Milk protein concentrates and isolates, recent developments focusing on improving solubility

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Milk is comprised of fat, protein, lactose, ash, and majorly water. With increased health awareness, consumers are more inclined towards protein-rich food and beverages. Among protein sources, milk proteins are excellent sources of essential amino acids with good PDCAAS (Protein Digestibility Corrected Amino Acid Score) and DIAAS (Digestible Indispensable Amino Acid Score scores) (Adhikari et al., 2022). Therefore, the demand for milk protein-rich food and beverages is huge. In earlier days, caseinates such as sodium caseinate and sodium caseinates were the choice of ingredients, however, their method of manufacture involves acidification and subsequent neutralization with strong acids and alkali, which is harsh on milk proteins and causes changes in their native form. In milk, milk proteins are ~3.0-3.5% which can be separated using the membrane processing technology without introducing any harsh acid or alkali. In addition, cold temperature separation also avoids any temperature-induced changes. So, different components of milk can be fractionated in separate streams, and concentrated as separate ingredients, and spray-dried to convert it into powders. It can be better explained using Figure 1.

In commercial manufacturing, fat is separated from milk using a cream separator, concentrating fat in the form of cream with little protein, lactose, and ash along with water. After cream separation, the remaining skim milk is subjected to ultrafiltration (UF). In UF, milk proteins are concentrated as retentate, while other components such as lactose, minerals, and water pass through the membrane. However, practically not all lactose, minerals, and water can be separated using UF. Therefore, to concentrate protein and reduce lactose, and minerals, diafiltration water is added to remove lactose and minerals. Diafiltration (DF) continues till the desired protein on a dry-matter basis is achieved. After that retentate is concentrated to desired solid ~22% and spay dried to get Milk protein concentrates and isolate (MPC/Iitre) powder. Based on protein on a dry-matter basis and protein as is, MPC/Iitre are classified as shown in Table 1 (as per American Dairy Products Institute classification).

MPC/I powders are used as concentrated sources of milk proteins, which are best for transport and storage. However, they need to be rehydrated/reconstituted for their use in dairy beverages, and protein enhancement. Rehydration of MPC/Iitre is challenging (Crowley et al., 2015). Unlike MPC/I, non-fat dried milk or skim milk powder which comprises ~50% lactose helps in better wetting, sinking, dispersing, and thereby good solubility. However, for MPC/Iitre, the main reason for the variation in the solubility of MPC is an aggregation of casein micelles during the manufacture of MPC and their subsequent storage (Corredig et al., 2019). Casein micelles have relatively rigid and stable micro-structure except for the C-terminal region of κ-Casein which is a hairy layer on the surface of micelles (Khalesi and FitzGerald, 2021). Stable molecular structure of casein changes during the processing and storage contributing to the insolublity of MPC/I (Mata et al., 2011). The hydrophobic interactions that occur between the

hydrophobic regions of the caseins, casein-casein, and casein-whey proteins crosslinking via divalent minerals (mainly calcium), and lactose-casein interactions play a major role in changing functionality. Therefore, casein micelles in MPC/Iitre show a high surface hydrophobicity index which hinders the rehydration of MPC and decreases solubility. (Cenini et al., 2020). In addition, casein aggregates formed due to the interaction of κ-casein with β-lactoglobulin and bovine serum albumin via S-S bond leads to the formation of skin-like coating on the MPC particles, which also contributes to insolubility.

As per the MPC/I process understanding, out of all the potential reasons, key reason for MPC's insolubility can be listed below.

- Protein concentration and protein-protein interaction- unlike milk where protein concentration is ~3-4%, during MPC/I manufacturing protein concentration reaches to ~18-20%, which promotes more protein-protein interaction. However, it is hard to avoid considering the economy of the process towards maximizing total solids before spray drying.
- Protein-protein interaction via S-S bond, which is mostly heat-induced interaction. However, recently most industry use a mild pasteurization, cold loop ultrafiltration process for protein concentration followed by reverse osmosis or vacuum evaporator to increase total solids before spray drying. This lowers the heat-induced protein-protein interaction.
- Mineral-induced aggregation: Minerals specifically calcium play a key role in the solubility of MPC/Iitre. Calcium exists in the soluble and colloidal form in milk in the ratio of 1:2.(Sikand et al., 2011), where colloidal calcium is mostly linked to phosphate molecules forming nanoclusters within casein micelles (Mata et al., 2011). Therefore, reducing calcium and shifting the equilibrium towards a higher proportion of serum calcium can reduce protein aggregation and enhance solubility (Eshpari et al., 2014). However, calcium removal needs to be controlled as calcium is responsible for maintaining the micellar structure of casein.

Therefore, several calcium reduction methods were reported in literature aiming to enhance the solubility of MPCs. Some of the selected approaches/methods are discussed here.

- *Acidification and ultrafiltration*: Control acidification as lower temperatures increase soluble calcium without coagulation, leading to more calcium coming into the serum. Using this milk chemistry, Luo et al. (2016) adjusted the pH of skim milk (to different levels 6.3,.5.9, 5.5) using 1M hydrochloric acid, followed by ultrafiltration and freeze drying to get MPCs. Compared to control MPC (as pH 6.7), solubility and heat stability of Treatment MPCs (at different native pHs 6.3,.5.9, and 5.5) were lower, however, once the pH of treatment MPCs was adjusted back to 6.7, they showed higher solubility and heat stability than control MPC. The results showed that partial removal of calcium increases the solubility and heat stability of MPCs.
- *Acidification, ultrafiltration, and ion exchange*: Khalesi and FitzGerald 2022, reported a method for manufacturing MPC with reduced calcium. Briefly, ultrafiltration concentrate ~20% of total solids were manufactured from skim milk using a 10 kDa ultrafiltration membrane. Ultrafiltration concentrate was diluted to 10% total solids and pH was adjusted to 6.0 using 0.5% citric acid at 10 °C, followed by preheating to 40 °C and passing through ion exchange resin (Purolite C100E cation exchange resins) column. The material eluted has calcium replaced with sodium and mixed with initial ultrafiltration concentrate (20% Total solid, 2.87% calcium) to 1.57%, 1.00%, and 0.36% calcium, keeping unmixed ultrafiltration concentrate as control. After, adjusting calcium, ultrafiltration concentrate was further concentrated to 20% total solids, followed by

spray drying (inlet:185°C and Outlet: 85°C) to get MPC powders. Control MPC showed the lowest overall solubility (65.82 ± 1.79%). For the calcium-reduced samples, MPC with 1.57% calcium had the lowest overall solubility (75.77 ± 0.43%), while MPC with 0.36% had the highest overall solubility (97.40 ± 2.01%). Similarly, Bhasker et al. (2007) also lowered the pH of ultrafiltration retentate using diluted citric acid followed by ion exchange to remove the calcium content of ultrafiltration retentate, then mixed with ultrafiltration retentate to produce MPC with different levels of calcium depletion to 33%, 50%, and 83%. All the calcium-depleted MPCs showed higher solubility than the control MPC. So, calcium reduction using acidification followed by ion exchange increases solubility.

- *Partial demineralization with carbon dioxide and ultrafiltration*: Marella et al. (2015) reported a method for partial demineralization of MPC using carbon dioxide, where they injected carbon dioxide in skim milk as well as ultrafiltration concentrate during the ultrafiltration process, followed by spray drying to get MPC. They were able to reduce calcium from 2.0% in control MPC to 1.3% in reduced calcium MPC. When reduced calcium MPCs were tested for solubility, they showed higher solubility (in warm and cold water) than control MPCs, further, they retained their solubility over the six-month storage study period than control MPC. Also, the reconstituted MPC (% protein) solutions showed higher heat stability (in terms of heat coagulation time) of reduced calcium than control MPC (Pandalaneni et al., 2018)
- *Calcium chelation using salts and ultrafiltration*: Rather than removing calcium from MPCs, calcium chelating salts can entrap calcium and improve functionality. Therefore, Meena et al. (2019) produce MPC with added disodium phosphate as a chelator. MPC with disodium phosphate showed significantly higher solubility than control MPC, freshly after production as well as after 90 days after storage. Also, heat stability (in terms of heat coagulation time) was reported higher than the control MPC.

In summary, it can be said that mineral reduction specifically calcium can improve the solubility of MPC/litre. Additionally, mineral reduction in MPC/Iitre, also helps in the shelf stability of products such as high-protein beverages, and high-protein ice cream formulations.

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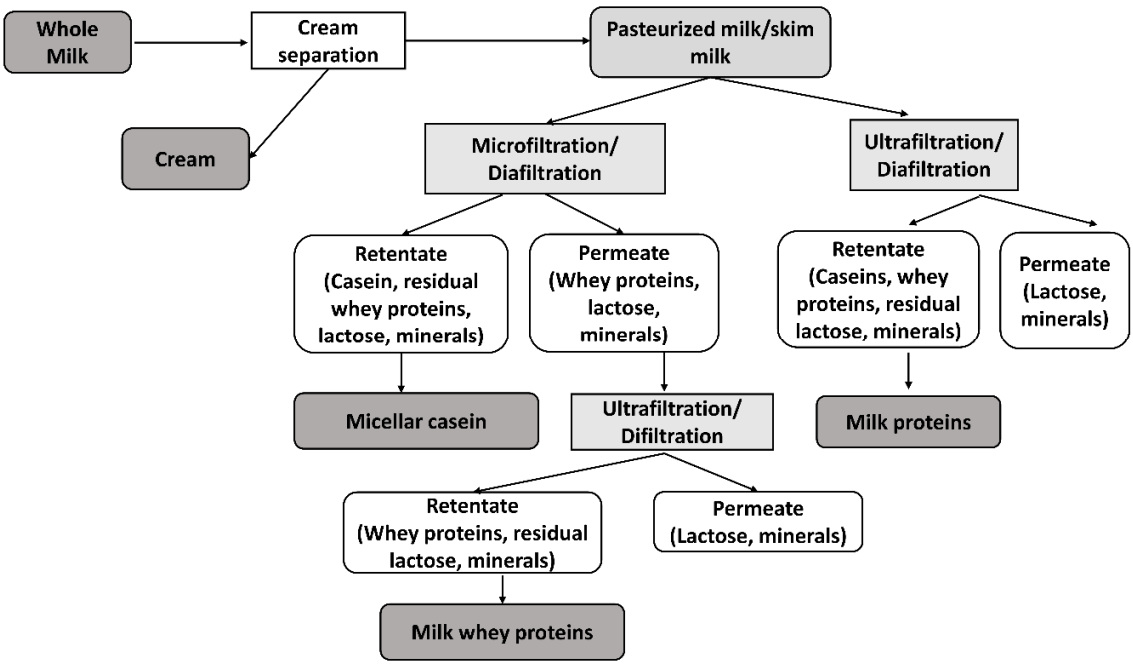

Figure 1 A schematic diagram of products obtained from milk using membrane separation.

Table 1 Proximate composition of milk protein concentrates and isolates (American Dairy Product Institute)

MPC (Milk protein concentrate) and MPI (Milk protein isolate)	Composition (%)				
	Protein	Moisture	Lipid	Carbohydrate	Ash
MPC 42	41.5 (min)	5.0 (max)	1.25 (max)	51 (max)	10 (max)
MPC 56	55.5 (min)	5.0 (max)	1.5 (max)	36 (max)	10 (max)
MPC 70	69.5 (min)	6.0 (max)	2.5 (max)	20 (max)	10 (max)
MPC 80	79.5 (min)	6.0 (max)	2.5 (max)	9 (max)	8 (max)
MPC 85	85 (min)	6.0 (max)	2.5 (max)	8 (max)	8 (max)
MPI	89.5 (min)	6.0 (max)	2.5 (max)	5 (max)	8 (max)