienced the greatest decrease in electrophoretic mobility, as compared to the caseins.

![Graph showing relative mobilities for major protein peaks in electrophoresed MPCs dried at various air outlet temperatures.](image)

**Figure 5.21:** Relative Mobilities for major protein peaks in electrophoresed MPCs dried at various air outlet temperatures.
Reduced electrophoretic mobilities indicate an increase in apparent molecular size. Hames (1981) notes that glycosylated proteins show an anomalous electrophoretic behavior under reducing conditions because the crosslinking of sugars to the protein molecules limits the binding of SDS, resulting in artificially high molecular weight estimates.

Matsuda et al. (1986) used a lactose-β-lactoglobulin model to investigate Maillard reactions. A dry mixture of lactose and β-LG was stored at 50°C and 65% relative humidity for ten days. SDS-PAGE electrophoregrams show a gradual decrease in electrophoretic mobility with increasing storage time. The protein bands also show smearing with increasing storage time. The authors attribute the increase in molecular weight mostly to the lactosylation of the protein and subsequent polymerization, but also to reactions with some lactose degradation products.

Increase in apparent molecular weight could also be partly due to polymerization. The fact that the reduction in electrophoretic mobility is consistent when SDS-PAGE is performed with and without a reducing agent rules out disulfide linkages as the main cause of possible polymerization. Therefore, it is likely that covalent bonds formed during accelerated storage at 55°C.

Several possibilities have been proposed as possible mechanisms for protein polymerization as the result of non-enzymatic browning reactions.
Cho et al (1986) studied glucose-induced polymerization by storing lysozyme with glucose in the solid state at 50°C and 75% relative humidity. They determined that 3-deoxyglucosone, an intermediate product of the Maillard reaction, was the cross-linker responsible for protein polymerization. Their experiment was confirmed by storing ovalbumin and bovine serum albumin with 3-deoxyglucosone at 50°C, which resulted in polymerization of these proteins.

Matsuda et al. (1991) stated that lactulose (4-O-\(\beta\)-D-galactopyranosyl-D-fructose) induced protein polymerization by cross-linking between lactulose-lysine amino carbonyl adducts from one molecule and free amino groups from other molecules. High temperature or alkaline pH can produce a rearrangement from aldoses to ketoses, resulting in the conversion of lactose to lactulose. However, these conditions were not likely in the production of the MPCs analyzed in this research.

5.6.2.1.8.3 Effect on Particle Size

Storage at 55°C resulted in the formation of large aggregates for all the samples. The particle size distributions are depicted in Figure 5.22 and the mean diameters are given in Table 5.13. All of the distributions appear to be monomodal and show a peak around 50-70 \(\mu\)m. Although the increase in particle size diameters after the accelerated storage test is consistent with the increase in apparent molecular weight, the differences between samples dried at different air outlet temperatures are not so evident as with electrophoresis.
Figure 5.22: Particle Size Distribution for MPCs dried at different air outlet temperatures after storage at 55°C for 15 days.

Table 5.13: Mean Particle Diameters for MPCs dried at different air outlet temperatures after storage at 55°C for 15 days
5.6.2.1.9 Storage Effect on Commercial MPC Samples

In order to validate whether accelerated storage tests could reflect possible protein polymerization reactions, commercial samples of MPC with different ages were analyzed using SDS-PAGE. The samples analyzed had a similar protein content to the MPCs produced in this research (70%) and had been stored at room temperature (ca. 25°C) for 4, 3 and 1 years. As can be seen from Figures 5.23 and 5.24, the older samples have reduced electrophoretic mobility as compared to the fresher sample. This provides evidence of lactosylation and polymerization during storage, possibly induced by Maillard reactions.
Figure 5.23: Electrophoregram of Non-Reduced SDS-PAGE for commercial 70% protein MPCs of different freshness. Lanes A and D: 3.5 years old; B and E: 4 years old; C and F: 1 year old.

*Note: For exact molecular weights, please see Figure 5.16.
Figure 5.24: Electrophoregram of Reduced SDS-PAGE for commercial 70% protein MPCs of different freshness. Lanes A and D: 3.5 years old; B and E: 4 years old; C and F: 1 year old.

*Note: For exact molecular weights, please see Figure 5.16.
5.6.2.2 Effect of Nozzle Orifice Diameter

As indicated at the beginning of this Chapter, due to only time and materials constraints, the spray dryer nozzle diameter was varied only for the MPC samples dried at 75°C air outlet temperature. Three different nozzle sizes were available: 0.5, 0.8 and 1.3 mm orifice diameter.

There was no statistically significant difference in terms of moisture and Aw for the samples produced with different nozzle sizes. The effect on particle size was also negligible. It can be seen on Table 5.13 that even though after one hour hydration the sample produced with the 0.5 mm nozzle seemed to have a larger particle size, the difference disappears after the second hour of hydration.

<table>
<thead>
<tr>
<th>Hydration Time (hours)</th>
<th>Nozzle Size (Orifice Diameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 mm</td>
</tr>
<tr>
<td>1</td>
<td>0.79\text{a}</td>
</tr>
<tr>
<td>2</td>
<td>0.43\text{a}</td>
</tr>
<tr>
<td>3</td>
<td>0.38\text{a}</td>
</tr>
<tr>
<td>4</td>
<td>0.40\text{a}</td>
</tr>
</tbody>
</table>

Note: Averages of 2 replicates. Subscripts indicate statistically significant differences.

Table 5.14: Sauter Mean Diameters (D[3,2]) for MPCs dried at 75°C air outlet temperature and different nozzle sizes.
When the particle size distributions are compared after ultrasonication, they are practically identical.

5.6.2 Relationship with Basic Functionality

Two basic functional characteristics were compared for the powders produced at low and high air outlet temperatures: foaming and emulsification. Foaming of a 5% protein solution was evaluated by the method of Phillips et al. (1990) for the MPCs dried at 65°C, 75°C and 90°C. All of the MPCs produced very unstable foams. Maximum overrun (ca. 340%) was obtained after 15 minutes whipping. No significant differences in overrun were found among the different MPCs. However, since they all produced unstable foams, this test might not provide a good comparison in terms of functional performance.

Emulsion Volume Index was evaluated only for the MPCs dried at the extreme air outlet temperatures (65°C and 90°C). This was done by mixing 3 grams of protein with 90 ml water and 10 ml vegetable oil. Both the water and the vegetable oil were preheated at 60°C. The mixes were homogenized twice using a bench-top homogenizer (A/S N. Foss Electronic, Denmark) and EVI was determined by the method of McDermott et al. (1981). EVI was 1.17 (±0.08) for the MPCs dried at 65°C and 1.21 (±0.08) for the ones dried at 90°C. The results were not significantly different at α=0.05.
CHAPTER 6

CONCLUSIONS

In the first stage of this research, it was shown that commercially available MPCs exhibit large differences in certain functional properties such as viscosity and foaming. These differences were not found to be correlated to basic product properties like protein content or protein solubility.

For the MPCs produced in the spray drying experiments, the samples did not show statistically significant differences in protein solubility both at pH 7 and pH 4.6. Therefore, for the drying temperatures used, the heat effect might not have been large enough to cause unfolding of the molecules and their subsequent aggregation.

The data obtained through laser diffraction and electrophoresis reflect different phenomena. Particle size distributions, as obtained with the instrument used in this research, cover a fairly wide range, and can provide information at a macromolecular level. SDS-PAGE, on the other hand, covers very small sizes and provides information
at the molecular level. On the other hand, while the samples are simply dispersed in water for particle size analysis, they have been purposely dissociated and denatured prior to electrophoresis.

No differences were apparent at the molecular level due to differences in drying temperatures, as shown by the fact that the electrophoretic patterns of the samples, both under reducing and non-reducing conditions, were practically identical.

Differences were found between the particle size distributions of the MPCs dried at low temperatures and the ones dried at high outlet temperatures. Since there were no changes in protein solubility or their electrophoretic separations, the differences in particle size distributions might reflect a macromolecular phenomenon attributed to differing spray drying conditions.

The marked differences in the susceptibilities of large particles to break down into small ones with respect to hydration time suggests that high air outlet temperatures can induce the formation of a case-hardened shell around a core of individual particles. This shell is relatively impervious to water. The samples dried at higher temperatures are, therefore, more difficult to hydrate. This could translate into decreased wettability and dispersibility. Eventually, the inability to hydrate rapidly could affect how the MPC interacts with other components of the food system, thus affecting its functionality.
However, the impact of particle size differences in MPC functionality remain to be evaluated.

The differences in particle size cannot be solely attributed to differences in spray dryer air outlet temperature. The large differences in moisture content and water activity between the samples dried at low and high outlet temperatures constitutes a confounding factor in the analysis. However, the fact that the particle size of the samples dried at 90°C air outlet temperature remains large, even after equilibration to high water activity, indicates that the temperature effect was the most important factor in the formation of large particles.

In order to isolate the effect of temperature, multiple stage drying would be needed. This would allow to remove additional moisture, even at low air outlet temperatures, minimizing the confounding effect of major differences in moisture content and water activity. However, multiple stage drying is typically not feasible at a pilot scale.

The effect of varying spray dryer outlet temperatures might also be more marked if the total protein content of the feed is increased. This can be achieved by diafiltration of the ultrafiltered skim milk retentate.

Changes at the molecular level can occur during storage, as shown by the differences in electrophoretic patterns for the samples produced at different air outlet temperatures after
storing them at 55°C for two weeks. These changes are likely the result of Maillard reactions, since a greater degree of browning was also observed for the samples whose electrophoretic mobility was most reduced. Specifically, lactosylation of milk proteins and cross-linking induced by intermediate products of the Maillard reactions have been pointed by various authors as possible ways by which the apparent molecular weight of some proteins can increase.

The differences in electrophoretic mobilities after the accelerated storage were attributed to the differences in water activity of the samples. As expected, MPCs that had been dehydrated at low air outlet temperatures and, consequently, had a higher water activity, underwent more intense browning and also showed the greater reduction in electrophoretic mobility of α-lactalbumin and β-lactoglobulin.

The changes during accelerated storage were validated by analyzing commercial MPC samples of varying freshness. Results showed that the older sample had a reduced electrophoretic mobility.

In summary, by varying the spray dryer outlet temperature, two factors are varied concomitantly: heat during drying and moisture content in the finished product. The temperature effect appears to be the main factor in the formation of large particles, while differences in moisture content and/or water activity are related to the keeping quality of the MPC powders.
Varying the nozzle orifice diameter did not seem to have a significant effect on the MPC characteristics studied. However, the nozzle orifice diameter was only varied under very specific conditions (air inlet temperature 200°C and outlet temperature 75°C). The absence of significant changes due to varying the nozzle diameter cannot be generalized to nozzle sizes outside the range used in this research. Also, droplet size may be more relevant when using a different kind of atomizer (i.e., rotary).
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