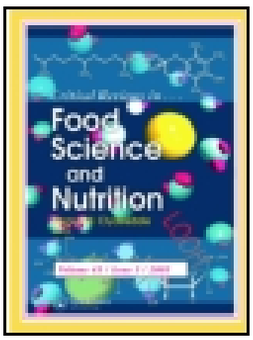

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REVIEW



# Insolubility in milk protein concentrates: potential causes and strategies to minimize its occurrence

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**ABSTRACT**

Milk protein concentrates (MPCs), which are produced from skim milk following a series of manufacturing steps including pasteurization, membrane filtration, evaporation and spray drying, represent a relatively new category of dairy ingredients. MPC powders mainly comprise caseins and whey proteins in the same ratio of occurrence as in milk. While bovine MPCs have applications as an ingredient in several protein enriched food products, technofunctional concerns, e.g., reduced solubility and emulsification properties, especially after long-term storage, limit their widespread and consistent utilization in many food products. Changes in the surface and internal structure of MPC powder particles during manufacture and storage occur via casein-casein and casein-whey protein interactions and also via the formation of casein crosslinks in the presence of calcium ions which are associated with diminishment of MPCs functional properties. The aggregation of micellar caseins as a result of these interactions has been considered as the main cause of insolubility in MPCs. In addition, the occurrence of lactose-protein interactions as a result of the promotion of the Maillard reaction mainly during storage of MPC may lead to greater insolubility. This review focuses on the solubility of MPC with an emphasis on understanding the factors involved in its insolubility along with approaches which may be employed to overcome MPC insolubility. Several strategies have been developed based on manipulation of the manufacturing process, along with composition, physical, chemical and enzymatic modifications to overcome MPC insolubility. Despite many advances, dairy ingredient manufacturers are still investigating technical solutions to resolve the insolubility issues associated with the large-scale manufacture of MPC.

**KEYWORDS**

Insolubility; micellar casein; milk protein concentrate; modification

## Introduction

Milk protein concentrate (MPC) is a source of high quality dairy proteins (Drake, Miracle, and Wright 2014). MPC is categorized as a casein (CN)-dominant ingredient (Yada 2018) which can be divided into low (≤42%), medium (60%–70%) and high (≥80%) protein content ingredients, while MPC with >90% protein is known as milk protein isolate (MPI) (Agarwal et al. 2015).

The applications of MPC in different food products have been reviewed by Agarwal et al. (2015). MPCs are used as a replacement for skim milk powder (SMP)/non-fat dry milk, for protein adjustment of commercial products such as neutral pH ready-to-drink protein beverages, ice-cream, yogurt, cheeses, spreads, coffee creamer, whipping cream, protein fortified products (e.g., different types of cheeses and yogurts), nutritional beverages and bars, in clinical nutrition, dietary supplements and in sports/performance nutrition (Moghaddas Kia et al. 2018; Singh et al. 2019; Chamberland et al. 2020; Gopirajah et al. 2020). Due to its wide range of applications and the demand for high quality MPC, numerous studies on MPC functionality, e.g., solubility, rehydration and wettability, dispersibility, flowability, viscosity, gelation, thickening, foaming/whipping, emulsification and heat stability have been performed (Crowley, Boudin, et al. 2015; Lin et al. 2018; Singh et al. 2019). Each of the applications outlined above require different MPC functionalities. For instance, high solubility MPC is required for the formulation of ready-to-drink beverages, while for yogurt formulation the water holding capacity (WHC) of the MPC ingredient is crucial. Therefore, it may be impractical to have one MPC ingredient with a wide range of functionalities suitable for all application areas.

MPC is manufactured through a series of processing steps starting with heat-treated skim milk (McSweeney et al. 2021). The application of membrane processing is a key step in MPC manufacture (Mistry and Maubois 2017). Utilization of ultrafiltration (UF) for the purpose of protein concentration over the temperature range 10–50 °C minimizes protein denaturation and preserves the nutritional and functional properties of the starting material. Furthermore, diafiltration (DF) is employed during the production of medium or high protein content MPCs. The final steps in the manufacture of MPC include (vacuum) evaporation of the UF/DF retentate followed by spray drying as schematically outlined in Figure 1 (Goulart and Hartel 2017; Patil et al. 2018). Proprietary processes used by different manufacturers may lead to variation in the technofunctional and

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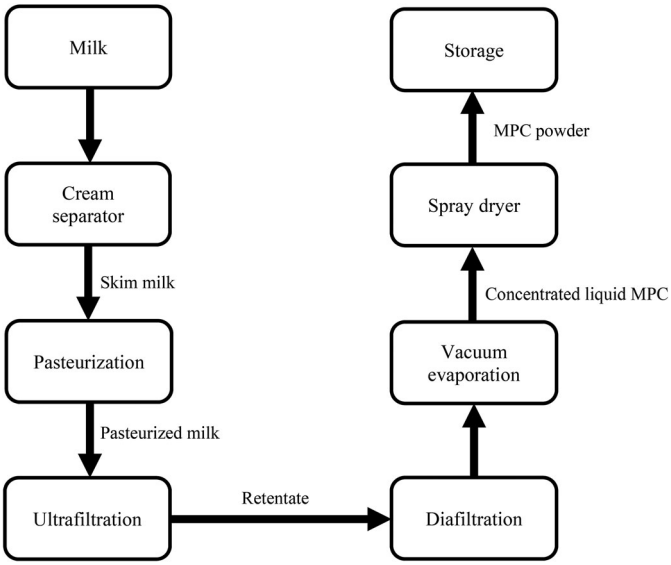


**Figure 1.** The processing steps involved in milk protein concentrate (MPC) manufacture.

nutritional properties between MPCs with similar protein content. This leads to a large variation in the properties of commercially available MPCs. Such variation may provide end-users with a range of functionalities which may be relevant for their specific application.

Solubility is key parameter during the formulation of many products containing MPC. However, in comparison to other milk protein ingredients (such as whey-based and whole milk products), MPCs can display poor solubility. This is mostly related to changes in CN micelles (CNMs) (Sikand et al. 2012) and the occurrence of chemical modifications such as protein crosslinking, deamidation and lactosylation during manufacture and storage (Le et al. 2012). Furthermore, the bulk and surface composition difference between MPCs were reported to be key to the difference in solubility between low and high protein MPCs. The insolubility of MPCs may adversely impact their viscosity, drinkability, shelf-life, along with the energy and time required to rehydrate MPC powders and accordingly, its target products such as cheeses, nutritional bars and protein fortified beverages (Eshpari et al. 2017).

Recent reviews on MPC have outlined some possible mechanisms associated with MPC insolubility (Mistry and Maubois 2017; Meena, Singh, and Panjagari 2017). This review specifically updates the advancements in regard to strategies employed to improve the solubility of MPC.

## Insolubility in MPC

### *Measurement of MPC solubility*

Different analytical methods are used to measure the extent of MPC solubility. While many authors report MPC solubility in the form of percent suspension stability (%SS) (Mimouni et al. 2010; Sikand et al. 2011; Haque et al. 2012), other terms such as insolubility index (ISI) (Fang, Selomulya, and Chen 2010), mass fraction of dissolved material ($\sigma$) (Fyfe et al. 2011), nitrogen solubility index (NSI) (Sikand et al. 2016) and protein solubility (Mao et al. 2012) have also been employed to express MPC solubility/insolubility. Furthermore, the use of particle size (PS) analyzers for assessment of the stability of reconstituted MPC particles and rate of particle flocculation has also been reported in the literature (Crowley, Gazi, et al. 2014; Eshpari et al. 2017).

### *Percent suspension stability*

Most of the studies on MPC solubility measure the SS% according to Havea (2006). This method is routinely used in industrial applications for dairy powders (Fang et al. 2011). SS% is defined as the ratio between the total solids (TS) content of the supernatant obtained after centrifugation of the reconstituted sample to the TS content (g) of the aqueous dispersion. Haque et al. (2015) filtered the supernatant under vacuum using GF/A microfiber filter paper (1.6 $\mu$m pore size, Whatman) prior to oven drying to eliminate possible dissolved aggregates. Therefore, the term "solubility" is considered as the non-sedimentable material retained under the above mentioned conditions. Rupp, Molitor, and Lucey (2018) decanted the supernatant and re-centrifuged it in order to ensure the removal of insoluble components. Havea (2006) noted that the soluble material obtained by the centrifugation method may include aggregated proteins, which could be classified as "insoluble" under different sets of dissolution/centrifugation procedures. SS% is also termed as the dispersibility (%) (Fang et al. 2011), powder solubility (%) (McSweeney et al. 2020) and the mass fraction of dissolved material (Mimouni et al. 2010). Fang, Selomulya, and Chen (2010) reported the percentage of sediment weight over the total solution (wt/wt%) as the ISI.

### *Nitrogen/protein solubility*

The nitrogen solubility of MPC has been determined in some studies using IDF method 173 (IDF 1995) where a mass of powder equivalent to 1 g of protein (using a nitrogen to protein conversion factor of 6.38) is dispersed in water (100 mL) at room temperature (22 °C) for 2 h and the pH is adjusted to pH 7·0. An aliquot (50 mL) of the sample is transferred to centrifuge tubes and the sample is then centrifuged (for 10 min at 3000 g). The supernatant is decanted and filtered through Whatman No 1 filter paper. The nitrogen content of the filtered supernatant is determined using the Kjeldahl nitrogen determination method (IDF 1993). The nitrogen content of the filtered supernatant as a function of the nitrogen content of the whole solution (%) is considered as the percentage nitrogen solubility (Augustin et al. 2012; Udabage et al. 2012). By calculating the protein content in the supernatant and dividing it by the protein content in the whole suspension (%), the protein solubility (%) is attained.

### Ultracentrifugation

Gazi and Huppertz (2015) analyzed the percentage solubility of $a_{S1}$-CN, $a_{S2}$-CN, $\beta$-CN, $\kappa$-CN, $\alpha$-lactalbumin ($\alpha$-La) and $\beta$-lactoglobulin ($\beta$-Lg) in MPCs using reverse-phase high performance liquid chromatography (RP-HPLC). The soluble proteins present in MPC have also been determined following ultracentrifugation (100,000 or 290,000*g*, room temperature, 1 h) of reconstituted MPCs using RP-HPLC (Xu et al. 2016; Cadesky et al. 2017). This approach allows detection and quantification of the non-sedimentable proteins (mostly whey proteins (WP) and minor levels of CNs) which are separated from the sedimentable proteins (mostly micellar CNs) following the ultracentrifugation step.

### PS analysis of re-constituted MPCs

Samples of reconstituted MPC are analyzed using a PS analyzer to show the stability of particles in suspension as a function of storage time. PS may be reported using different terms such as the volume weighted mean (D[4,3]), the surface weighed mean (D[3,2]), the average PS (d(0.5)) and the size of the particles within which 10% d(0.1) and 90% d(0.9) of the sample lie. The specific surface area (SSA) is another factor which determines the stability of particles in suspension. Crowley, Gazi, et al. (2014) reported the d(0.1), d(0.5) and d(0.5) as well as SSA for a range of MPC samples (MPC35, MPC50, MPC60 and MPC70, MPC80, MPC85 and MPC90) showing a significant difference between the MPC particles, while they observed similar SSA for MPC35, MPC50, MPC60 and MPC70 (0.32–0.44 $m^2.g^{-1}$) and similar SSA for MPC89, MPC85 and MPC90 (0.74–0.77 $m^2\ g^{-1}$). Eshpari et al. (2017) used a dynamic light scattering instrument for measurement of the mean hydrodynamic diameter value of particles of MPC80 which were in the range of 170–200 nm.

In addition to these methods, assessment of the WHC may also be measured as an indication for the ability of MPC to entrap water molecules. For the determination of WHC, a volume of $dH_2O$ (e.g., 10 mL) is added to MPC powder (e.g., 0.5 g) in a pre-weighed centrifuge tube, the sample is gently mixed for 30 s and the mixture is then centrifuged. The supernatant is removed and the weight of the residue minus the mass of the initial powder is calculated to report the WHC which is expressed as g water/g protein (Meletharayil, Patel, and Huppertz 2015). Shilpashree, Arora, Chawla, and Tomar (2015) reported the WHC of MPC85 to be ~3.0 g water/g protein.

Furthermore, the wettability of MPC powders (the ability of MPC powder surfaces to absorb water) may be also determined using conventional or instrumental techniques. For the former method, a known weight of MPC powder (e.g., 10 g) is dispersed evenly in 250 mL $dH_2O$ in a 600 mL beaker and the time for the MPC particles to submerge in the water is determined. Using this method, McSweeney et al. (2020) determined the wettability of a range of MPC samples with different protein contents showing that during 20 min around 50% of particles of low protein MPCs (MPC40, MPC55 and MPC65) were submerged in $dH_2O$ while for MPC85 this value was only 15%. For the instrumental method, the contact angle ($\theta$) of MPC powder disks prepared using an hydraulic compressor (8000 kg) and water droplets is measured as an indication of wettability (where higher $\theta$ values represents lower wettability). Crowley, Desautel, et al. (2015) reported $\theta$ values for a range of MPC samples with different protein contents showing that by increasing the protein content from 35 to 85%, the $\theta$ varied from 60 to 75°.

The use of different terminology and analytical methods may lead to different results concerning the reported solubility values of various MPCs. Therefore, it is often difficult to directly compare the reported solubility of MPCs arising from different publications. Furthermore, since solubility is a pH dependent factor, adjusting the pH to a constant value (ideally pH 7.0) prior to analysis may allow for better comparison between different MPCs.

## The process of MPC rehydration

In comparison with some other CN-rich ingredients, the reconstitution of MPC is sometimes rate-limiting in terms of its applications. The composition of the concentrated milk, along with the thermal and physicochemical alterations induced during the manufacture and storage of MPCs significantly impact the microstructure and thereby the process of MPC powder reconstitution (Anema et al. 2006; Babu et al. 2018; Kieferle et al. 2019). Forny, Marabi, and Palzer (2011) segregated the rehydration of MPC into a number of steps which include wetting and immersion of the particles, followed by dissolution of the soluble particles. The poor wettability of MPC compared to other ingredients such as SMP and buttermilk powder at a same protein content may be attributed to its higher CN content (Silva and O'Mahony 2017). Many efforts have been made to enhance the rate limiting dissolution step of MPCs using physical post-manufacturing treatments such as the application of high temperature and intense agitation (Fang et al. 2011; Richard, Toubal, et al. 2012).

Exposure to high temperatures during MPC reconstitution may help solubilization of MPC powder. Reconstitution of MPC80 at 37 °C was found to help improve the solubility in comparison with dissolution at 4 °C (Sikand et al. 2012). A reconstitution temperature of 50 °C has been suggested as optimal to achieve complete aqueous solubility in a short time (Fang et al. 2011). A 40% improvement in dissolution of MPI was observed when the dissolution temperature was elevated from 25 to 50 °C (Crowley, Megemont, et al. 2014). McCarthy et al. (2014) suggested a two-step dissolution protocol, i.e., 10-min stirring at 50 °C followed by 1 min high intensity ultrasonication (20 kHz) at 50 °C in order to obtain complete solubilization of MPC. Nevertheless, for some MPC applications, such as the formulation of ready-to-drink beverages, cold solubility is an important parameter.

The application of intense mechanical agitation to MPC induces shear stress associated with vigorous turbulence which shortens the time required for rehydration (Richard, Toubal, et al. 2012). High pressure homogenization

(100–900 bar) of MPC50 and MPC85 disintegrated the aggregates of micellar CN, leading to an improvement in the rehydration (Warncke and Kulozik 2020). The application of homogenization (13,800 kPa) for reconstitution of MPC80 (3.5% (wt/vol) protein suspension) improved rehydration (Sikand et al. 2012). Deshpande and Walsh (2018) employed a sonication treatment (70% amplitude for 30 s) on a reconstituted MPC70 and observed a 50% reduction in the viscosity from an initial value of 500 mPa.s. This viscosity reduction may be attributed to modification of the protein network and the enhanced solubility of MPC powders (Richard, Toubal, et al. 2012). The application of ultrasonication (20 kHz, 70.2 W, 1 min) in combination with 10 min stirring was also shown to yield complete dissolution of MPC80 (5% wt/vol) (McCarthy et al. 2014). Dispersion (at 50 °C) of MPC80 (20% dry matter) in combination with hydrodynamic cavitation (flow rate of 850 L/h, motor frequency of 48.8 Hz, electrical current of 30.7 A and rotor speed of 2914 rpm) was reported to yield complete hydration (Pathania et al. 2018).

Moreover, addition of 5–25 mM sodium hexametaphosphate (SHMP) as a calcium chelating agent to reconstituted MPC80 has been shown to improve its rehydration (Power et al. 2020). However, addition of SHMP may also increase the viscosity. It was shown that the addition of transglutaminase to SHMP treated MPC greatly reduced the viscosity. Addition of sodium carbonate (0.5 g/100 mL) to reconstituted MPI (4% (wt/vol) on a powder basis) has been shown to increase the pH of the solution and improve its dissolution ability (Wu et al. 2020a). Addition of citric acid to neutralize the pH has been shown to generate colloidal particles containing both micellar and non-micellar CNs (Wu et al. 2020b). Addition of 5 mM calcium to reconstituted MPC80 (13.5% (wt/vol) protein) at pH 6.7 reduced 53% of the protein solubility. This reduction was only 10% at pH 7.2 (Gaspard et al. 2017).

### *Interactions contributing to limited solubility/diminished rehydration properties*

The main cause of the insolubility in MPC appears to be associated with the structures formed by the CNMs. CNMs have a relatively rigid structure except for the C-terminal region of κ-CN located on the surface of CNMs which is flexible (Foster, Baer, and Mistry 1990). CNMs in their native state represent a stable, supramolecular sponge-like structure. Changes in the surface and internal structure of the CNM during processing and storage contribute to the insolubility of MPC (Mata, Udabage, and Gilbert 2011). The main chemical interactions which impart the functional properties of MPCs include hydrophobic interactions which occur between the hydrophobic regions in the CNs, [CN–CN] and [CN–WP] crosslinking via minerals (mostly calcium), and [lactose–CN] interactions (Holt 1992; Molnar et al. 2004; Mimouni et al. 2009). Thus, the CNMs in MPC display a high surface hydrophobicity index which in turn has been associated with its insolubility (Cenini et al. 2020). Furthermore, aggregated CNs (in particular κ-CN) attached to β-Lg and bovine serum albumin (BSA) via disulfide-linkages contribute to the development of a skin-like coating on the surface of MPC particles (Mao et al. 2012; Eshpari, Tong, and Corredig 2014). This in turn can also lead to increased insolubility in MPCs.

## Strategies to minimize insolubility of MPC

Different approaches have been employed during and immediately post-manufacture to help improve the solubility of MPC. These strategies have been categorized into: *a) modification of the composition*, e.g., addition of salts (Sikand, Tong, and Walker 2013), addition of calcium chelators (Pandalaneni et al. 2019) and calcium replacement via ion exchange (Sikand et al. 2016); *b) physical treatments*, e.g., the application of high pressure processing (HPP) (Cadesky et al. 2017), microfluidization (Augustin et al. 2012) and ultrasonication (McCarthy et al. 2014); *c) optimization of the manufacturing conditions*, e.g., changing the time–temperature programme during thermal treatments (Cao et al. 2016); changing the operating temperature of the UF process (Arunkumar and Etzel 2018); optimization of the drying conditions (Augustin et al. 2012); *d) optimization of storage conditions*, e.g., optimization of the temperature and relative humidity (RH) during storage (Haque et al. 2011; Fang et al. 2011); *e) chemical treatments*, e.g., using lactosylation (Le et al. 2012) and succinylation (Shilpashree, Arora, Chawla, Vakkalagadda, et al. 2015) and *f) enzymatic modification*, e.g., using different protease and alkaline phosphatase treatments (Ryan et al. 2018; Chen et al. 2018; Power et al. 2019).

### *Modification of composition*

A number of strategies have been proposed to overcome the insolubility of MPCs through composition modification to alter the CNMs in MPCs. The composition of raw milk depends on several factors such as animal breed, season, stage of lactation, feeding, milking interval, etc (Phelan et al. 1982; Heck et al. 2009). While there appears to be no available data concerning the effects of the above mentioned variables on MPC properties, it is universally accepted that the composition of raw milk is crucial for the technofunctional properties of the resultant MPC, as several studies have explored the impact of altering the content of different components on MPC solubility. In addition, the composition of skim milk varies with the operating parameters employed during the different manufacturing steps (i.e., membrane filtration). Furthermore, the structure of the components may vary by changing the conditions of the heat treatment, spray drying and storage, which in turn influence the solubility of MPC (Sikand et al. 2011). These variations result in the presence of commercially available MPCs having different technofunctional properties. The main components of MPC include CN (as the main protein) and WP, lactose, calcium and lipid molecules. Figure 2 summerizes the representative proximate composition of MPCs having different protein contents.

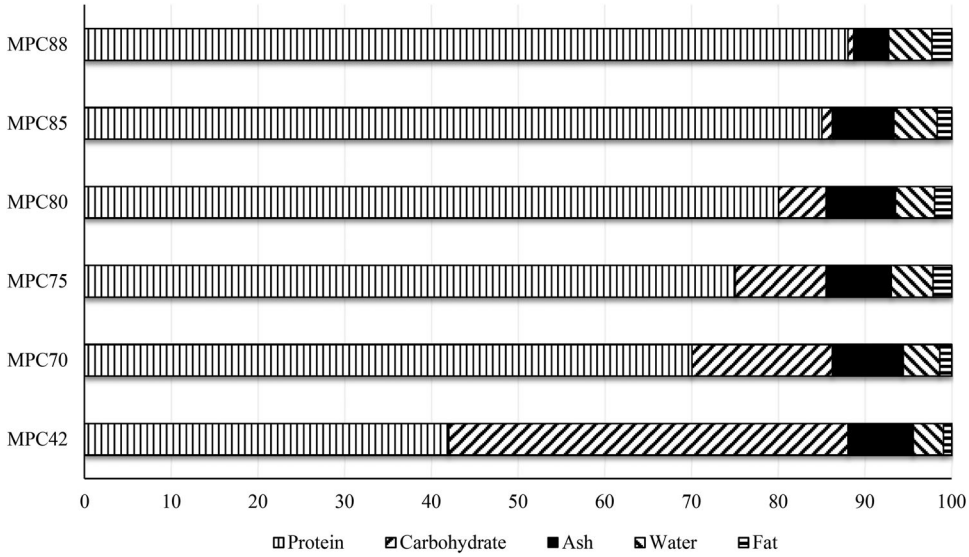


**Figure 2.** Diagrammatic representation of the mean proximate composition (%wt/wt) of different milk protein concentrates (MPC) (Data taken from Agarwal et al. 2015).

### *Protein*

The protein fraction of bovine MPC consists of CNs and WPs in the same ratio as found in milk (CN:WP, 4:1, McSweeney et al. 2020). The protein content plays a major role in the solubility behavior of MPC powders. It has been reported that high protein MPCs have powder particles with larger sizes (McSweeney et al. 2020) which may be associated with the lower solubility. By employing a novel technique of broadband acoustic resonance dissolution spectroscopy, it was shown that higher protein contents resulted in obstruction of water penetration into MPC particles (Vos et al. 2016). The lower ionic strength of higher protein content MPCs may also be part of the reason for this observation (Chew et al. 2014; Crowley, Desautel, et al. 2015). In addition, the ratio of micellar and non-micellar CN seems to be a key parameter in terms of MPC solubility.

### *Lactose*

Lactose is mostly located in the core of dairy powder particles (Nijdam and Langrish 2006; Fang et al. 2012; Kelly et al. 2015). As this molecule is water soluble, it could be postulated that by increasing the lactose content in MPCs that the degree of solubility should be enhanced and water transfer to the powder should be facilitated (Richard, Le Page, et al. 2012). However, during the MPC manufacturing process (especially where high temperature is applied) and also during storage, lactose-protein interactions (Maillard reactions) can occur leading to a decrease in solubility.

The lactose level in bovine milk generally varies between 4.4 to 5.2%. The lactose level in MPC may influence the subsequent reconstitution characteristics of MPCs. During the manufacture of MPC, UF treatment removes the majority of the lactose (Agarwal et al. 2015). The lactose level varies depending on the extent of protein enrichment, e.g., the lactose content in MPC42 is about 46% while it is <5% in MPC85 (Uluko et al. 2016). Low protein MPCs (e.g., MPC55) have a high level of surface lactose (>20% of total powder particle surface composition) while high protein MPCs (e.g., MPC80) have a low level of surface lactose (<5% of total surface composition) (Hogan and O'callaghan 2010) as the surface composition of high protein MPCs mostly contains protein and lipid molecules. MPCs with a higher lactose content are generally more soluble. This can be because:

1. The hydrophilic nature of lactose may help improve the solubility of MPCs.
2. The high level of surface located lactose facilitates the penetration of water molecules into the powder particles.
3. Lactose acts as a spacer material which reduces the extent of CN interactions (Anema et al. 2006).
4. Lactose can participate in hydrogen bonding with protein molecules and thereby enhances solubility (Baldwin 2010).

Reconstitution of MPC80 (5% (wt/vol) on a solids basis) with added lactose (2%, wt/wt) followed by spray drying (inlet T = 150 °C, outlet T = 65–76 °C) was shown to increase the extent of cross-linked CNs in the MPC powder (Le, Bhandari, and Deeth 2011). This has been associated with the promotion of the Maillard reaction and the generation of lactulosyllysine during spray drying. This leads to greater insolubility. On the other hand, it has also been reported that addition of lactose (5.6 and 11.2%) to high protein MPCs during rehydration enhances hydrophilic interactions and decreased intramicellar destabilization of the CNMs, leading to improved WHC which may have an enhancing effect on MPC solubility (Meletharayil et al. 2016). Limited studies are available regarding the effects of the addition of lactose on the physicochemical and techno-functional properties of MPCs, thus further studies are required for an in-depth understanding of these effects.

The state of lactose (amorphous or crystallised) in the MPC is also an important parameter for the solubility of

**Table 1.** Summary of various studies on solubility enhancement of milk protein concentrates (MPC) through $Ca^{2+}$ depletion.

| Sample | Treatment description | Effects | Reference |
|---|---|---|---|
| Concentrated skim milk | 600 mM NaCl was added to UF permeate. | Reduction in pH from 6.5 to 6.3 on addition of 600 mM NaCl, increase in $Ca^{2+}$ from 2.7 mM to 6.2 mM and increase in soluble $Ca^{2+}$ from 9.7 mM to 12.0 mM observed in concentrated skim milk. No change in soluble phosphorus and in CNMs sizes reported. | Huppertz and Fox (2006) |
| MPC80 | 50–150 mM NaCl added during DF. | Increasing [NaCl] caused enhanced solubility, increased free sulfhydryl groups and reduced disulfide bridges, particle size and surface hydrophobicity. | Mao et al. (2012) |
| MPC 80 | UF milk supplemented with 150 mM NaCl or KCl. | Highest solubility (100%) observed for samples treated with both salts in comparison to control sample (53%). Lowest turbidity observed for samples supplemented with NaCl (128 Nephelometric Turbidity Unit (NTU)) and KCl (131 NTU) in comparison to control sample (531 NTU). Lowest fat content observed for samples supplemented with KCl (i.e., 3.7%) and with NaCl (i.e., 3.9%) in comparison to control (i.e., 4.2%). Lower moisture content observed for samples supplemented with NaCl (i.e., 3.6%) and with KCl (i.e., 4.0%) in comparison to control (i.e., 5.2%). Highest ash observed for samples supplemented with KCl (i.e., 12.4%) and NaCl (i.e., 10.8%) in comparison to control (i.e., 6.9%). | Sikand, Tong, and Walker (2013) |
| MPC85 | Sample batch (1 L) treated with 160 g Amberlite SR1L $Na^{+}$ resin and stirred at 1300 rpm for 2 h to decalcify MPC up to 83.6%. | Increase in pH from 6.8 to 7.2 observed. Increase in $Na^{+}$ from 0.17 to 1.42% observed. Reduction in turbidity and mean particle diameter observed. | Xu et al. (2016) |
| MPC80 | NaCl or KCl (150 mM) added during DF. | Nitrogen solubility index (NSI) at 4 °C increased from 53.3 (control) to 100% for both treated samples. NSI at 25 °C increased from 46.5 (control) to 97.5% for both treated samples. NSI at 55 °C increased from 39.5 (control) to 58.0% for Na-treated sample and to 51.0 for K-treated sample. | Sikand et al. (2016) |
| MPC60 | Addition of 150 mM disodium phosphate (DSP) after UF and adjustment of the pH to pH 6.6, and also addition of NaCl and KCl (75 mM:75 mM) during DF. | Reduction of calcium content improved the dispersability of MPC via prevention of the protein–protein interaction and aggregation by enhancing repulsive forces. | Meena, Singh, Arora, et al. (2017) |
| MPC70 | Adjustment of UF retentate to pH 7.10 using KOH and adjustment of UF retentate to pH 5.85 using $NaH_2PO_4$ | Alkalization of UF retentate caused reduction in calcium (34%), thus improved the solubility by 32% after 60 days storage at 15 °C. This may be attributed to the reduced crosslinking and interactions of calcium with caseins. Acidification of UF retentate caused reduction in calcium (27%) via dissociation of casein micelles, thus improved the solubility by 20% after 60 days storage at 15 °C. | Meena et al. (2018) |
| MPC80 | Injection of $CO_2$ into pasteurized skim milk before UF resulted in 20 or 30% reduction of $Ca^{2+}$ in MPC. | Longest heat coagulation observed for MPC with 1.5% $Ca^{2+}$ and short coagulation observed for untreated MPC having 2.1% Ca. | Pandalaneni et al. (2019) |
| MPC80 | Injection of $CO_2$ into pasteurized skim milk before UF caused pH reduction and also 20 or 30% reduction of $Ca^{2+}$ in MPC. | Minimum apparent viscosity observed for MPC with 1.5% $Ca^{2+}$ and maximum apparent viscosity was observed for untreated MPC having 2.1% Ca. | Pandalaneni et al. (2019) |

MPCs. Both amorphous and crystalline forms of lactose are present in MPCs (Forny, Marabi, and Palzer 2011). Generally, the presence of amorphous lactose may lead to powders with undesirable stickiness, while crystallization of lactose results in less hygroscopic powders (Belletti et al. 2009). Kelly et al. (2015) exposed different MPCs ranging from MPC35 (with a lactose content of 49.6%) to MPC90 (with a lactose content lower than 1%) to room temperature storage at different RH values. They observed that lactose crystallization occurred at low RH for MPCs with a high lactose content. Furthermore, the development of crystals results in higher viscosity and greater insolubility. They stated that water absorption by the internal layers of MPC powders was inhibited due to the impenetrability of the surface, where extensive lactose crystallization occurred.

### *Lipid*

During the manufacture of MPC, the various membrane filtration steps do not remove the lipid molecules (Liu et al. 2020). However, since MPC is obtained from skim milk, its lipid content is naturally low, i.e., 1–2%. Products with low

**Table 2.** Comparison of the mineral contents in milk protein concentrates (MPC) and milk protein isolates (MPI) with different solubilities (Adapted from Sikand et al. 2011).

| Sample | Solubility (%) | Ca | Mg | K | Na | P | Cl | Total |
|---|---|---|---|---|---|---|---|---|
| | | (mg/100 g) | | | | | | |
| MPC80 | ~25 | 1500 | 68 | 226 | 45 | 1100 | 70 | 3000 |
| MPC80 | ~45 | 1400 | 80 | 350 | 100 | 400 | 286 | 2600 |
| MPI | ~35 | 1500 | 66 | 266 | 64 | 1100 | 153 | 3000 |
| MPI | ~55 | 1500 | 69 | 199 | 52 | 1100 | 183 | 3200 |
| MPI | ~95 | 600 | 6 | 503 | 167 | 400 | 223 | 1900 |

lipid contents are generally expected to be highly soluble (Vignolles et al. 2007). Furthermore, the interaction between lipid and protein molecules is limited. Nonetheless, lipid molecules generally migrate (mostly during spray drying and storage) to the surface of MPC powder particles and this is also anticipated to contribute to lowering the solubility of MPCs due to the corresponding increase in surface hydrophobicity. The surface located lipid is also susceptible to oxidation which may result in the development of MPC insolubility (Liu et al. 2020).

### *Minerals*

The ash content does not vary greatly in MPCs having different protein contents. It remains in the range of 6.0–8.0%, which is higher than the ash content in WP concentrates (<4% for WPC80 and WP isolate) (Agarwal et al. 2015). As outlined earlier, the poor solubility of MPCs is considered to be primarily associated with CNMs interacting with each other via hydrophobic interactions along with the interaction of CNMs with calcium (Sikand et al. 2011; Corredig et al. 2019). Thus, the presence of calcium ions which enhance crosslinks between CNs is also a contributory factor for MPC insolubility. Mineral equilibrium in the MPC evaporate is therefore a factor that has been extensively considered for the improvement of the MPC solubility. Modification of the mineral content can beneficially alter the solubility of MPCs. The mineral equilibria of milk are related to a number of intrinsic and extrinsic factors (Holt 2004; Boiani et al. 2018). Reducing the overall mineral content in milk protein concentrate prior to spray drying and changing the equilibrium of the calcium (from an initial ratio of serum to colloidal calcium of 31:69 to higher proportion of serum calcium) has been reported as a promising strategy to improve MPC solubility (Table 1) (Horne and Lucey 2009; Sikand, Tong, and Walker 2013).

Around two-thirds of the milk calcium is linked to phosphate in the form of colloidal calcium phosphate (CCP) while the remaining portion is in the serum phase (Horne and Lucey 2009; Luo, Vasiljevic, et al. 2015). Specific treatments can alter the calcium equilibrium between the soluble and colloidal phases in MPC (Deeth and Lewis 2015). Environmental changes (e.g., pH, ionic strength, temperature) which cause the release of calcium phosphate from the colloidal to the serum phase are reported to improve the solubility of MPC (Yanjun et al. 2014). Therefore, calcium depletion from the colloidal phase prior to spray drying has been considered as one of the main approaches to enhance the solubility of MPC (Eshpari, Tong, and Corredig 2014). While the CNM system (colloidal phase) of MPC mostly consists of Ca, Mg, P and citrate, the aqueous phase contains these minerals in lower amounts compared to the colloidal phase. On the other hand, the aqueous phase mostly contains Na, K and Cl in high amount. Soluble calcium is distributed through the serum phase of milk, while colloidal calcium is specifically attached to $PO_4^{-3}$ inside the CNM network in the form of calcium phosphate (Vaia et al. 2006). Micellar stabilization during processing and storage is promoted via the interaction of CCP with the CNMs (Sikand et al. 2011).

Most of the current studies on the compositional modification of MPCs focus on the removal of calcium using a) the replacement of calcium with monovalent salts, e.g., Na and K, during the DF steps, b) acidification of milk prior to or during membrane filtration or c) using calcium chelators. It was postulated that depletion of calcium reduced CN aggregation and, accordingly, led to the formation of loose structures which are subsequently more readily hydrated.

Sikand et al. (2011) showed that different high protein MPCs had different mineral profiles, indicating that the DF step employed during MPC manufacture has a significant influence on changing the abundance of minerals in MPCs (Table 2). The total amount of calcium in MPC40 samples was in the range of 0.9–1.0 g/100 g while the total amount of calcium in MPC80 ranged between 1.4 and 1.5 g/100 g (Sikand et al. 2011). It has been shown that the solubility of MPC80 with a total mineral content of 3.0 g/100 g and a calcium content of 1.5 g/100 g was lower than the MPC80 having 2.6 g/100 g total minerals and 1.4 g/100 g calcium. Similarly, MPI with a total mineral content of 3.0 g/100 g and a calcium content of 1.4 g/100 g showed lower solubility than an MPI with a total mineral content of 1.9 g/100 g and a calcium content of 0.6 g/100 g (Sikand et al. 2011). The solubility properties of protein enriched beverages containing MPC85 with three levels of calcium (1.5, 1.7 and 2.1% (wt/wt%)) was studied (Pandalaneni et al. 2019). After 30-day storage at room temperature, the minimum apparent viscosity ($\mu_{app}$) of the beverage having maximum solubility was in the sample with the lowest calcium content. The possibility of replacing calcium with monovalent ions (Na and K) has also been examined (Carr, Bbhaskar, and Ram 2005; Tessier and Rose 1958). Decalcifying MPC80 (using Na- or K-cation exchange resins) was shown to decrease the ionic strength and to prevent extensive insolubility in MPC80 (Dybing et al. 2007). A high solubility in MPC85 (equal to 96%) after 7 weeks storage was observed where calcium was partially replaced with $Na^+$ (Bhaskar, Singh, and Blazey 2002). The complete dissolution of MPC80 by the addition of NaCl (150 mM) during DF was reported by Mao et al. (2012). Significant disaggregation of CNMs in MPCs occurred when calcium was removed (up to 83%) using ion exchange treatment. The solubility of the MPC and the mean PSs of the CNMs increased three-fold and decreased by 50%, respectively (Xu et al. 2016). In addition, a positive effect of the ionic environment on the solubility of high protein content MPC powders has been reported by Hussain, Gaiani, and Scher (2012). The inclusion of rapidly dissolving

minerals, such as KCl and NaCl, during DF increased the solubility of MPC by increasing the ionic strength (Sikand et al. 2016). It has been reported that addition of $CaCl_2$ and NaCl may restructure CNMs and unfold whey proteins, thereby helping to improve the solubility of MPC. Addition of NaCl (500 mM) to the concentrated milk protein has also been shown to increase its viscosity from ~2.8 to 3.5 mPa.s (Zhao and Corredig 2015). Low levels of salt (0.75%–1.50% wt/vol) resulted in faster wetting and swelling, while higher levels (3%–6%) increased the time required for MPC powder particle dissolution (Hussain et al. 2011). Furthermore, addition of 150 mM disodium phosphate (DSP, $Na_2HPO_4$) during DF led to enhanced solubility of MPC55 following a period of 2 months storage at 25 °C (Meena, Singh, and Gupta 2020).

At neutral pH, the level of $PO_4^{3-}$ will be very low and most will be in $HPO_4^{2-}$ and $H_2PO_4^-$ forms. Thus, changing the pH and supplementation with other minerals such as Na and K prior to spray drying may also modify the mineral equilibrium in MPCs leading to enhanced solubility. Acidification of skim milk prior to membrane filtration is an approach which has been used to induce structural modifications in CNMs (Liu et al. 2018). This strategy promotes the partial dissociation of CCP and the release of calcium ions to the serum phase (Ahmad et al. 2008; Ezeh and Lewis 2011). Consequently, this results in a loosening of the micelle structure and a reduction in mean micelle size, resulting in enhanced solubility of MPC (Liu et al. 2017). Reducing the pH of milk to pH 6.0 led to a 15% decrease in the final calcium content of MPC80 in comparison to control (pH 6.95), thereby improving solubility (Eshpari, Tong, and Corredig 2014). The structure of CNMs was not significantly changed when the pH was reduced to pH 6.0 by the addition of glucono-$\delta$-lactone (GDL) to the concentrate prior to spray drying of MPC (Liu et al. 2018). Further addition of GDL to reach a pH in the range 5.4–5.7 caused dissociation of the structure of the micelles by loosening protein–protein interactions (Liu et al. 2018). Eshpari et al. (2015) added GDL (3.25 g/L) to skim milk before membrane filtration and observed a reduction in pH to 6.0, leading to a reduction in CCP and enhanced solubility of the resultant MPC. Marella et al. (2015) injected gaseous $CO_2$ (2200 ppm) as an acidulant to skim milk before and during (1.5 to 2.0 L/min) UF and observed a pH reduction to pH 5.7. The MPC obtained by this method was found to be more soluble due to a 34% reduction in calcium content (Marella et al. 2015), it was also more stable to thermal treatment (Sunkesula et al. 2021). Luo, Vasiljevic, et al. (2015) also reported that addition of HCl (1.0 M) to skim milk to obtain different pHs (6.3, 5.9, or 5.5) resulted in an increase in soluble calcium in UF permeates from 16.1 mM for the control (pH 6.7) to 24.8 mM for the sample at pH 5.5. The total calcium in the retentate was reduced by more than 15%, leading to increased solubility of the MPC.

Removal of calcium from the CNM system using calcium chelating agents may, at least partially, dissociate the CNMs by inducing electrostatic repulsive forces between the micelles. This can lead to the development of CN enriched dairy ingredients with improved solubility. Addition of chelating agents including sodium phosphate, trisodium citrate and SHMP to chelate calcium has also been successfully examined to improve MPC solubility (McCarthy et al. 2017).

Overall, it seems that modification of the calcium equilibrium in the MPC evaporate prior to spray drying is key to improving solubility. This can be performed by changing the pH, addition of minerals (including NaCl and KCl) to the serum phase or by the removal of calcium. However, it should be noted that on removal of calcium other parameters such as the gelation properties may be reduced (Eshpari et al. 2015).

## Physical processes

Modification of the structure of the CNMs prior to spray drying using different physical treatments has been investigated in several studies as a means of enhancing the solubility of MPC. The main objective of these approaches is dissociation of the CNMs (Patil et al. 2018). The main physical approaches studied include the application of HPP, ultrasonication, microfluidization and cavitation. The specific details of the starting material, the conditions employed and the outcome of these treatments are summarized in Table 3.

### HPP

HPP has been applied for improvement of MPC solubility by loosening the non-covalent bonds between macromolecules in CNMs. HPP treatment for 15 min at 250 MPa resulted in maximum solubility of MPC; while increasing the pressure to 350 MPa led to significant protein denaturation, leading to losses in solubility (Cadesky et al. 2017). HPP (200 MPa) of the evaporate prior to spray drying increased MPC solubility in the resultant powder (Sikand et al. 2012). HPP (200 MPa) in combination with heating the concentrate at 40 °C before spray drying was shown to enhance the solubility of MPC powders (Udabage et al. 2012).

### Ultrasonication

In several studies, ultrasonication (20–24 kHz) has been successfully applied to the concentrate prior to spray drying to disaggregate CNMs during MPC (60%–80% protein) manufacture to increase solubility (Augustin et al. 2012; Yanjun et al. 2014). Ultrasonication treatment (20 kHz, 41 W, 30 min) has previously been shown to enhance the solubility of skim milk powder particles (Shanmugam, Chandrapala, and Ashokkumar 2012).

### Microfluidisation

The application of high shear stress to the concentrate before spray drying has been shown to partially disaggregate CNMs, thereby altering the surface composition of powders, inducing a viscosity reduction in the reconstituted MPCs,

**Table 3.** Strategies studied to help improve the solubility of milk protein concentrates (MPCs).

| Principle | Sample | Method | Description | Reference |
|---|---|---|---|---|
| Composition modification | MPC40 and MPC80 | Changing the protein content | Lower protein content increased solubility. | Sikand et al. (2011) |
| | MPC80 | Addition of NaCl | Addition of NaCl (150 mM) increased solubility. | Mao et al. (2012) |
| | MPC80 | Addition of Na and K | Addition of Na and K (150 mM) to the MPC prior to spray drying increased solubility. | Sikand, Tong, and Walker (2013) |
| | MPC65 and MPC80 | Addition of Glucono-$\delta$-lactone (GDL) | Acidification by GDL (3.25 g/L) increased solubility. | Eshpari, Tong, and Corredig (2014) |
| | MPC80 | Injection of $CO_2$ | Increasing the acidity by injection of carbon dioxide at flow rates of 2,200 ppm before and 1.5 to 2 L/min during UF) enhanced solubility. | Marella et al. (2015) |
| | MPC85 and MPI | Changing the protein content | Increasing the protein content from 85 to 90% decreased the solubility. | Hauser and Amamcharla (2016) |
| | MPC | Addition of HCl | Lowering the pH to pH 5.9 using HCl (1 M) increased solubility. Lower than pH 5.9, reduced solubility. | Luo, Ramchandran, et al. (2015) |
| | MPC35–90 | Changing the protein content and addition of KCl | Lower protein content and addition of KCl (80 mM) increased solubility. | Crowley, Desautel, et al. (2015) |
| | MPC80 | Addition of NaCl and KCl | Addition of NaCl and KCl (150 mM) increased solubility. | Sikand et al. (2016) |
| | MPC | Employing Na-exchange column | Ca replacement up to 83.6% with Na increased solubility. | Xu et al. (2016) |
| | MPC80 | Addition of nanovesicles | Addition of 1, 5 and 10% wt/wt (total solids) lecithin nanovesicle dispersions to the concentrate of MPC80 led to 90% solubility of MPC during 180 days of storage at 4 °C. | Bansal, Truong, and Bhandari (2017) |
| | MPC | Addition of chelating agents | Addition of ethylenediamine tetra-acetic acid (EDTA) (200 mM) and citrate (200 mM) to skim milk increased solubility. | Ramchandran, Luo, and Vasiljevic (2017) |
| | MPC40–85 | Addition of GDL | Acidification by GDL (34.5 mM) increased solubility. | Liu et al. (2017) |
| | MPC65 and MPC80 | Addition of GDL | Acidification by GDL (33.8 mM) increased solubility. | Liu et al. (2018) |
| | MPC35 and MPC90 | Protein content | Lower protein content increased solubility. | Babu et al. (2018) |
| | MPC80 | Blending MPC with proline-rich whey peptides | Blending MPC with of 5, 10 and 25% (wt/wt) proline-rich whey peptides led to 200%–500% improvement in MPC solubility. | Torres-Hernandez, Howell, and Bennett (2018) |
| | MPC40, MPC55, MPC70 and MPC85 | Protein content | Samples with higher protein content had higher insolubility after for 45 days storage at 50 °C. | Fan et al. (2018) |
| | MPC55 | Addition of DSP | Addition of 150 mM DSP during DF enhanced MPC solubility over 2 months. | Meena, Singh, and Gupta (2020) |
| Physical modifications | MPC82 | Microfluidization, ultrasonication and homogenization | Applying micro fluidization (400–1200 bar), ultrasonication (24 kHz, 600 W) or homogenization (350/100 bar), prior to spray drying increased solubility. The former was more effective. | Augustin et al. (2012) |
| | MPC80 | | Applying homogenization with pressure of 200 MPa at 40 °C prior to spray drying improved solubility. | Udabage et al. (2012) |
| | MPC80 | Ultrasonication | Ultrasonication (24 kHz, 12.50 W) prior to spray drying increased solubility. | Yanjun et al. (2014) |
| | MPC | Applying HPP | HPP with pressures $\leq$350 MPa increased solubility and with pressures $\geq$350 decreased solubility. | Cadesky et al. (2017) |
| Optimization of manufacturing parameters relevant to the solubility of MPC | MPC85 | Storage time and temperature | Shorter storage time and lower storage temperature (20 °C) increased solubility. | Anema et al. (2006); Havea (2006); Hunter et al. (2011) |
| | MPC85 | Storage time, temperature and $a_w$ | Shorter storage time, lower storage temperature (25 °C) and lower $a_w$ (0.23) increased solubility. | Haque et al. (2011) |
| | MPC85 | Dryer inlet temperature | Lower inlet temperature (77 °C) during spray drying increased solubility. | Fang et al. (2012) |
| | MPC85 | NF | NF (instead of evaporation) following UF increased solubility. | Cao et al. 2014) |
| | MPC | Temperature during UF | | |

(*continued*)

**Table 3.** Continued.

| Principle | Sample | Method | Description | Reference |
|---|---|---|---|---|
| | | | Lower UF processing temperature (15 °C) increased solubility. | Luo, Ramchandran, et al. (2015) |
| | MPC65 | NF | Employing nanofiltration instead of evaporation after UF increased solubility. | Cao et al. (2016) |
| Optimization of storage parameters relevant to the solubility of MPC | MPC | Storage time and $a_w$ | Shorter storage and lower $a_w$ (0.23) increased solubility. | Haque et al. (2015) |
| | MPC80 | Storage temperature | Lower storage temperature (4 °C) increased solubility. | Sikand et al. (2016) |
| | MPC80 | Storage temperature | MPC stored at higher storage temperature (40 °C) for 2 weeks had lower solubility compared to MPC stored at lower temperature (25 °C). | Gandhi, Amamcharla, and Boyle (2017) |
| | MPC70–90 | Storage temperature | Lower temperature of storage (25 °C) increased solubility. | Babu et al. (2018) |
| Chemical modification | MPC70 | UV photo-oxidation | Shorter/non exposing to UV increased solubility in comparison to long exposing to UV (6 W, 302 nm UV lamp). | Semagoto et al. (2014) |
| | MPC85 | Succinylation | Succinylation by addition of 4 mol of succinic anhydride/mole of lysine increased solubility. | Shilpashree, Arora, Chawla, and Tomar (2015) |
| Enzymatic modification | MPC80 | Hydrolysis | Subjecting MPC to chymotrypsin and papain and particularly pepsin and trypsin increased solubility. | Banach, Lin, and Lamsal (2013) |
| | MPI | Hydrolysis | Treatment of MPC using commercial enzymes including Flavourzyme, Neutrase and Protamex decreased pH and increased solubility. | Ryan et al. (2018) |

leading to enhanced solubility (Trinh, Haisman, and Trinh 2007). Microfluidisation treatment may also disintegrate the CMNs via the applied shear stress (Bucci et al. 2018). It was reported that microfluidization of the skim milk concentrate (TS= 22%) at 400, 800 and 1200 bar prior to spray drying successfully retained the solubility of MPC80 over a period of 8 months storage. The application of microfluidisation of an MPC evaporate at 800 bar led to the generation of MPC with almost 30% higher solubility compared to that of MPC which was homogenized (350/100 bar) or sonicated (24 kHz, 600 W) (Augustin et al. 2012). Applying a constant shear rate (either 100 or 1000 $s^{-1}$) at low pH also increased the disaggregation of CNMs and reduced the extent of insolubility in the resultant MPC powders (Ranadheera et al. 2019).

### Cavitation

Hydrodynamic cavitation (set at 25 or 50 Hz) prior to spray drying did not lead to a significant improvement in the solubility of MPC80 (Li et al. 2018). However, this pretreatment may help reduce the energy requirements of the drying process by reducing the viscosity of the feed entering the spray dryer.

The physical methods outlined herein have mostly been employed prior to spray drying in order to loosen and avoid aggregation in the CNMs. However as outlined earlier, physical treatments have also been shown to enhance the solubility of MPC powder.

## Manufacturing parameters relevant to the solubility of MPC

Modification of the processing parameters during MPC manufacture can influence the functionality of MPCs. During the heat treatment of milk, e.g., pasteurization, sterilization and evaporation, the applied thermal stress may denature WPs and CNs. Denatured WPs may deposit on the surface of the CNMs and interact specifically with $\kappa$-CN. This promotes attractive forces, particularly disulfide linkages between $\beta$-Lg and $\kappa$-CN and accordingly, aggregation is enhanced (Kieferle et al. 2019; Lin et al. 2018). Several studies have considered the effects of different time–temperature combinations during evaporation and spray drying on subsequent MPC solubility. Furthermore, reducing the temperature during membrane processing and reducing the pore size of the membranes has been highlighted as a means of improving MPC solubility in a number of studies. Nevertheless, the main efforts have focused on thermal treatments during the spray drying process. The spray-dryer inlet and outlet temperatures, the size of the nozzles and the properties of the feed sample (including protein concentration, TS, viscosity, etc.) have also been studied with respect to enhancing the solubility of the resulting MPC powders.

### Factors prior to spray drying

The effect of the application of high temperature during thermal treatment of skim milk for the production of MPC80 has been investigated in terms of MPC solubility (Gazi and Huppertz 2015). It was shown that the solubility of MPC80 decreased by increasing the extent of heat treatment from 72 °C − 15 s to 95 °C − 45 s. The conditions employed during membrane processing (UF and DF) may also influence the solubility of MPCs by changing the properties of the MPC evaporate which is mostly associated with the extent of filtration. Furthermore, the application of higher temperatures during UF (15, 30 and 50 °C) led to a

lower solubility in the final MPC (Luo, Ramchandran, et al. 2015). Furthermore, recent studies proposed the use of low heat nanofiltration (NF) (instead of evaporation) following UF during the manufacture of MPC60 to improve solubility without inducing significant changes in the compositional profile (Cao et al. 2014, 2016).

Another critical factor is the viscosity of the concentrated skim milk prior to the drying process. Filtration and evaporation lead to an increase in viscosity of concentrated milk. After cooling the concentrates, the viscosity of the concentrated proteins is further increased as a function of storage time. This phenomenon termed "age thickening" occurs as a result of protein–protein interactions (Bienvenue, Jiménez-Flores, and Singh 2003). The mean diameter of the final powder particles may also be changed (from <1 to 100 µm) by increasing the viscosity of the concentrate (Schuck et al. 2007; Crowley, Gazi, et al. 2014; Ho et al. 2019). This may lead to a decrease in the solubility of the MPC powders. An apparent viscosity ($\mu_{app}$) value of ~100 mPa.s has been proposed as the maximum for dairy concentrates prior to spray drying (Enríquez-Fernández, Camarillo-Rojas, and Vélez-Ruiz 2013; Zisu, Schleyer, and Chandrapala 2013).

### Spray drying conditions

Denaturation of some milk proteins occurs during spray drying. This increases the hydrophobicity on the surface of the powder particles as a result of moving the hydrophobic amino acids (AAs) from the core to the particle surface, while increasing their association with surface located κ-CN (Anandharamakrishnan, Rielly, and Stapley 2008; Fang et al. 2012). The application of different spray dryer inlet and outlet temperatures results in powders with different surface properties (e.g., hydrophobicity and specific surface area), which in turn resulted in different rehydration kinetics (Birchal et al. 2005; Fang et al. 2012). At high inlet/outlet temperatures, protein denaturation is increased, giving powders having non-spherical shapes with large particle diameters (Fang et al. 2012). The loss in solubility for β-Lg (around 20%), α-La (around 40%) and CNs (higher than 50%) has been reported on drying at inlet/outlet temperatures of 178/103 °C. In addition, increased levels of surface fat, as a result of increased inlet temperatures, has been shown to induce higher powder particle hydrophobicity leading to reduced solubility (Fang et al. 2012). DeCastro and Harper (2001) reported that outlet temperatures in the range of 65–90 °C did not significantly influence the structure of CNMs. This was attributed to the high thermal stability of CNs at low moisture level. Similar findings were reported by Oldfield, Taylor, and Singh (2005) who did not observe a significant change in protein solubility when SMP was dried at inlet/outlet temperatures within the range 160/89 °C and 200/101 °C.

Moreover, the humidity of the air inside the spray dryer may cause water absorption by the particles and lead to lactose crystallization resulting in reduced solubility (Kelly et al. 2015).

Therefore, in order to avoid the development of insolubility in MPC, the manufacturing parameters (related to thermal treatments, membrane filtration and spray drying) employed should be chosen to minimize the formation of interactions between CNMs and with other components such as WPs, lactose molecules and calcium ions.

### Storage parameters relevant to the solubility of MPC

Once produced MPCs may be stored for several weeks to a few months prior to distribution to end-users. The insolubility in MPC is promoted on storage, especially at elevated temperatures (Augustin, Oliver, and Hemar 2011). During storage, MPCs are susceptible to protein aggregation, lactose crystallization, lactosylation and lipid oxidation (Crowley, Gazi, et al. 2014). These phenomena occur as a consequence of covalent and non-covalent interactions among protein, lactose, salt and to a lesser extent lipid molecules. During storage, protein-protein interactions in CNMs and also the interactions between CNMs and WPs are increased. This mostly occurs by calcium crosslinks which enhance CNM aggregation and promotes the development of insolubility and also hydrophobic interactions between the hydrophobic surfaces of proteins (Minanath and Meena 2020). Furthermore, during storage, lactose may react with Lys residues in a process known as the lactosylation (Maillard) reaction. The bioavailability of Lys residues is consequently reduced (Anema et al. 2006). Although the amount of lactose in high protein MPCs is low, the Maillard reaction (which induces significant crosslinking in MPC during storage) has been reported as one of the main reasons for the insolubility in MPCs (Anema et al. 2006). Therefore, the solubility of MPCs decline as a function of storage time (Anema et al. 2006). This effect may however be delayed by reducing the temperature and the RH during storage.

The contribution of storage parameters including storage duration, temperature and RH on the solubility of MPC have been assessed (Table 3). While MPC82 had 70% solubility immediately after manufacture, this declined to 30.0 and 7.5% after 3 and 11-week storage (T = 25 °C, RH = 85%), respectively (Haque et al. 2015). Higher temperatures during storage promotes lower solubility (Haque et al. 2011; Fang et al. 2011). Storage of MPC70–90 over 12 weeks at 40 °C led to a 40–50% greater loss in solubility, in comparison to storage at 25 °C (Babu et al. 2018). According to Hunter et al. (2011), the solubility of MPC85 after 10 days of storage at 20 °C was 50% which further decreased to 48% after 40 days storage. A similar sample stored at 40 °C had a solubility equal to 22% after 10 days which reduced to 10% after 40 days storage. A similar trend was reported for storage of MPC85 at temperatures between 20–50 °C during 60 days (Anema et al. 2006). Increasing the temperature from 25 to 40 °C at a constant RH (i.e., RH = 44 and 88%) was shown to reduce the solubility of MPC80 (Le, Bhandari, and Deeth 2011). Increasing the RH from 44 to 88% at a constant temperature (i.e., 25, 30, 35 and 40 °C) also reduced the solubility of MPC80 (Le, Bhandari, and Deeth 2011). Moreover, exposure of MPC to UV light during storage has been reported to decrease solubility by significantly increasing the concentration of protein carbonyls (from 2 to

15 nmol/mg protein) and the generation of high molecular weight aggregates via increased formation of non-disulfide crosslinks in CNMs (Semagoto et al. 2014).

### Chemical modification

Chemical modification is a promising approach for improving the technofunctional properties of proteins. The effects of chemical treatments such as succinylation on the solubility of MPC have been investigated (Table 3).

The addition of succinic anhydride to milk proteins in order to alter protein charge and to decrease hydrophobicity has been examined in several studies (Shilpashree, Arora, Chawla, Vakkalagadda, et al. 2015; Yang et al. 2016; Wan, Liu, and Guo 2018). This modification has been reported as a promising approach for improvement of the solubility, emulsification and foaming characteristics of CNs (Vidal, Marchesseau, and Cuq 2002). Succinic anhydride reacts with the $\epsilon$-amino groups of AA residues (specifically Lys). This promotes intermolecular electrostatic repulsive forces and improves solubility. Shilpashree, Arora, Chawla, and Tomar (2015) showed that addition of 4 mole of food-grade succinic anhydride/mole of lysine in MPC85 led to solubility enhancement of the powder (up to 50%), especially at alkaline pH (pH 8.0). Furthermore, the average size of the CNM particles was reduced from 700 to 200 nm and the zeta-potential became more negative (i.e., from −15 to −30 mV).

Photo-oxidation using a UV lamp (6 W, 302 nm, 25 °C, 150 h) has also been shown to reduce the solubility of MPC70 by around 15% (Semagoto et al. 2014).

Limited data on other chemical modifications (e.g., acylation and phosphorylation) on the solubility of MPC is available. However, the enhancement effect of these methods on the solubility of CNs has been demonstrated. The acylation of CNs (at pH 5.0) resulting in the attachment of acetate groups to the amino groups of Lys has been reported to enhance CN solubility (Santos and Tomasula 2000). Phosphorylation of CNs at a pH near the isoelectric point (pH 4.6) has been reported to reduce the surface hydrophobicity of CNMs, accordingly, improving the solubility (Medina et al. 1992). These methods require further investigation for their ability to enhance the solubility of MPC.

### Enzymatic modification

A limited number of studies exist on the enzymatic modification of MPC and its effects on the technofunctional properties. The application of some enzymes catalyzing protein hydrolysis may be used to modify overall protein structure and thereby improve the solubility of MPC (Table 3).

Four different proteinases, i.e., pepsin (pH= 2.0, T = 37 °C), trypsin (pH= 8.0, T = 37 °C), chymotrypsin (pH= 8.0, T = 50 °C) and papain (pH= 6.8, T = 60 °C) were used to hydrolyze MPC80 (Banach, Lin, and Lamsal 2013). All enzymes (especially pepsin and trypsin) were reported to improve solubility by reducing protein surface hydrophobicity. Ryan et al. (2018) characterized some attributes of MPI hydrolysates obtained following hydrolysis with commercial proteinases, i.e., Flavourzyme, Neutrase and Protamex. They reported that enzymatic hydrolysis significantly increased the solubility of MPI, while the thermal stability was reduced on hydrolysis.

Enzymatic dephosphorylation of serine residues in the CNs by incubating MPC80 with bovine alkaline phosphatase (pH 6.5, 37 °C, 3 h) has been examined as an enzymatic approach to loosen the structure of CNMs (Power et al. 2019). This method has been successfully employed to lower the viscosity of MPC solutions (10% (wt/wt) protein) from 125 to 40 mPa.s, leading to improved solubility. This suggests the important role of phosphate residues in preserving the rigidity of micelle structures. $\beta$-CN was more susceptible to enzymatic dephosphorylation than $\alpha$- and $\kappa$-CN.

The drawbacks in the application of enzymatic hydrolysis include the increased cost of processing, potentially associated with increasing processing times, modification of the sensory properties of the product (especially the taste), the need for additional thermal processing to inactivate the enzyme, changes in the physico-chemical properties of proteins and protein denaturation as a result of heat deactivation of enzymes, difficulty in controlling the hydrolysis process, and changes to other technofunctioal properties including gelation and emulsification properties, etc.

## Conclusion

A broad range of applications have been suggested for MPCs. The occurrence of the insolubility in MPCs as a result of manufacturing parameters and storage conditions restricts its usage, especially for sports/performance nutrition and beverage applications. The potential reasons for the insolubility of MPC and the parameters involved therein have been reviewed herein.

Several studies have focused on enhancement of MPC solubility. These can be categorized into: a) alteration of the mineral equilibrium in MPCs which aims to alter the ratio of micellar and non-micellar CN; b) optimization of the manufacturing steps, aimed of adjusting parameters associated with processes such as duration of the treatments and the temperature applied, and the conditions of storage (duration, temperature, RH%); c) application of different physical treatments (which is the most examined approach) mainly by employing shear stress, ultrasonication, microfluidization and cavitation in order to disrupt the integrity of the CNM network; d) chemical modification (e.g., succinylation) which aim to alter the properties of the CNMs, the central element involved in MPC insolubility; and e) enzymatic modification which breaks covalent bonds within and between proteins or removes phosphate groups.

In summary, it appears that a knowledge of the factors contributing to the insolubility of MPC and the ways to modify CNMs (either through avoiding the formation of CNM aggregates or disaggregation of CNM aggregates) are well advanced in recent years. Nevertheless, it has to be pointed out by improving one functional property of MPC, e.g., solubility, that other functionalities such as gelation, emulsification and thermal stability may change. Therefore,

there is a need to consider the contribution of different processing strategies on the overall functionality of MPC's.

## Declaration of interest statement

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

## Abbreviations

| | |
|---|---|
| AA | amino acid |
| $\mu_{app}$ | apparent viscosity |
| BSA | bovine serum albumin |
| CN | casein |
| CNM | casein micelle |
| CCP | colloidal calcium phosphate |
| DF | diafiltration |
| DSP | disodium phosphate |
| GDL | glucono-$\delta$-lactone |
| HPP | high pressure processing |
| ISI | insolubility index |
| MPC | milk protein concentrate |
| MPI | milk protein isolate |
| NF | nanofiltration |
| NSI | nitrogen solubility index |
| RH | relative humidity |
| SMP | skim milk powder |
| SHMP | sodium hexametaphosphate |
| TS | total solids |
| UF | ultrafiltration |
| WHC | water holding capacity |
| WP | whey protein |
| WPC | whey protein concentrate |
| $\alpha$-La | $\alpha$-lactalbumin |
| $\beta$-Lg | $\beta$-lactoglobulin |

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