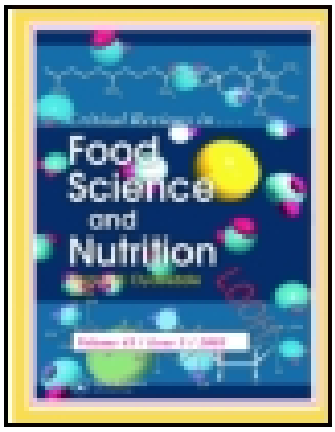

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Functional Characteristics of Milk Protein Concentrates and Their Modification

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SUMMARY

A major deterrent to the usage of milk protein concentrate (MPC), a high protein milk product with increasing demand as a food and sports drink ingredient, has been its poor functional characteristics when compared to other milk protein products such as whey protein concentrate

and sodium caseinates. This review discusses the recent research on functional properties of MPC focusing on factors that may contribute to the poor functional characteristics before, during and after production. Current research, methods employed and new understanding on the causes of poor solubility of MPC at mild temperatures (about 20^{o}C) has been presented including loss of solubility during storage as these areas have received unprecedented attention over the past decade and also affects other useful functional properties of MPC, such as emulsifying properties, gelation and foaming. Processing methods, which include heat treatment, high pressure application, microwave heating, ultrasound application, enzyme and salts modification, have been used or have potential to modify or improve the functional properties of MPC. Future research on the effects of these processing methods on the functional properties including effects of enzyme hydrolysis on bitterness and bioactivity has also been discussed.

Keywords: milk protein concentrate, solubility, functional properties, processing

1. Introduction

High milk proteins are increasingly being used as ingredients in several dairy and nondairy food products such as cheese, yoghurt, ice creams, fruits, beverages and specialized health products because of their properties including solubility, viscosity, gelation, emulsification, foaming, Maillard reaction and bioactivity. Bioactive compound mining from milk proteins is a growing area of research with several commercialized products basing on the claims of antioxidative, antihypertensive, immunomodulating, antimicrobial, antiulcerogenic and anti-inflammatory properties. Such claims have been well reported and overviewed (Martinez-Maqueda et al., 2011; Contreras et al., 2011; Tsopmo et al., 2011; Eriksen et al., 2008; Darewicz et al., 2006; Clemente, 2000, Meisel and Schlime, 1996).

One such high milk protein product is the milk protein concentrate (MPC), a relatively new dairy product as compared to the other high milk protein products such as whey protein concentrates and sodium caseinates. Milk protein concentrates are also known as “aggregated proteins” and are described as concentrated forms of milk proteins that contain both the caseins and the whey proteins in the same proportions as whole milk (Euston and Hirst, 1999; Ye, 2011).

MPC is finding increasing usage in food as an ingredient because of its high protein to low fat/low sugar characteristics as well as its physicochemical characteristics (Augustin et al., 2011), making it a choice for dietetic and special low sugar foods. Several studies have been conducted to show improved characteristics in products in which MPC has been incorporated (Francolino et al., 2010; Rehman et al., 2003a, b; Gonzalez et al., 1999) particularly cheese (**Table 1)**. However,

it is widely reported that the characteristics of this high protein content milk powder varies with production method and age in storage emphasis being on solubility (Thomas et al., 2004, Havea, 2006; Fyfe et al., 2011a). A great number of studies have dwelled on quantifying these changes mainly solubility, foaming and emulsifying properties employing methods such as spectroscopy, nuclear magnetic resonance (NMR), rheology and proteomics (Le et al., 2012; Haque et al., 2011, Sikand et al., 2011). Despite these efforts, it is far from conclusion on what really causes these changes in functional properties. Castro-Morel and Harper (2002) reported correlation between MPC solubility and protein content in high protein powders (82-86% protein content) but reported lack of it in other samples. It is not clear if these characteristic changes would include changes in the bioactivity of the milk protein concentrate.

This review gives an overview of the current research on functionality of milk protein concentrates (solubility, emulsification, gelation and foaming) and attempts to highlight changes that may be brought about through application of emerging technologies such as microwave, high pressure and ultrasound. Highlights have also been presented on the potential of enzyme modification and associated bioactivity as areas which MPC has received little attention when compared to the other milk proteins such as whey protein concentrate and sodium caseinates such as bitterness research.

2. Milk protein concentrates production

The process of manufacturing milk protein concentrate powders involves pasteurization, ultrafiltration and diafiltration, followed by water removal by vacuum evaporation and spray

drying (Mistry and Hassan, 1991a). The protein content in MPC ranges from 36% to 85% depending on the degree of ultrafiltration and diafiltration of which further categorization gives low-protein powder (≤40% protein content), medium-protein powder (60-70%) and high-protein powder (≥80% protein content)(Sikand et al., 2011). During ultrafiltration, water, lactose, and mineral salts are removed in the permeate stream and protein is concentrated (Mistry and Hassan, 1991a). The retentate is enriched in protein but depleted in lactose and soluble milk salts, and the milk minerals associated with the casein micelle remain. By using ultrafiltration alone, milk protein concentrates of up to 65% protein can be obtained. For production of milk protein concentrate powder with more than 65% protein, diafiltration is required. By adding water in the filtration process, the lactose and soluble milk salts can be further washed out, leading to an increase in protein content of the retentate and therefore a higher protein powder (Getler et al. 1997). Milk protein concentrate powders are produced by spray drying and the processing factors have effects on the functional properties of the powders produced (Chen and Patel, 2008).

3. Spray drying effects

During spray drying several parameters may affect the physical characteristics of the MPC powders. Such characteristics would include the size, shape, porosity and surface wrinkles. The parameters may differ depending on equipment used and the process parameters during spray drying such as inlet temperatures, outlet temperatures and type of atomizers among others. Several studies have been conducted to understand the effects of spray drying conditions on the resultant functionalities of dairy powders and recently overviewed (Chen and Patel, 2008; Thomas et al., 2004). The drying temperatures have been associated with the characteristics of

the surface of the particles which exhibit increased fat and protein content (Gaiani et al., 2007). This has a direct influence on the wettability of the powder as it depends on surface compositions. Although outlet temperature range of between 65 and 90 ^{o}C has been found to have no effect on protein denaturation, it affects the moisture content and rehydration rate of the resulting particles (Fang et al., 2011).

In a recent study, Gaiani et al. (2010) studied the effect of differing spray drying temperatures on surface competitive adsorption. It was reported that at lower drying temperature, casein was over expressed on the particle surface compared with bulk composition, whereas higher drying temperature, the casein content at the surface was similar as in the bulk. In a study by Millqvist-Fureby et al. (2001), a series of experiments were conducted to examine the surface composition of whey protein isolate particle with different heat treatments using electron spectroscopy for chemical analysis. It was reported that with increasing degree of denaturation (insoluble proteins), the fat coverage increased slightly, while protein coverage decreased and lactose coverage remained relatively constant on the particle surface. It showed that heat treatment could alter the composition of the particle surface. Fang et al. (2012) studied the relationship between spray drying temperature and the resulting powder solubility on mono-disperse MPC droplets dried under different inlet air temperatures in a pilot scale drier. A direct relationship between spray drying temperatures and the resultant particle functionality was determined. The particle morphologies obtained from lower inlet air temperature appeared spherical whereas the one from higher inlet air temperature appeared deflated (**Figure 1**).

From the previous studies, the spray drying conditions have an effect on the functionality of the milk protein concentrates including the drying time (Baldwin, 2010). Inlet and outlet temperature effects have been the widely studied parameters due to the certainty of control during experimentation.

4. Functional properties of MPC

The functional properties of milk powders such as milk protein concentrates are generally attributed to their milk proteins. However, some functional properties, like wettability, can be attributed to powders themselves. Interfacial properties of milk proteins and milk powders appear very important for the use of milk powders as food ingredients. The present review will only focus on them. First, properties at the powder/water interface (solubility, dissolution, wettability, etc.) will be discussed since they are a prerequisite for the reincorporation of milk powders into foods. Then three interfacial properties of milk protein concentrates are described: the emulsifying properties at the oil/water interface, gelation and foaming properties at the air/water interface. Compared with other milk protein powders, MPC has poor functional properties which necessitate considerable research to improve its functional characteristics so that it can gain widespread use among processors and consumers (Singh, 2011).

4.1 Solubility

Solubility of milk protein powders remains the most important functional property as it affects the expression of the other properties. Solubility, either true solution or colloidal dispersion, is required as a prerequisite for obtaining optimum functional performance of components in foods

(Anema et al., 2006; Baldwin and Truong, 2007; Mimouni et al., 2010). The solubility of milk powder depends on different dissolution steps in water such as wettability, sinkability, and dispersibility. The definitions for these terms have been given elsewhere (Thomas et al., 2004; Schuck, 2011).

The issue of poor solubility of milk protein concentrates in ambient temperature water is well known (Zwijgers, 1992; Schuck et al., 2002; Baldwin, 2010) which limits their use as ingredients in cold milk and consumer reconstitution in cold water. Solubility of MPC increases with increasing temperature (Mistry and Hassan, 1991b, Baldwin and Truong, 2007) and decreasing protein concentration (Sikand et al., 2011). This has resulted in several studies trying to elucidate the factors causing insolubility in MPC at room temperatures employing different methods (Baldwin, 2010). In most of these studies, the solubility of MPC has been measured by an insolubility test of milk powders, modified from the method originally developed by the American Dry Milk Institute (Baldwin and Truong, 2007). The experimental conditions for determining solubility in MPC are different which makes comparative analysis of different studies difficult (**Table 2)**. Standardization of the experimental procedure for solubility tests in milk protein concentrates would be a good idea for easy comparison of results. Similar solubility tests applied to MPC have been used in whole milk powders (Baldwin and Ackland, 1991; Van Mil and Jans 1991). Solubility has also been determined as nitrogen solubility index (NSI) in whey protein (Groubet et al., 1999) and caseinates (Szpendowski et al., 1997) following attempts for a standardized solubility test procedure proposed by Morr et al. (1985). NSI is determined

from the protein content in a supernatant after centrifugation as a percentage of the total protein content.

4.1.1. Methods used to study dissolution kinetics in MPC

The technological advancement in various fields has seen the application of different methods to characterize the dissolution of MPC. Although "centrifugation and fractionation" of MPC into soluble and insoluble fractions remains popular in determining solubility, methods such as static light scattering microscopy, nuclear magnetic resonance (NMR), proteomic approach and focused beam reflectance measurement (FBRM) have been recently employed to explain dissolution kinetics. These methods monitor either the physical or the chemical properties of the MPC during dissolution.

Mimouni et al. (2009) used static light scattering (SLS) microscopy to monitor rehydration of MPC while Haque et al. (2011) successfully used an integration of results obtained from characterization of MPC during storage using Fourier transform infrared (FTIR) spectroscopy for protein conformational modifications and NMR relaxometry for water–protein interactions to determine a link between minor protein unfolding/refolding and loss of solubility upon ageing of MPC. Sikand et al. (2011) also employed FTIR spectroscopy coupled with high performance liquid chromatography (HPLC) while Le et al. (2012) managed to use a proteomic approach combining gel electrophoresis and mass spectrometry to detect lactosylation, deamidation and protein cross-linking (chemical changes) in stored MPC which were related to solubility changes. Another approach developed by Fang et al. (2010, 2011) used focused beam reflectance

measurements. All these methods have managed to give insights into the process of solubility, factors and interactions involved in rehydration process and subsequently draw correlations with loss of solubility in MPC as also done by Mata et al. (2011) using small angle X-ray scattering method. However, more studies need to be done in order to ensure repeatability of the results.

4.1.2. Causes of low solubility in MPC

Different studies have tried to explain the reasons behind the poor dissolution property of MPC powder before, during and after production. Milk protein concentrates (MPC) have shown to be more sensitive to drying temperatures than other milk products (Baldwin and Truong, 2007) which might cause particles of low solubility. In a study of the effect of dryer type on functionality of MPC, Fyfe et al. (2011b) found significant differences between laboratory scale and commercial dryer-produced powders suggesting that dryer type has influence on functionality of MPC. This may explain why Castro-Morel and Harper (2002) found inconsistency in correlation between protein content and solubility in their study as their samples would have come from different dryers and produced with different conditions.

Sikand et al. (2011) also found out that difference in mineral compositions resulting from differences in processing conditions have an effect on solubility of MPC. **Table 3** shows the mineral composition of various MPC40, MPC80 and MPI (>85% protein content) powders used in the study by Sikand et al. (2011)**.** There was significantly higher total mineral content and levels of potassium in MPC40 than in MPC80 and MPI which would initially explain the higher solubility values in MPC40 (>95%). Analysis of the mineral composition showed low potassium

content of high -protein powders which was attributed to a high rate of washing process (diafiltration) to achieve high protein content; however, calcium depletion was less sensitive to diafiltration, as milk protein content is associated with calcium content. Samples 9 and 10 (high protein powders) also exhibited high solubility values comparable to MPC40. The study observed that samples 9 and 10 had increased levels of monovalent cations and decreased levels of divalent cations as compared with other MPC80 and MPI samples; contained roughly one-third the calcium as compared with other MPC and MPI samples, and they had a proportional decrease in phosphorus content. Sample 9 displayed higher potassium content, whereas sample 10 displayed higher sodium content. It therefore appeared that the high solubility of samples 9 and 10 could be associated with their mineral composition particularly calcium and phosphorus contents, which were lower than those of other samples, and also to the potassium content for sample 9, which was higher than the other samples. Thus mineral content would be a great suspect to solubility differences among milk protein concentrates and may be manipulated to enhance the solubility of the high-protein powders. A recent study by Mao et al. (2012) confirmed that addition of sodium chloride during diafiltration process in MPC production affects its solubility with a concentration of 150mM giving the highest solubility. It would therefore appear that minerals affect the protein aggregation and hence the solubility. This was also in agreement to other findings by Gualco (2010) and Havea (2006). Even after production, salts addition would definitely affect solubility as Hussein et al. (2011) found out that total rehydration time in native micellar casein was reduced from 467 min to about 217 min and 187 min when NaCl (12% concentration) and $CaCl_2$ (2.25% concentration) were added, respectively. This suggests that salts may change the casein structure and hence solubility.

In another dimension, Mistry and Hassan, (1991b) showed that particles of the high milk protein powders as examined by scanning electron microscopy were characterized by smooth surface and dents while particles of skim milk powder prepared in the same spray dryer had a wrinkled surface. This suggested that the smooth surface acts like a barrier to water entry during dissolution. Fang et al. (2012) showed that particle morphologies differed with inlet drying temperatures used. Studies in skim and whole milk powders also found that surface composition has an effect on water adsorption (Nijdam and Langrish, 2006; Murrieta-Pazos et al., 2011, Rogers et al., 2012). The role of the casein micelle structure and milk protein composition has also been speculated (Havea, 2006, Sikand et al., 2011). Optimization of the spray drying conditions for production of MPC would be an area of possible research as has been done for other protein products such as soy milk (Telang and Therat, 2010) and whey protein concentrate (Bernard et al., 2011).

After production, MPC solubility decreases with storage time and storage temperature (Havea, 2006). Here, a diverse number of possible reasons have been suggested due to the different methods employed in monitoring the kinetics of dissolution of MPC. One possible explanation for this particular phenomenon is that the proteins on the surface of MPC powder continuously form cross-linked networks with neighbouring proteins during storage particularly hydrophobic casein molecules and some minor whey proteins. This cross-linked network could act as a barrier for water to penetrate, thus inhibiting the rehydration of MPC particles. However, it is noted that the increasing number of cross-links adversely affects the solubility of the powder only after a

certain threshold limit of the crosslink (Havea, 2006). Fyfe et al. (2011a) reported the formation of a "crust" consisting of a thin layer of fused casein micelles on the surface of stored powder and attributed this to the decrease in solubility. In general, the predominant factor in solubility loss in storage is the casein to casein interaction on the surface of the MPC powders with a minor contribution of minor whey proteins.

Mimouni et al. (2010) disputed that the formation of insoluble material is behind the loss of solubility in stored MPC. According to this study, the loss of solubility during storage is due to changes in rehydration kinetics. The results suggest that the release of micelles from powder particles is the rate-limiting step of the MPC rehydration process and is inhibited upon storage. The study also excluded effects of lactolysation or other Maillard reaction products on solubility loss which are detected in stored MPC (Le et al., 2012). Although, this remains to be confirmed by other studies, there is one fact that all studies agree that whey protein do not significantly contribute to the solubility loss of MPC.

Haque et al. (2011) observed protein conformational modifications and water–protein interactions and suggested that there is a link between minor protein unfolding/refolding and gradual loss of solubility upon ageing of MPC. Storage at higher water activity resulted in more water molecules in close proximity to the protein surface and also slightly higher protein unfolding, which could eventually lead to loss of solubility.

The use of different techniques in determination of solubility loss in MPC has resulted in differing speculations on the causes of solubility loss in MPC. More research needs to be done to conclusively establish these factors and improve the solubility of MPC so that it is attractive as an ingredient.

4.2 Gelation

Another important functional property of milk powders is gelation. Gels are formed when proteins interact and produce an elastic network. A recent review has given the general direction of research in milk protein emulsion gels (Dickinson, 2012). A number of studies have been conducted on gelation in MPC. Kuo and Harper (2003) indicated that gelation is directly related to the dispersability of MPC after showing that increasing dispersability of MPC 85 increased the rennet gels formed. The gels formed by MPC 56 were stronger that those formed by MPC 85. Ferrer et al. (2007) encountered the same phenomenon and attributed it to difference in ionic strength as the low protein MPC (MPC 56) had higher calcium to protein ratio than MPC 85. Martin et al. (2010) studied the rennet gelation behaviour of reconstituted MPC which was compared with raw skim milk. Reconstituted MPC did not coagulate unless supplemented with approximately 2 mM calcium chloride, which was attributed to the mineral removal during ultrafiltration/diafiltration. Addition of sufficient calcium restored rennet coagulation kinetics and gel strength of reconstituted MPC to approximately that of raw skim milk. Mizuno and Lucey (2007) found that when gels are formed from MPC with addition of emulsifying salts, the resulting gel characteristics would depend on the pH and concentration of the added salts. **Figure 2** shows that gels with the highest breaking force were formed when tetrasodium pyrophosphate

(TSPP) was added at a concentration of 6.7 mM and no gel was formed when TSPP was added at concentrations of ≤2.9 or ≥10.5mM. In the same study, other phosphate-based emulsifying salts were tested but for these emulsifying salts, gelation only occurred after several days or at greater gelation temperatures whereas no gelation was observed for trisodium citrate. Gelation induced by tetrasodium pyrophosphate (TSPP) was dependent on pH, and the breaking force of gel was greatest at pH 6.0. The importance of minerals and salts in gelation of MPC is therefore strongly established and has been recently reviewed (Hussein et al., 2012).

Matia-Merino and Singh (2007) studied acid-induced gelation of MPC and partly concluded that there exists a potential to manipulate the generation of different types of gel matrices, by controlling rate of acidification, casein micelle integrity, calcium levels and pectin concentrations to obtain different synergistic or antagonistic effects. A further explanation indicated that the possible formation of casein–calcium–pectin complexes with the casein micelles or in the serum phase, along with casein–casein and pectin–pectin interactions (through calcium) and casein–pectin complexes formed during acidification, are responsible for the development of the acid-induced three-dimensional network. The work on understanding the formation of gels in MPC is still on-going with the predominant factor still not yet determined.

Briscoe et al. (2002) also studied the gelation of aqueous MPC (13% w/w) and attributed the enhanced gelation upon pressurization (up to 1000 bar) on the disruption of hydrophobic groups and ionization of charged groups caused by an increase in electrostriction and to a smaller extent on the disruption of hydrogen bonds.

4.3 Foaming

Foams consist of a discrete gas or bubble phase dispersed in either a liquid or solid continuous phase. Proteins play an important role in forming and stabilizing foams in aerated dairy products such as ice cream. Foam formation and characteristics in milk has been recently reviewed (Huppertz, 2010; Dickinson, 2003) and several studies have shown that the foaming behavior of milk proteins is dependent on factors such as heat treatment, pH and ionic environment influence (Ward et al., 1997; Hagolle et al., 2000; Zhang and Goff, 2004). The addition of EDTA has been reported to improve the foamability of milk protein dispersions due to dissociation of casein micelles (Ward et al., 1997; Zhang and Goff, 2004).

Although foamability and foam stability of milk proteins have been well studied, the actual composition of air–water interfaces in protein-based foams is much more difficult to study than the composition of fat–water interfaces in protein-stabilized emulsions, due to the difficulty in separating adsorbed from non-adsorbed proteins, i.e., separating air bubbles from foams is much more difficult than separating fat globules from emulsions (Dickinson 2003; 2006). The foaming properties of milk powders are useful for products in which air-water dispersions are desirable (cakes, whipped toppings, etc.).

Two characteristics describe foaming properties. The foaming power (or foam volume) of milk proteins corresponds to their ability to stabilize air-water interface to create a foam. The foaming power of a protein solution corresponds to the increase in volume after whipping in set

conditions. It is expressed as foam volume (*FV*) or foam overrun (*FO*) (Yankov and Panchev, 1996; Singh, 2011), which is a percentage of the volume after whipping to the initial volume.

Foaming power is also evaluated from the electrical conductivity of the foam (Thomas et al., 2004). Foaming stability is defined by the time before powder collapses. Foaming stability is generally desired, except in some food processes, like ice-cream production. Foaming stability is determined following foam volume, air release, serum drainage, or electrical conductivity as a function of time (Britten and Lavoie 1992). In a study by Yankov and Panchev (1996), milk protein concentrate was inferior to whey protein concentrate in both foam overrun and stability. Whey protein concentrates generally have very good foaming powers (Singh, 2011), however, their foaming stability depends on the characteristics of whey proteins. Foaming power is higher when proteins are fully soluble.

Apart from protein concentration, level of denaturation and preheat treatment, foaming power of MPC also depends on pH as evidenced from the results of Mistry and Hassan (1991b), in which foam expansion doubled when pH was raised from 7 to 10, because the pH treatment solubilizes whey proteins. Viscosity of the MPC dispersion is also directly related to foam stability (Yankov and Panchev, 1996). There have been studies on the effects of salt addition on foaming characteristics particularly for sodium caseinates and whey protein concentrates (Marinova et al., 2009). The findings suggest that addition of salts does not affect foaming characteristics of WPC but for sodium caseinates. This is not peculiar as salts affects solubility and solubility affects

foaming. In comparison to foaming properties research in other milk products, MPC has received little attention in this area.

4.4 Emulsifying properties

Over the years, emulsifying properties have been used in the food industry to create many food products, including milk, cream, coffee creamer, soft drinks, nutritional beverages, sauces, dips, deserts, dressings, mayonnaise, ice cream, margarine, and butter (Singh, 2011; McClements, 2010, Friberg et al., 2004). These products may be in an emulsified state in their final form (e.g., milk or cream) or they may have been in an emulsified state in the course of manufacturing (e.g., powdered soup, sauce, or coffee creamer) (McClements, 2010; Schuck, 2011). The two major categories of food emulsions in the food industry are oil-in-water (O/W) and water-in-oil (W/O) emulsions. O/W emulsions consist of oil droplets dispersed in an aqueous medium (e.g., milk, cream, beverages, and dressings), whereas W/O emulsions consist of water droplets dispersed in an oily medium (e.g., margarine and butter). In milk protein concentrates, the O/W emulsions are the type encountered (Ye, 2011).

There are three characteristics that describe emulsifying properties which are the emulsifying capacity, the emulsifying activity and the emulsifying stability of a protein. The emulsifying capacity, which is the most reported (Hill, 1996), is the maximum quantity of oil (gram of oil per gram of emulsifier) that can be dispersed in a protein solution before phase inversion. The emulsifying activity is given as the surface area of the interface stabilized by a given concentration of emulsifier. It is expressed in m^2/g while the emulsifying stability corresponds to

the time before phase inversion (Thomas et al., 2004). Generally, the emulsifying activity index (EAI) and the emulsifying stability index (ESI) are calculated from the spectroturbidity (observed at 500 nm) (Manoi and Rizvi, 2009) or from the electrical conductivity of the emulsion.

In milk protein concentrate studies, different conditions have been used in the determination of emulsion properties as summarized in **Table 4**. The types of oil (corn, soy and rapeseed), protein concentration and the homogenization process (Horn et al., 2012) have all contributions on the emulsifying properties that would be obtained. This should be borne in mind when making comparative analysis of the behavior of emulsions. Apart from the properties mentioned above, particle size distribution of protein dispersion (Ye, 2011; Dybowska, 2008, Euston and Hirst, 2000), creaming tests and rheological tests are also used to study emulsifying stability in milk protein concentrates (Ye, 2011; Hemar et al., 2005; Euston and Hirst 2000) as in other milk proteins (Manoi and Rizvi, 2009; Thomas et al., 2004; Euston and Hirst, 1999).

It has been shown that in simple O/W emulsions, the emulsifying ability of MPC is much lower than that of whey protein and sodium caseinate (Euston and Hirst, 1999; Ye and Singh., 2000). Both sodium caseinate and whey protein products (WPC and WPI) show excellent emulsifying ability, and it is possible to make stable emulsions at a relatively low protein-to-oil ratio (about 1:60) (Ye and Singh, 2001). Conversely, much higher concentrations of MPC are required to make a stable emulsion and larger droplets are formed in these protein-stabilized emulsions under similar homogenization conditions. The relatively low emulsifying ability of MPCs has

limited their applications in some food formulations. Ye (2011) found that there was improvement in emulsion properties when low calcium MPC was used. However, there was a decrease in emulsion stability at low protein concentration indicating a complex mix of factors contributing to emulsion properties. In order to improve some of the emulsion properties of MPC different methods have been tested with mixed results including preheating (Dybowska, 2008) and use of disassociating buffer (Euston and Hurst, 2000).

Hemar et al. (2005) studied the rheology and the microstructure of emulsions stabilized with milk protein concentrate (MPC) using different MPC and oil concentrations. The results indicated that emulsions were Newtonian at low oil concentrations (<20 wt%), pseudoplastic and thixotropic at high oil concentrations (>20 wt%). This was in agreement with previous studies in other emulsion systems (Campanella et al., 1995; Dybowska, 2004). The rheological behavior of the emulsions coupled with confocal microscopy showed consistency with bridging flocculation mechanisms, whereby more than one droplet is shared by adsorbed casein aggregates/micelles, a view shared by the study by Ye (2011).

It has been well studied that protein and lipid present on hydrocolloids play very important roles in their emulsifying properties. Emulsion properties in other low emulsifying milk products such as sodium caseinates have been improved by the use of gum (xanthan gum, corn fiber gum, etc) (Hemar et al., 2001; Dickinson, 2006; Yadav et al., 2010). In MPC, the effect of adding carrageenans was studied by Mena-Casanova and Totosaus (2011) reporting increased emulsifying stability. Hemar et al. (2001) showed that MPC/xanthan gum mixtures (up to 1%

xanthan) resulted in phase separation as seen from confocal micrographs but not in mixtures with sodium caseinate or whey protein isolate systems (**Figure 3**). This indicated a depletion flocculation mechanism leading to formation of large particles and hence better emulsion stability. It would be interesting to study the effects of addition of other gums and other stabilizers to the emulsion properties of MPC.

5. Effect of storage on functional properties

The effect of storage on functional properties of milk protein concentrate has received great attention over the past decade. Storage of MPC85 at temperatures greater than 20^{o}C reduced its solubility in water, suggesting that storage period and storage temperature are responsible factors in solubility loss (Mistry and Hassan, 1991b; Anema, et al., 2006). The results could be evolved onto a single curve, indicating that the same physical process affects the solubility at all temperature/time combinations. This was confirmed recently in a study by Hunter et al. (2011) in which a master curve of temperature/time and solubility loss as well as loss in renneting gelation was developed. Haque et al. (2011) added water activity as another factor.

Recent studies have also proposed that rehydration of MPC85 is due to the slow dissolution kinetics and that, given sufficient hydration time, MPC85 could be almost fully dispersed (Mimouni et al., 2009). However, the powders used in these studies were not stored under particularly severe temperature conditions and would not have attained minimum solubility.

Electrophoresis results showed that it was only the casein micelles that became insoluble, and that the whey proteins and other small molecules were in the solution phase (Anema et al., 2006; Havea, 2006). It was proposed that the casein micelles may cross-link at the surface of the powder particles through either covalent or non-covalent (hydrophobic) interactions, forming a particle that allows small molecules including whey proteins to diffuse out but retains the casein micelles (Anema et al., 2006). This proposal has been supported by recent studies that show that the casein micelles are involved in the insolubility and that a network of casein micelles forms at the surface of the particles, producing a porous barrier that allows diffusion of small molecules but retains the casein micelles (Fang et al., 2011; Fyfe et al., 2011a).

Le et al. (2011) have investigated the solubility and chemical changes due to the Maillard reaction in (MPC80) during storage at temperatures and relative humidities in the ranges of 25-40 °C and 44-84%, respectively. The results indicated that Maillard reaction (observed by Furosine production and browning) in MPC80 increased during storage, whereas the solubility decreased suggesting that the Maillard reaction may also be a cause of solubility loss in MPC powder. Further, Le et al. (2012) employing 2D gels indicated that the products of Maillard reaction were dependent on storage temperature, time and humidity. The milk protein/lactose interaction needs conclusive studies on their effect on solubility during storage of MPC. The general process of Maillard reaction in milk powders has been overviewed by others (Thomas et al., 2004; Oliver, 2011).

More studies to elucidate the causative agents of solubility loss in stored MPC need to be carried as are processing methods for reducing this phenomenon. The effects of storage of MPC on the other functional properties (foaming, emulsification) have not received much attention as the changes in solubility. As previously discussed, this might be because solubility affects the other functional properties of MPC. The factors that affect solubility would definitely impact emulsifying and foaming properties. For example, controlled Maillard reaction has been used to improve emulsifying properties of whey protein isolate (Hunt and Dalgleish, 1994).

6. Modification of functional properties by processing

Different processing methods have been studied in order to improve or modify the functional characteristics of milk proteins and reviewed with the common methods being enzymatic modification (Panyam and Kilara, 1996; Augustin and Udabage, 2007; Buchert et al., 2010; Foegeding and Davis, 2011) and heat application (Raikos, 2010). Other processing methods such as ultrasound application (Soria and Villamiel, 2010; Arzeni et al., 2012), microwaving (Zhang and Wang, 2008), high pressure application (Galazka et al., 2000; Lopez-Fandino, 2006; Considine et al., 2007) and chemical modification (Augustin and Udabage, 2007) have also been applied but to a smaller scale. The discussion here will concentrate on the application of these methods to milk protein concentrate as results obtained from studies of other milk protein products have been reviewed elsewhere (Augustin and Udabage, 2007). **Figure 4** shows the theoretical intervention points for changing functionality of MPC based colloidal structures.

6.1. Effects of heat treatment and high pressure

Heat treatment has been the choice of processors in modifying functional properties of milk proteins. For instance, studies have found that the solubility of MPC could be improved by raising the reconstitution temperature, resulting in an increase in water transferral towards the interior of the powder particles (Zwijgers, 1992; Davenal et al., 1997). This has also been reported in sodium caseinates (Jahaniaval et al., 2000). Dybowska (2008) studied the effect of preheat treatment on oil-in-water emulsions (30% rapeseed oil) stabilized with MPC at 60^{o}C and homogenization pressure of 10 and 2 MPa. Emulsions formed from unheated proteins were less stable (42.4%) and an increase in preheating temperature increased emulsion stability up to 50.4% (**Figure 5A**). Preheating of oil at 60^{o}C gave more stable (52.2%) emulsions than unheated oil (42.4%); however, the emulsion stability decreased from 52.2% to 48.2% when the oil preheating temperature was increased from 60 to 95^{o}C (**Figure 5B**).

Udabage et al. (2011) studied the effects of high pressure (HP) and heat treatment (100-400 MPa at 10-60^{o}C) on the solubility of milk protein concentrate (MPC) powders. After twelve months of storage, the solubility, measured at 20^{o}C, of fresh MPC powders made with no HP treatment decreased from 66% to 50%. The combinations of pressure and heat (200 MPa and 40^{o}C) applied to the concentrate before spray drying was found to be the most beneficial for improved solubility of MPC powders. This combination of pressure/heat improved the initial cold water solubility to 85%. The solubility was maintained at this level after 6 weeks storage at ambient temperature and 85% of the initial solubility was preserved after 12 months. The improved solubility of MPC powders on manufacture and on storage are attributed to an altered surface composition arising from an increased concentration of non-micellar casein in the milk due to

HP treatment prior to drying. The results suggest that increasing the non-micellar casein content by HP treatment of milk is a strategy that may be used to obtain high protein milk powders with enhanced solubility. Improved gelation of MPC has been reported by Briscoe et al. (2002) after application of moderate pressures (up to 1000 bar).

6.2. Application of ultrasound and microwave technology

Ultrasound (US) technology involves the use of sound frequency above the human hearing threshold (>16 kHz) and a huge amount of research has gone into the application of ultrasound at both high power (low frequency; 16 - 100 kHz) and low power (high frequency; 100 kHz - 1 MHz) as recently overviewed by Vilkhu et al. (2008), Soria and Villamiel (2010) and Kwiatkowska et al. (2011). The use of the latter is mainly confined to medical application whilst the former finds application in sonochemistry. When US is applied to an aqueous solution or suspension an increase in mixing, shearing and mass transfer is observed. Under certain conditions cavitation is produced which manifests itself in the production of tiny bubbles which implode to produce so called “hotspots” which tend to generate highly reactive hydroxyl radicals and unusual chemical transformations (Kwiatkowska et al., 2011; Bremner, 1990). Generally, the ultrasonic equipment used in modification of functional properties fall into three types: an ultrasonic probe system (usually 20 kHz, an ultrasonic bath (around 40 kHz), or an ultrasonic transducer fitted to a glass reactor (from 300 to 1100 kHz).

The effects of ultrasound on functional properties of proteins largely depend on the nature of the protein (Soria and Villamiel, 2010). Arzeni et al. (2012) observed that the effect of ultrasonic

application was different in whey protein concentrate, egg protein and soy protein. Chandrapala et al. (2012) also observed differences in effect on surface hydrophobicity of a-lactalbumin, b-lactoglobulin and their mixture. Application of ultrasound at 20kHz showed greater effect on α-lactalbumin than on β-lactoglobulin. Jambrak et al. (2008) found that ultrasound (using 20 kHz probe) affected solubility and foaming ability of whey proteins which was attributed to sample exposure to high temperatures caused by sonication. In milk protein concentrate, effect of ultrasonic application on functional properties has been scantly done or not done at all. This is an area of interest looking at the problems of solubility loss which might be reduced by the cavitation effects of ultrasound (Soria and Villamiel, 2010) as well as temperature increase associated with it (Arzeni et al., 2012).

Microwave irradiation is another treatment that it is known to affect protein structure. Microwaves (MW) are electromagnetic waves and the heating of proteins by microwave energy is achieved both by the absorption of microwave energy by rotation of the bipolar water molecules and translation of the ionic components of the proteins. This energy is converted into heat (Ohlsson and Bengtsson, 2001). The use of microwave irradiation in modifying functional properties has been done in various proteins. For example, Zhang and Wang (2008) reported that application of microwave energy at 600W for 2 minutes increased the solubility, emulsification and stability of soy protein concentrate by 32.15 %, 58.87 % and 56.54 % respectively. MW has been used in combination with high pressure to improve the enzyme hydrolysis of b-lactoglobulin with success (Izquierdo et al., 2005). Proteolysis of dairy whey proteins with different enzymes in combination with MW treatment has also shown the potential to more

efficiently produce hypoallergenic dairy hydrolysates (Izquierdo et al., 2008). The application of MW in modification of functional properties of MPC is also scanty.

6.3. Enzyme modification

Enzyme hydrolysis of proteins causes changes (positive or negative) in functionality of proteins. This has been reported in several studies of proteins and reviewed (Panyam and Kilara, 1996; Chobert et al., 1996). Severin and Xia (2006) studied Alcalase and Protamex hydrolysates of whey protein concentrate (WPC 80) at different degree of hydrolysis (DH). They reported improved protein solubility due to formation of small particles but poor emulsifying and foaming properties except Alcalase produced hydrolysates (5% DH) with varying effects on gelation. Limited hydrolysis would in principle help in producing hydrolysates with improved functional characteristics. Gelation in whey proteins was also increased by 50% after storage of resulting a-chymotrypsin hydrolysates (Rabiey and Britten, 2009). Such determination of hydrolysis effects on MPC hydrolysates is scanty in literature. Enzyme modification is also possible through protein cross-linking using transglutaminase and other related enzymes. The results of which depend on the substrate type (Hiller and Lorenzene, 2009). Chemical modification of functional properties is possible through acetylation, succinylation of proteins with acid anhydrides, phosphorylation, lipophilization, glycosylation, thiolation, reductive alkylation and covalent attachment of amino acids, but it is not been favored due to increased costs, use of chemicals, consumer acceptability and need for meeting regulation standards (Panyam and Kilara, 1996).

Addition of salts has also been used to change the functionality of MPC before and after production. Mizuno and Lucey (2007) used tetrasodium pyrophosphate (TSPP) to improve gelation at 25^{o}C and hardest gels were formed when 6.7mM salt was added. Less or more salts did not form gels. It is thought that the salts act with calcium as a cross-linking agent between dispersed caseins and when the balance between (a reduced) electrostatic repulsion and (enhanced) attractive (hydrophobic) interactions becomes suitable for aggregation and eventual gelation of casein molecules. This seems to agree with finding by Babella (1989) which showed that increasing the calcium to total mineral ratio decreased the solubility of MPC. However, an increase in the potassium or sodium to total mineral ratio did not change its solubility.

6.3. 1 Enzyme hydrolysis and bioactivity

Milk mining for bioactive compounds has received considerable attention by researchers. This has been reviewed by several authors (Clemente, 2000; Nekluydov et al., 2000; Lopez-Fandino et al., 2006; Korhonen and Pihlanto, 2006; Elias et al., 2008; Ricci et al., 2010; Mills et al., 2011). Most of the bioactive compounds are from hydrolysates of milk proteins using different enzymes. Since milk protein concentrate is considered as aggregated milk, it has not received much attention to bioactive compounds or peptides mining, the focus being on pure protein fractions. But other researchers have hydrolysized milk protein concentrates and determined the bioactivity of the resulting peptide fractions. Using two enzymes (trypsin and chymotrypsin), Amiot et al. (2004) showed that fractions from milk protein concentrate had the ability to promote growth of skin cells (by 108%) and a patent to the same effect exists (US 6506732**).** The peptide fractions were from both the casein and whey fraction of the milk protein (less than 800Da) containing

high concentrations of both hydrophobic and aromatic amino acids. The potential to use MPC as a nutraceutical is therefore presented.

A recent study by Lacroix and Li-Chan (2012) compared the dipeptidyl peptidase (DPP)-IV inhibiting activity (indicator for type 2 diabetes therapy) of different milk protein hydrolysates including milk protein concentrate (MPC 80). In the investigation, MPC gave the lowest inhibiting activity. It should be mentioned that enzyme hydrolysis products depend on several factors such as pH, temperature, enzyme-to-substrate ratio, total solids, time and degree of hydrolysis and enzyme type (Cheung and Chan, 2010; Otte et al., 2007; Spellman et al., 2005; Neklyudov et al., 2000). Thus, comparative studies would be needed to establish whether the bioactivity claimed in the pure milk proteins would be the same, increased or decreased in MPC. Analytical and statistical tools, such as response surface methods and Taguchi designs (Contreras et al., 2011; Tavares et al., 2011; Chen et al., 2011; Cheung and Chan, 2010) would also help in determining optimal hydrolysis conditions for particular functional characteristic requirement. It would not be surprising that there would significant differences due to protein-protein interaction, peptide-peptide interaction or even enzyme-peptide interaction (Amiot et al., 2004).

6.3.2. Bitterness

It has long been observed that bitterness is associated with enzyme hydrolysis of proteins which would in effect hinder consumer acceptability of its products despite improved functional and bioactive characteristics. This triggered a series of research to isolate the bitter compounds and in most studies, it was concluded that there are low molecular and hydrophobic peptides

responsible for bitterness in the hydrolysates. This has been reported (Spellman et al., 2005; Spellman et al., 2009; Cheung and Chan, 2010) and comprehensively reviewed elsewhere (Lemieux and Simard, 1992). Efforts have been made to counter the bitterness through such means as masking, use of peptidases, controlled hydrolysis and manipulation of bitter receptors (Maehashi and Huang, 2009). As previously stated, enzymatic modification studies of MPC are few and it follows that bitterness studies on MPC are not many if any. Combined effects of processing methods (heat treatment, high pressure, ultrasound and microwave) and enzyme hydrolysis on modification of functional properties offers a great opportunity for tailor made functional foods with reduced bitterness.

7. Future perspective

In conclusion, this review has given the recent activities in the study of functional properties of MPC. Functional properties of MPC and its hydrolysates are very important and need to be studied in detail in order to improve on them. Although a lot has been done on understanding the solubility issues in MPC, more has to be done on the other properties such as gelation, emulsification and foaming. Enzyme modification also presents an opportunity to change the functional properties of MPC. Although most studies are concerned with bioactivity of proteolysis products, this method has not been fully explored and it is possible to optimize the composition of hydrolysates by varying or controlling the location of the bond hydrolyzed. Such an approach would give rise to peptides with high functionality. Moreover, enzymes other than proteases, for example transglutaminase, may be used to give to proteins new properties. The fate of the resulting bioactive and functional foods *in vivo* needs thorough exploration.

It is important to study the effects of new technologies on the functional properties of MPC. This includes high pressure, microwave and ultrasonic application whether acting alone or in combination. Apart from that, enzyme modifications would also have to be studied applying optimization tools to improve products emanating from MPC including less bitter hydrolysates. The development of testing standards for easy comparative analysis is also imperative.

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Table 1: Some applications of MPC in food processing

Usage	Form	Effect	Reference
Pizza cheese	Dry MPC 63.5	Improved yield with meltability increasing during one month storage	Rehman et al., 2003a
Oaxaca cheese	MPC 40	Increased yield	Caro et al., 2011
Reduced fat cheddar cheese	MPC 63.5	Increased yield	Rehman et al., 2003b
Ice cream	MPC56 and MPC85	Higher mix viscosity, larger amount of fat destabilization, narrower ice melting curves, and greater shape retention	Alvarez et al., 2005
Italic citric Mozzarella cheese	Alapro^{TM}4861 (MPC83)	Increased yield	Francolino et al., 2010
Ice cream	MPC70	Improved protein content	Patel et al., 2006
Yoghurt	Not given	No significant difference in viscosity and syneresis index	Gonzalez et al., 1999
Bing cherries	Alaplex 1235 (MPC92.6)	Prolonged shelf life	Certel et al., 2004

Table 2: Methods used to determine solubility in milk protein concentrates

Term used	Specifics of the method	Reference
Insolubility index (ISI)	The sediment amount after drying divided by the weight before centrifugation (modified Niro method A 3a at www.niro.com). 50ml solution centrifuged at 1000rpm for 5 minutes and supernatant discarded. The sediment is dried overnight at 50^{o}C.	Fang et al., 2010
% Solubility	$\%Solubility = \frac{Solids\ in\ the\ supernatant}{Solids\ in\ the\ solution} \times 100$ Sample of 40ml is centrifuged at 1000 x g for 10 min at 20^{o}C, supernatant filtered under vacuum, 5g aliquot of filtrate dried in aluminium dishes with 20g acid washed sand at 105^{o}C for 24hrs	Haque et al., 2012; Haque et al., 2011
Solid content	50ml sample centrifuged at 4400 x g for 5min at 24^{o}C, the supernatant filtered under vacuum and dried at 102^{o}C for 24hrs (Equation not given)	Mimouni et al., 2010
Percent suspension stability (%SS)	$\%SS = \frac{TS\ content\ of\ supernatant}{TS\ content\ of\ original\ dispersion} \times 100$ Where: TS is the total solids	Sikand et al., 2011

	(modified from Anema et al., 2006) Sample is centrifuged at 700 x g for 10 minutes	
Amount of soluble material, σ	$\delta = \frac{weight\ of\ dry\ material}{weight\ of\ solution} \times 100$ Sample centrifuged at 700 x g for 10 minutes and dried overnight at 105^{o}C and cooled in a dessicator	Fyfe et al., 2011a; Havea, 2006; Anema et al., 2006

Table 3: Effect of mineral composition on solubility (Source: Sikand et al, 2011)

Sample	Sample type	Concentration expressed in mg/100g sample							%SS*
		Calcium	Magnesium	Potassium	Sodium	Phosphorus	Chloride	Total mineral	
1	MPC40	903	83	1134	284	816	1059	4279	99
2	MPC40	956	89	1177	303	882	1049	4456	98
3	MPC80	1423	80	350	100	375	286	2614	43
4	MPC80	1486	76	217	50	1085	136	3050	28
5	MPC80	1496	68	226	45	1085	70	2990	24
6	MPC80	1493	69	366	89	1109	213	3339	27
7	MPI	1506	66	266	64	1118	153	3173	33
8	MPI	1436	69	199	52	1083	183	3022	55
9	MPI	571	6	503	167	434	223	1904	97
10	MPI	553	17	116	400	569	269	1924	98
11	MPI	1644	82	161	52	1206	103	3248	55

*values approximated from graph

Table 4: Examples of emulsifying characteristics that are determined in emulsion studies of MPC

Author	Type of MPC and oil used	Method
Mena-Casanova and Totosaus (2011)	Alapro 4560 (MPC from New Zealand Milk Products) and corn oil	15ml of protein solution added with 10 ml oil for 1 min then used burette to add more oil at 9ml/min and emulsions were formed by homogenizer. Electrical conductivity of solution was monitored until phase inversion. Emulsion capacity was reported as the total emulsified ml of oil per gram of protein before phase inversion and emulsion work was the area under conductivity – time graph while emulsion stability (s) was given as: $s=(C_s-C_e)\frac{\Delta t}{\Delta c}$ where C_s is the conductivity of the protein emulsion after 1 min (before adding more oil), C_e is the conductivity of emulsion before slope change and $\frac{\Delta t}{\Delta c}$ is the reciprocal of initial slope of the conductivity curve during the first minutes when oil was added.

Dybowska (2008)	Commercial MPC75 and rapeseed oil	3wt% solution and 30 vol wt oil was mixed by stirring. Homogenisation involved two stages at 10MPa and 2MPa at 60^{o}C and the cooling to 25^{o}C. Emulsifying capacity was based on electrical resistance. Ten ml of the protein dispersion, 1 ml of 1 N NaCl and 5 ml of rapeseed oil were poured to the measuring cell. The mixture was homogenized for 10 min at room temperature to produce emulsion. Then, rapeseed oil was delivered over the mixer blades at a constant rate 3.6 ml/min using a peristaltic pump. The delivery of oil was stopped at the point of emulsion collapse and the capacity expressed as the amount of oil (ml) emulsified by 100 mg of milk protein concentrate. Dynamic viscosity of the dispersion was measured using a viscometer at 25^{o}C. Forty five ml of the emulsions was poured to a glass tube and centrifuged at 1467 x g and 25 ^{o}C. Under high centrifugal force the sample separates into two layers: upper oil phase and lower continuous phase. Stability was expressed as a percentage volume of the oil phase to the volume of the sample. Emulsion particle size distribution was done by a Malvern Zetasizer NanoS

Euston and Hirst (2000); Euston and Hirst (1999)***	MPC85 from New Zealand Dairy Board and soya oil	0.5 wt % protein solution was held for 2.5hrs at 55°C before adding oil which was heated to 55°C to make a 0.4 wt % final emulsion. Homogenization was done at 200bar. Particle size distribution for each emulsion was determined by Malvern Mastersizer E. Emulsifying stability was estimated as the emulsion ratio given as the ratio of the volume of the cream phase to the theoretical volume of a totally stable emulsion. ***The accelerated creaming stability of the emulsions was determined from a sample of the emulsion stored in a centrifuge tube for 24 hrs at 20°C. After storage the sample was centrifuged at 180 *g* for 30 min. The bottom 5 ml of the emulsion was removed, and the fat content of this aliquot was determined using the Rose-Gottlieb method. The creaming stability index for the emulsion was defined as the ratio of the fat in the bottom 5 ml to the total fat content of the whole emulsion.

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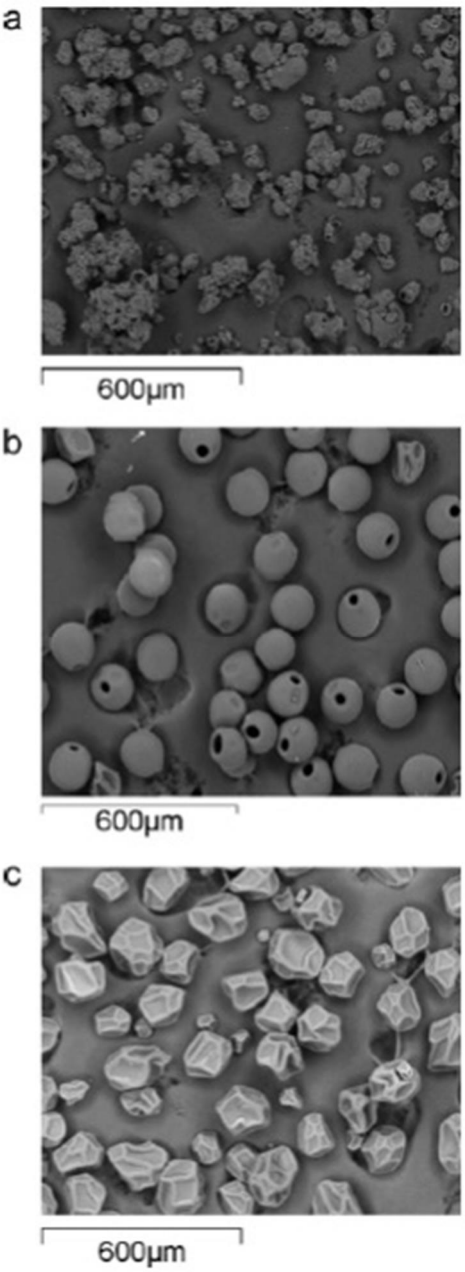

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Figure 1: Scanning electron micrographs of MPC particles with different morphology as a result of different drying conditions (**a**. control; **b**. 77 ^{o}C; **c**. 178 ^{o}C) (source: Fang et al., 2012).

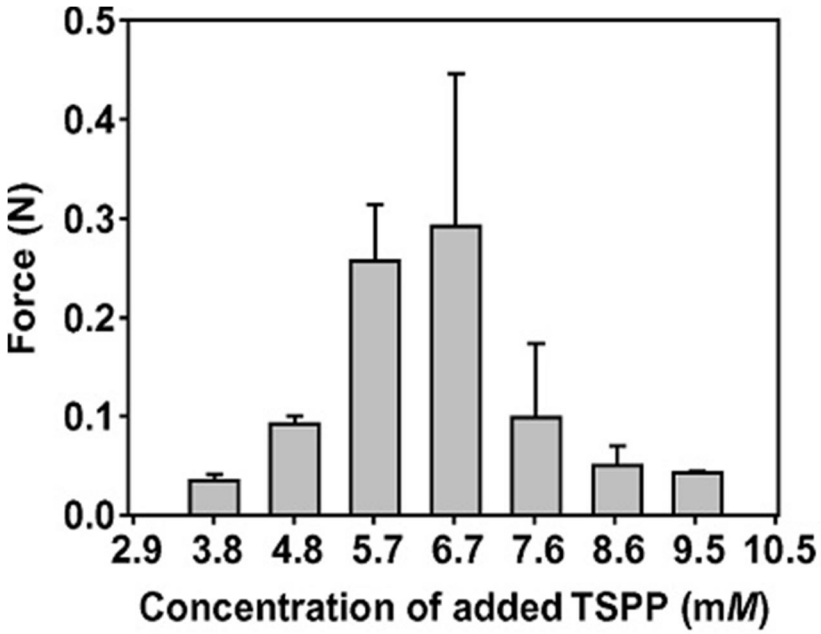

Figure 2: Effect of tetrasodium pyrophosphate (TSPP) salt addition on the strength of MPC gels (source: Mizuno and Lucey, 2007).

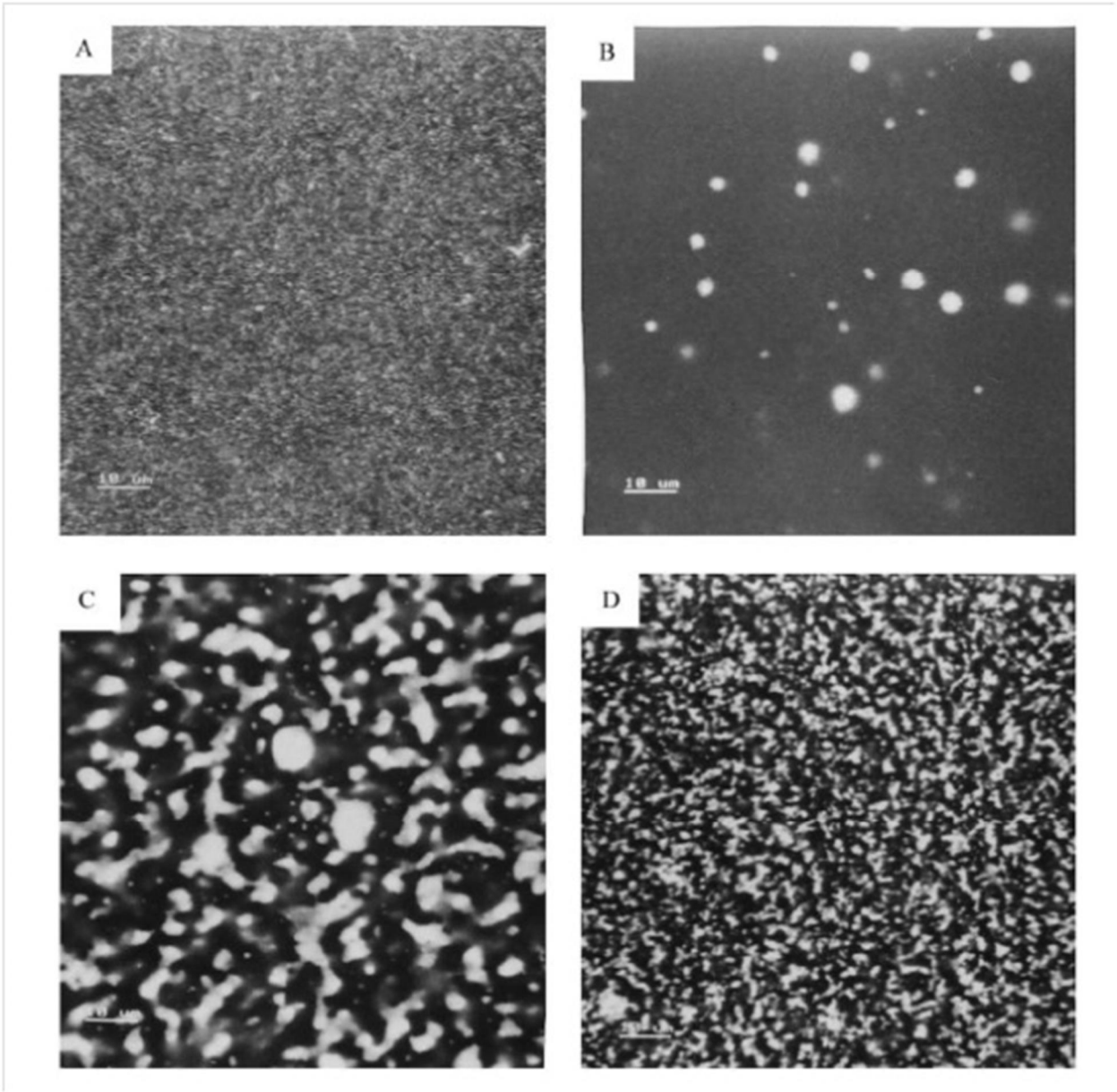

Figure 3: Confocal micrographs of MPC (5%)/xanthan mixtures containing: (A) no xanthan, (B) 0.1% xanthan, (C) 0.5% xanthan and (D) 1% wt xanthan (source: Hemar et al., 2001).

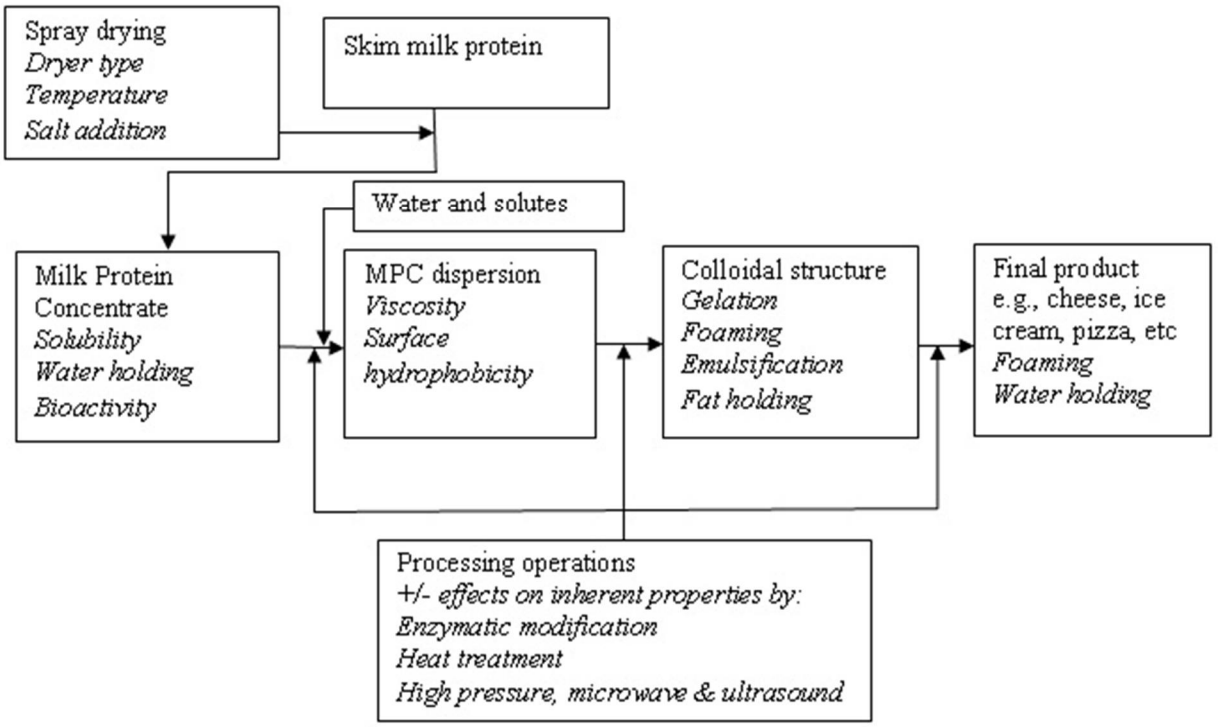

Figure 4: Processes involved from the production of milk protein concentrate to the formation of MPC based colloidal structures. Processing can be done along the chain to change the functional properties.

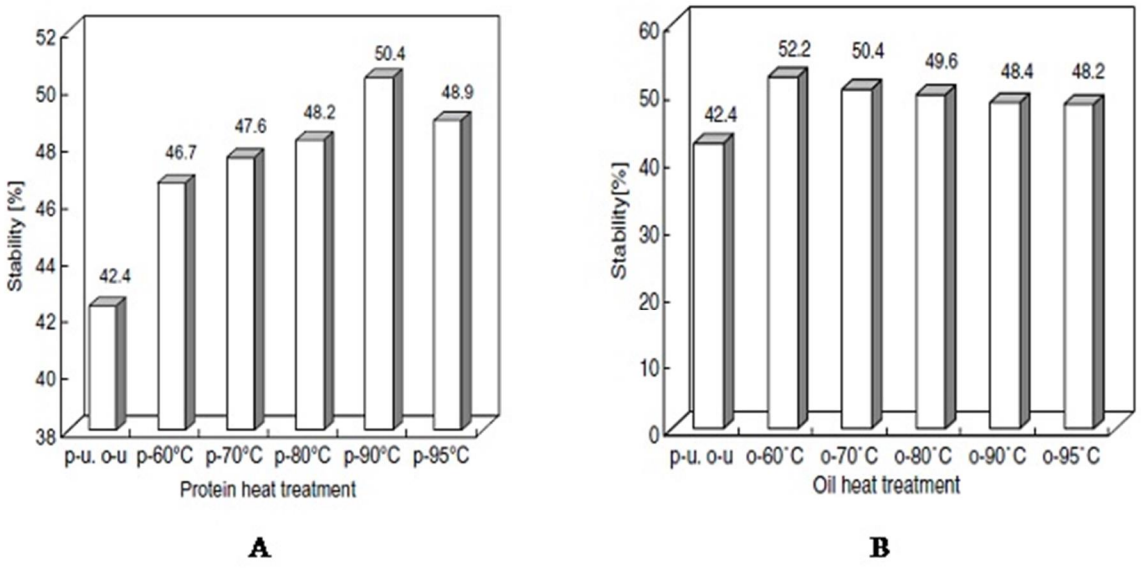

Figure 5: Effect of preheating on emulsion stability of MPC, A: emulsion stability after protein heating, B: emulsion stability after oil heating (source: Dybowska, 2008).