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Short review

Interactions of milk proteins during the manufacture of milk powders

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Abstract – The manufacture of milk powders involves heating the milk, usually known as preheating, then concentrating the milk solids by evaporation and spray drying. In the manufacture of high protein milk powders, ultrafiltration/diafiltration is normally applied prior to evaporation and drying. These processes cause interactions between the various components in milk that influence powder functionality. The structure and stability of casein micelles are fairly well known under conditions as in milk. However, the details of the effects of milk powder processes on casein micelle structure and interactions are not much understood. Preheat treatment applied to milk prior to evaporation aims to produce powders with specific functional properties and applications. A major effect of preheating is the denaturation of whey proteins and their association with casein micelles. The nature and extent of association of whey proteins with the casein micelles, involving κ-casein, influence how the casein micelles behave during further processing. During evaporation, casein micelle size increases due mainly to aggregation of some of the micelles and increased association of whey proteins with the micelles. There has been little work examining the specific effects of spray drying on casein micelles in milk. In the manufacture of high protein powders, concentration of milk by ultrafiltration, in particular diafiltration prior to drying, can cause dissolution of colloidal calcium phosphate, resulting in loosening of the casein micelle structure, and possibly swelling of casein micelles. Increasing the degree of concentration causes progressive breakdown of micellar structure from an intact micelles to a swollen diffuse micelle and finally to a smaller fragmented micellar structures. These changes in casein micelles predispose the milk system to further protein-protein interactions during spray drying, and consequently impact on the functionality of the product.

milk powder / protein / interaction / micelle / denaturation

摘要 – 乳粉生产过程中乳蛋白之间的相互作用。乳粉生产过程需要先对乳进行预热处理, 然后经过蒸发和干燥来浓缩乳中的固体。在制备高蛋白含量的乳粉时, 在蒸发和干燥之前通常采用超滤或全滤的方法。这些过程能够影响乳成分之间的相互作用进而影响乳粉的功能性。在乳中酪蛋白胶束的结构和稳定性非常重要。乳粉加工过程对酪蛋白胶束结构影响以及存在着蛋白质之间的相互作用, 关于这方面内容有过详细的研究。在蒸发之前采用预热的目的是使生产出来的奶粉具有特殊功能性和用途。预热的主要作用就是使乳清蛋白变性后能够与酪蛋白胶束聚集在一起。乳清蛋白与酪蛋白胶束之间固有的和外在的聚集作用, 包括对κ-酪蛋白的聚集, 在进一步加工中会对酪蛋白胶束的性质产生一定的影响。在蒸发过程中, 主要是由于一些酪蛋白胶束的聚集, 使得酪蛋白胶束变大, 也增加了乳清蛋白与酪蛋白胶束的聚集作用。关于干燥对乳中酪蛋白胶束具有特殊影响的报道非常少。在生产高蛋白含量的乳粉时, 干燥之前使用超滤, 特别是使用全滤, 可以使得磷酸钙胶体分散, 导致酪蛋白胶束的结构松散, 并有可能使得酪蛋白胶束膨胀。增加浓缩程度会加剧酪蛋白胶束结构从完整的胶束到膨胀分散的胶束以至于最后形成小的酪蛋白胶束碎片。由于酪蛋白结构上的变化容易导致喷雾干燥过程中发生蛋白-蛋白之间的相互作用, 最终影响产品的功能性。

乳粉 / 蛋白质 / 相互作用 / 胶束 / 变性

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Résumé – Interactions entre les protéines laitières au cours de la fabrication de poudre de lait. La fabrication de poudre de lait implique un traitement thermique du lait, qu'on appelle préchauffage, puis sa concentration en matière sèche par évaporation ou séchage par atomisation. Pour les poudres à teneur élevée en protéines, l'ultrafiltration/diafiltration est normalement utilisée avant évaporation ou séchage. Ces procédés provoquent, entre les différents composés du lait, des interactions qui influent sur les propriétés fonctionnelles des poudres. La structure et la stabilité des micelles de caséine sont relativement bien connues dans le lait. Cependant, les effets des procédés de fabrication de poudre sur la structure et les interactions des micelles de caséine sont bien compris. Le but du traitement de préchauffage appliqué au lait avant évaporation est de produire des poudres ayant des propriétés fonctionnelles et des applications spécifiques. Un effet majeur du préchauffage est la dénaturation des protéines de lactosérum et leur association avec les micelles de caséine. La nature et l'amplitude de l'association des protéines de lactosérum avec les micelles de caséine, impliquant la caséine κ, influent sur le comportement des micelles de caséine au cours des traitements ultérieurs. Pendant l'évaporation, la taille des micelles augmente principalement à cause de l'agrégation de certaines micelles et de l'association accrue des protéines de lactosérum avec les micelles. Il y a eu peu de travaux sur les effets spécifiques du séchage par atomisation sur les micelles de caséine dans le lait. Dans la fabrication de poudres à teneur élevée en protéines, la concentration du lait par ultrafiltration, en particulier la diafiltration, avant séchage, peut provoquer la dissolution du phosphate de calcium colloïdal, résultant dans un relâchement de la structure de la micelle et potentiellement un gonflement des micelles. L'élévation du taux de concentration provoque la rupture progressive de la structure de la micelle qui passe de l'état intact à un état diffus gonflé pour finalement s'émietter en plus petits fragments. Ces changements dans les micelles de caséine prédisposent le système du lait à des interactions protéines/protéines ultérieures au cours du séchage par atomisation et ayant un impact sur les propriétés fonctionnelles du produit.

poudre de lait / protéine / interaction / micelle / dénaturation

1. INTRODUCTION

Milk powders are defined by the Codex Standard 207-1999 [6] as "milk products that can be obtained by the partial removal of water from milk". There are mainly two types of commercial milk powders, that is, skim milk powder (SMP) and whole milk powder (WMP) that are further classified as either instant or regular. SMPs are commonly classified as low-, medium- or high-heat powder on the basis of their whey protein nitrogen index (WPNI). In recent years, a number of high protein milk powders with protein content in the range 50–85% have been developed, using membrane technologies. These powders are commonly referred to as milk protein concentrates (MPC), and may be classified as MPC56, MPC70 and MPC85; the number denotes approximate protein concentration. The compositions of various types of milk powders are given in Table I.

Milk powders are used in a number of applications [3, 14]. In recombining applications, SMP and anhydrous milk fat are mixed together by dissolving them in water (reconstitution) and homogenisation. The products that can be made include recombined evaporated milk, recombined milks, recombined sweetened condensed milk, yoghurt, cheese and fermented milk beverages. Milk powders, both SMP and WMP, can be also used as functional ingredients to a wide variety of foods, for example chocolate, bakery, beverages and confectionery.

MPCs are commonly added to milk or cheese formulations to enhance the protein content and/or the yield of the final product. The MPC can be used to enhance the textural characteristics of yoghurts. The use of MPCs in nutritional drinks is growing. In these application, MPC provides both casein and whey proteins in the same ratio and milk, but without the high lactose content [3].

Milk powders may be used by consumers directly at home by mixing milk powder and water. Alternatively powder

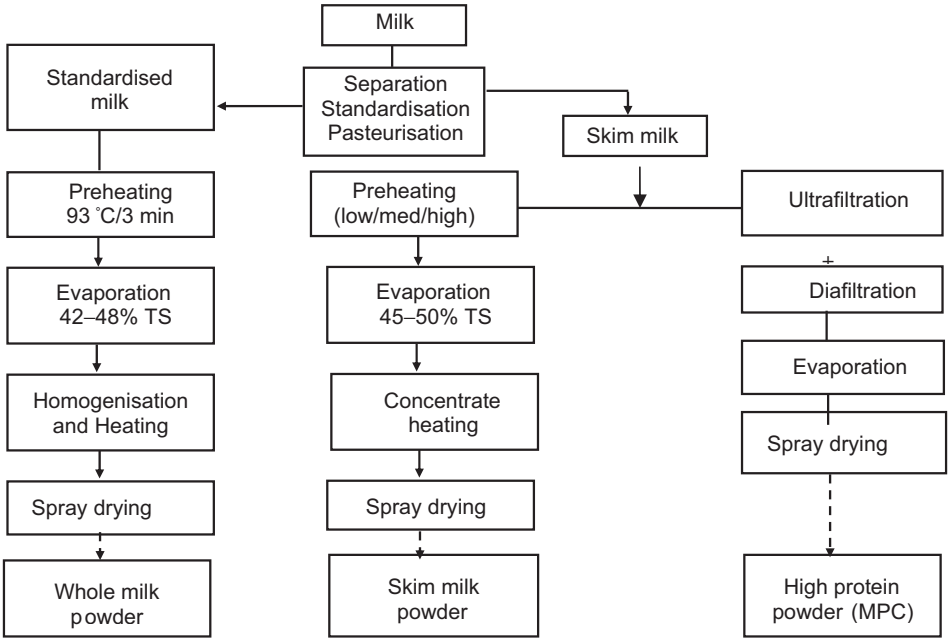

Figure 1. General manufacturing processes for a typical whole milk powder (WMP), skim milk powder (SMP) or milk protein concentrate powder (MPC).

Table I. Typical compositions of milk powders.

Product	WMP	SMP	MPC56	MPC70	MPC85
Milk fat	26.8	0.8	1.3	1.4	1.0
Protein	25.0	34.0	57.1	70.0	85.0
Lactose	39.1	53.5	30.1	17.0	3.0
Minerals (ash)	5.8	7.9	7.7	7.2	7.0
Moisture	3.3	3.8	3.8	4.4	4.0

WMP = whole milk powder; SMP = skim milk powder; MPC = milk protein concentrate powder.

can be used as a whitener by adding it directly to hot coffee or tea. For household use the powders are instantized during spray drying by lecithination and agglomeration, so they dissolve rapidly in water at room temperature [14].

The manufacturing processes for milk powder consist of several distinct stages (Fig. 1): (i) separation and pasteurization of the raw milk into desired components for final product specifications (for SMP most of the fat is removed, whereas, for WMP only a portion of fat is removed); (ii) preheat treatment of milk for a set time/temperature combination for the desired functional characteristics of the powder; (iii) concentration of milk by multiple-effect evaporator to remove water; (iv) homogenisation of the concentrate is the case of WMP manufacture to protect the fat globules from coalescence during spray drying; (v) heat treatment of the concentrate to reduce the viscosity prior to its entry into the spray drier; and (vi) spray

drying of the concentrate to remove most of the water.

In the manufacture of MPC, skim milk is concentrated by ultrafiltration to increase the protein content and remove lactose and salts (Fig. 1). In the manufacture of higher protein powders (normally above 70% protein on a dry powder basis), diafiltration is normally applied. Generally, there is no preheating step involved. After ultrafiltration and diafiltration of the skim milk, the retentate is evaporated to remove more water and then spray dried.

Milk powders, intended for various end uses, require consistency of composition, desirable physical attributes and functional properties. Functional properties of milk powders aligned with specific end uses, to some extent, can be induced by manipulating the milk compositions and processing techniques (including heat treatment, homogenisation and ultrafiltration). Although the factors controlling the physical attributes, such as solubility, dispersibility, etc., are fairly well documented, relatively little is known about interactions of proteins that may occur during the powder manufacturing processes and how these interactions affect the functionality of the final powder. This paper provides an overview of the important interactions of proteins during the manufacture of milk powders, including high protein powders. The paper also considers possible consequences of these interactions in the functional properties and applications of milk powders.

2. INTERACTIONS OF PROTEINS DURING SKIM MILK POWDER MANUFACTURE

2.1. Preheat treatment

Preheat treatment, prior to evaporation, is the most important tool in the manufacture of SMPs. Preheating temperatures vary widely, ranging from pasteurization (72 °C for 15 s), through low-temperature, long-time heating (e.g. 85 °C for up to 30 min) to high-temperature, short-term heating (120 °C for 2 min) using direct (steam injection) or indirect (plate heat exchanger) heating [20]. There are a large number of studies on heat-induced interactions of milk proteins, but they have largely been carried out by heating milk in water or oil baths under laboratory scale conditions [7, 16]. The commercial heating equipment, as that used in milk powder manufacture, can produce quite different levels and types of proteins interactions compared to heat treatments on a laboratory scale.

The most important effects of preheating on milk proteins are the denaturation of whey proteins and their association with the casein micelles [19]. The minor whey proteins, serum albumin and immunoglobulins, begin to denature at ~65 °C, but the major whey proteins, β-lactoglobulin (β-LG) and α-lactalbimin (α-LA), show significant denaturation only at temperatures >70–75 °C [9, 16]. The order of sensitivity of various whey proteins to heat-induced denaturation has been reported to be: immunoglobulins > serum albumin > β-LG A > β-LG B > α-LA. In conditions that exist in milk, the denaturation of whey proteins is immediately followed by aggregation reactions. The denatured whey proteins can interact with the casein micelles, more specifically with κ-casein in the micelles and/or simply self-aggregate to form polymeric products. β-LG has been shown to form complexes with α-LA, which can subsequently interact with κ-casein [8, 13].

The principal interaction between β-LG and κ-casein has been shown to involve the formation of disulfide bonds between β-LG and κ-casein. Two disulfide bridges and a free thiol group in the native structure of β-LG play an important role in this interaction. Our recent work [18], using 2-D PAGE methods, has identified several

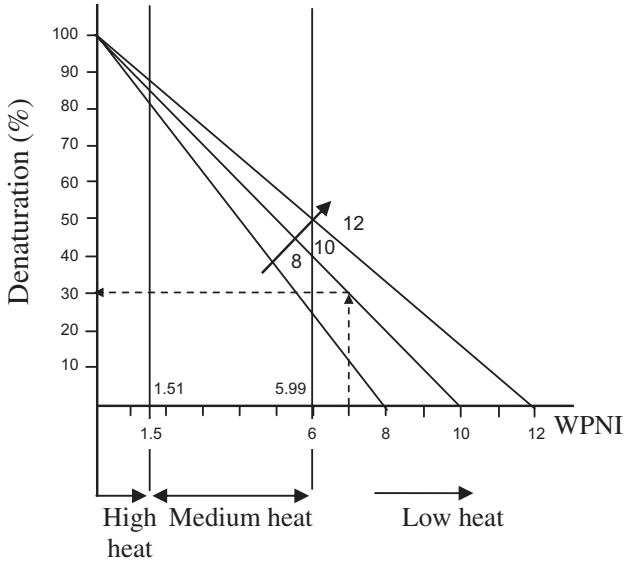

Figure 2. Denaturation and WPNI at varying levels of whey proteins in raw milk.

interaction products that are generated during heat treatment of skim milk. Heat treatment causes native β-LG to interchange into non-native monomer and non-native disulfide-bonded dimer which then interact with α-LA or κ-casein. It is interesting that α_{s2}-casein, which has one disulfide bridge, does not interact with β-LG in milk systems [18]. This may be because α_{s2}-casein is located inside the micelles and is not accessible to β-LG or that α_{s2}-casein is a particularly stable entity, especially as a dimer [18].

The interactions of whey proteins with the caseins micelles have been shown to be related to changes in casein micelle size during heat treatments. Anema and Li [1] reported that on heating milk at temperatures up to 100 °C, the change in micelle size was dependent on the pH of milk at heating, which also influenced the extent of association of denatured whey proteins with the micelles. At pH 6.5, where about 70% of the denatured whey proteins were associated with the micelles, the average micelle size increased by about 35 nm. At pH 6.7, where the level of whey protein association was about 30%, the micelle size increased only about 5 nm.

In the dairy industry, WPNI is a common measure for denaturation of whey proteins in skim milk powders. Basically, the powder is dissolved in water and then saturated with NaCl solution, followed by filtration which retains denatured whey proteins and casein. The filtrate is analysed for its protein content, and the value is expressed as WPNI, which is the quantity of undenatured whey proteins per gram of powder. Thus, as the extent of denaturation of whey proteins increases, the WPNI decreases (Fig. 2). Strong positive correlation ($R^2 > 0.95$) between WPNI and the extent of denaturation of β-LG or total whey proteins as determined by polyacrylamide gel electrophoresis (PAGE) analysis have been shown [2]. A major limitation of the WPNI is that it does not take into account the original whey protein content of milk. Therefore, the higher the whey protein content of the raw milk prior to heat treatment, the higher is the WPNI of the powder (Fig. 2).

As the level of whey proteins in the raw milk varies with lactation and/or season, use of the WPNI is satisfactory only when the original whey protein content of the raw milk is shown. On the basis of WPNI, SMPs can be classified into low-, medium- and high-heat types (Fig. 2). WPNI is also a gross indicator, with certain limitations, of suitability of the SMP for different applications.

The extent of protein interactions that occur during preheating also affects powder solubility and shelf life. Generally, the higher the degree of denaturation and aggregation, the poorer the powder solubility and better the oxidative stability of WMPs [14].

2.2. Evaporation

The purpose of evaporation is to remove as much water as possible without adversely affecting the quality of the powder. As the milk becomes more concentrated, its viscosity increases, making it difficult to remove water. Therefore, the evaporation process is limited to concentrating milk only up to ~50% total solids. The evaporation of milk is normally carried out at temperatures between 50 and 70 °C with a residence time in each stage of about 1 min [20]. Under these conditions, whey proteins denaturation is considered to be minimal [19]. Recently, Oldfield et al. [17] showed that evaporation of skim milk to 47 to 49% total solids in a multiple effect evaporator, and subsequent heat treatment of the concentrate in the range 64–74 °C had no significant effect on the denaturation of β-LG, α-LA, serum albumin or immunoglobulin G. This was considered to be partly due to the increased stability of whey proteins at high total solids concentrations.

The pH of milk decreases during concentration from an average initial value of 6.7 to approximately 6.3 at 45% total solids. This is partly due to changes in salt equilibria as more calcium phosphate is transferred from the soluble to the colloidal state, with a concomitant release of hydrogen ions. Le Graet and Brulé [11] have shown that, when the milk is concentrated about fivefold by evaporation, soluble calcium and soluble phosphate increase by a factor of about two, the remainder of the soluble calcium and phosphate being transformed into the colloidal state. The activity of calcium ions increases only slightly but the ratio of monovalent to divalent cations increases markedly [13]. Casein micelles may increase in size due to the increase in colloidal calcium phosphate or due to coalescence of the micelles [20]. While numerous studies have been carried out on the behaviour of proteins in normal milk, research into evaporated milk is very difficult due to its high viscosity and tendency towards age gelation.

2.3. Spray drying

The changes induced in milk systems by spray drying are not well understood. The heat exposure of milk during spray drying may vary considerably, depending on the design of the drier, the operating conditions and the length of time the powder is held before cooling. The native properties of the milk components are essentially unmodified by moderate drying conditions [20]. The normal size distribution of the casein micelles and their heat stabilities and renneting characteristics are substantially recovered on reconstitution of spray-dried milk.

During spray drying, the increases in protein denaturation and aggregation are likely to depend on the temperature of the air into which the milk is sprayed (inlet air temperature), the degree of concentration and the temperature of the concentrate prior to drying, the size of the drying droplets and the temperature of the

air/powder mixture exiting from the drier (outlet air temperature). Drying is usually very rapid and the temperature of the milk droplets does not exceed 70 °C until they have lost almost all their water [20]. The temperature of the droplets approaches that of the outlet air as the drying process nears completion. For this reason, the outlet air temperature is a critical parameter controlling heat damage to dry milk products. It has been shown recently that the denaturation of whey proteins during spray drying was minimal with no apparent loss of immunoglobulin G and only a small loss of serum albumin (3–7%) [17]. Varying the inlet/outlet air dryer temperature (200/100 °C–160/89 °C) appears to have no significant effect on whey proteins denaturation [17].

Changes may occur in the salts equilibria during spray drying. The process of drying would be expected to produce the same types of changes in salts equilibria as does evaporation, i.e. an increase in colloidal calcium phosphate and a decrease in pH. It has been shown that the concentrations of soluble calcium and soluble phosphate in reconstituted skim milk are about 20% lower than those in the original milk [15].

3. INTERACTIONS OF MILK PROTEINS DURING THE MANUFACTURE OF MILK PROTEIN CONCENTRATE POWDERS

In the manufacture of MPC, skim milk is concentrated by ultrafiltration prior to evaporation and spray drying. As the process involves no preheating (except pasteurization), whey protein remain largely in their native state. The ultrafiltration process allows water, lactose, non-protein nitrogen compounds and soluble salts to pass through the membrane while retaining casein micelles and whey proteins. Some calcium, magnesium, phosphate and citrate are associated with the casein micelles in milk, and hence are retained in the concentrate. The ratio of casein to whey proteins in the MPC is similar to that in the SMP.

Electron microscopy studies [21] have shown that the size of the highest proportion of casein micelles (80–100 nm) in milk was reduced (to 60–80 nm) at a volume concentration factor of about 5. The volume distribution and the average diameter of casein micelles decreased upon ultrafiltration of skim milk. These changes in micelle size were considered to be due mainly to the changes in the levels of milk salts, particularly calcium and phosphate. In contrast, McKenna [12] found that there was little change in particle size at the early stages of ultrafiltration, but at the end of the ultrafiltration process, and particularly during diafiltration the average micelle size increased significantly.

Examination of casein micelles by electron microscopy during the course of ultrafiltration and diafiltration revealed progressive swelling of casein micelles and formation of non-micellar material [12]. Figure 3 shows a thin section micrograph of skim milk; roughly spherical particles, i.e. casein micelles, of different sizes are clearly observed (Fig. 3A). After ultrafiltration there appears to no considerable change in casein micelle size, but more non-micellar material could be seen in the micrographs (arrows) (Fig. 3B). After diafiltration, there appeared to be significant change in the structure of casein micelles. There is an increase in non-micellar material, and casein micelle appears to be swollen and less dense (Fig. 3C). After evaporation, the micelles appear to pack together, resulting in aggregation of some of the micelles (Fig. 3D). The non-micellar material appears to link these micelles together.

It is likely that the loss of serum calcium and phosphate during ultrafiltration and diafiltration results in the dissolution of

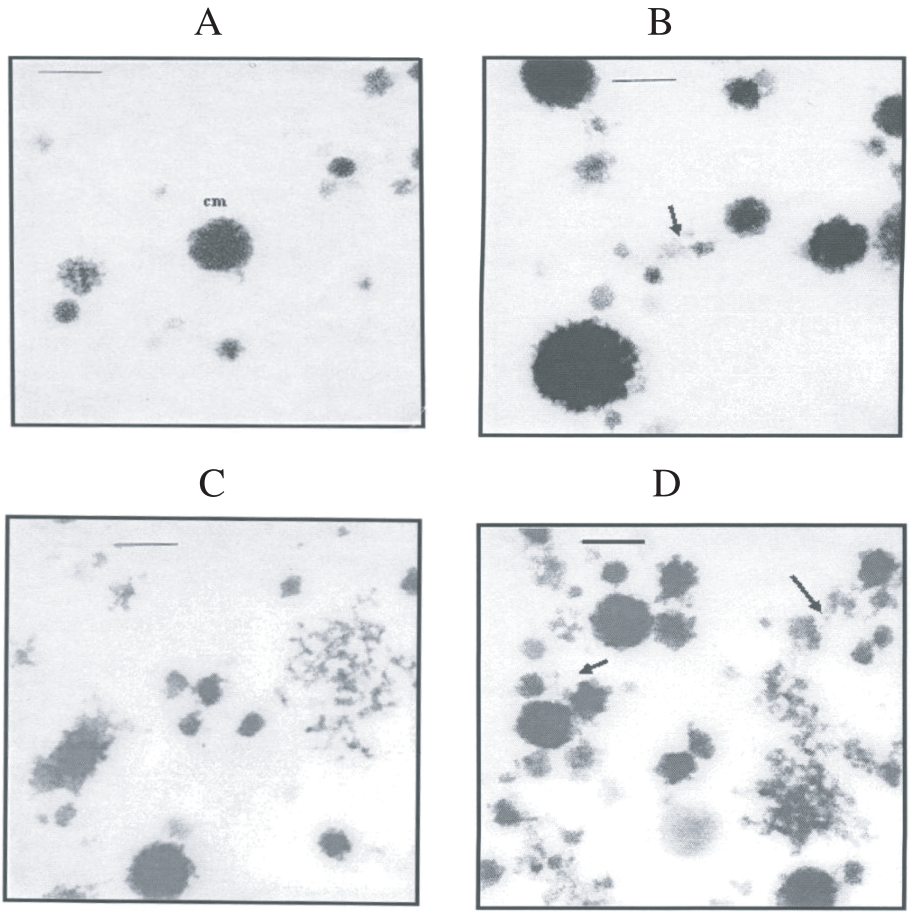

Figure 3. Thin sectioning transmission electron microscopy of skim milk (A), ultrafiltrated skim milk (B), diafiltrated skim milk (C) or evaporated skim milk (D) (from McKenna [12]).

colloidal calcium phosphate. Consequently this continual loss of colloidal calcium and phosphate results in loosening of casein micelle structure and is thus responsible for swelling of the micelles [12]. During evaporation, there appears to be an increase in micelle aggregation, involving the non-micellar material or dissociated casein micelles. Spray drying probably induces further protein-protein interactions, but the nature of these interactions is not known.

MPC powders with very protein content (e.g. MPC85) are generally known to have poor solubility upon reconstitution in water at 20 °C, but the solubility improves at higher reconstitution temperatures. The solubility of these powders decreases with storage time at elevated temperatures. The major factor affecting the solubility behaviour of MPC85 appears to be related to the rate of water transfer into the powder particle rather than to the thermal processes during manufacture. Various processes have been proposed for manufacturing MPC powders with improved solubility in cold water, involving the addition of a monovalent salt to the ultrafiltered retentate prior to drying [5] or partial

Table II. Functional requirements of milk powders in selected food products.

Product	Type of powder	Functionality
Recombined milk	Low- and low-medium-heat	Solubility, flavour, emulsion stability
UHT recombined milk	Low- and medium-heat	Solubility, heat stability, emulsion stability
Recombined evaporated milk	High-heat	Heat stability, viscosity
Recombined cheese	Low-heat	Rennetability
Ice cream	Medium-heat	Emulsification, foaming, water absorption
Confectionery	High-heat	Water absorption, texture
Baked foods	High-heat	Water binding, texture

replacement (~30%) of calcium content of ultrafiltered retentate by sodium ions [4]. The insoluble material found in the reconstituted MPC powders has been characterised by McKenna [12] and Havea [10]. Using electron microscopy McKenna [12] showed that insoluble material in MPC85 consists of large particles (up to 100 μm) formed by fusion of casein micelles, involving some kind of protein-protein interactions. These fused casein micelles appear to form a skin-like structure on the outside of a powder particle, inhibiting the movement of water into the particle. Upon reconstitution in water, large parts of these particles remain intact.

Havea [10] confirmed these findings, and reported that the insoluble material consists predominantly of α_s- and β-caseins, as revealed by PAGE analysis. This material was held together by weak non-covalent interactions that were easily disrupted under SDS-PAGE conditions. Although some disulfide-linked protein aggregates consisting of κ-casein and β-LG were present in MPC powders, these aggregates were not considered to play an important role in the formation of insoluble material. Further work is required to understand the nature of protein-protein interactions involved in the formation of insoluble material, and develop methods that can be used to minimize protein interactions during the manufacture of MPC.

4. FUNCTIONAL PROPERTIES OF MILK POWDERS

The functional properties of milk powders are essentially the manifestation of the physical and chemical properties of milk proteins. These include emulsification, foaming, water absorption, solubility, viscosity, gelation and heat stability. The preheat treatment of milk prior to evaporation and drying has been the most widely used method to produce milk powders with different functional properties. Membrane technology has also been exploited to modify the functional properties of milk powders. Table II lists the commonly used milk powders in selected food products and the major functional attributes required of milk powder. Milk proteins provide a number of key functions in a number of food applications. Casein micelles, which largely retain their integrity during manufacture, provide their unique functional properties, e.g. curd formation, heat stability, and emulsification. The whey proteins may be more or less denatured, depending on the heat treatment during manufacture.

The denaturation of whey proteins may alter the properties of casein micelles by interacting with κ-casein at the surface of the casein micelles. This interaction increases the rennet clotting times and impairs curd formation, thus making milk powder unsuitable for used in recombined cheese. On the other hand, this interaction increases the heat stability of milk proteins when powders are used in the manufacture of recombined evaporated milk. Casein micelle/denatured whey protein complexes are able to imbibe a considerable quantity of water which increases the viscosity and gel strength of yoghurt and other cultured milk products. There is limited information available in the literature on the relationship between functional properties and the process-induced changes in proteins. In order to produce new ranges of milk powders with specific functional properties, quantitative understanding of the fundamental basis of functionality, and its relationship to the various changes in milk components during manufacture, will need to be developed.

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