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Technical Report:

Milk Fractionation Technology and Emerging Milk Protein Opportunities

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The range of expertise required by food product developers today makes it challenging to stay informed about new developments regarding dynamic dairy ingredients. This report summarizes the emerging fractionation research on advances in the wide variety of rich nutrients found within cow's milk, as well as the latest research on dairy ingredient processing and applications. The Dairy Research Institute®, established under the leadership of America's dairy farmers through the dairy checkoff program, has supported research to help in the discovery of new dairy ingredients and processing technologies. The goal is to assist food formulators in developing protein-packed food and beverages that consumers demand.

Milk Fractionation Techniques

Basics of Milk Composition

Protein is an essential dietary component, and sufficient intake is important in a healthy and balanced diet. Consumers are becoming increasingly aware of and knowledgeable about the role of protein in the diet. In addition, consuming a higher-protein diet can help people maintain a healthy weight, curb hunger, enhance exercise recovery and maintain muscle mass as they age. Dairy products not only provide protein but also high-quality protein (i.e., protein rich in all the necessary amino acids). Dairy proteins are versatile, multifunctional proteins, which is evident by their wide use in a variety of foods and beverages. Dairy proteins also are now widely used in protein-enhanced foods of all types.

Milk has a unique composition of nutritional components, including proteins, fats, lactose, minerals and other minor compounds (Table 1).[1] Each of these components differs in size, structure and physical properties. The proteins in milk consist of a group of proteins called caseins and another group called whey proteins. Of the approximately 3.6 percent protein in milk, roughly 80 percent is casein and 20 percent is whey protein. Caseins and whey proteins have very different structures and physical properties. Processing techniques have been developed to modify the composition of milk according to the unique characteristics of its components. These processes have given the dairy industry the ability to make a wide variety of dairy ingredients manufactured from milk. Some of these processes involve the use of filtration methods that can separate milk according to the molecular weight of its components.[2]

Table 1[1]

Nutrient	(%)
Water	87.4
Protein	3.6
Caseins	2.95
α_{s1}-casein	1.2
α_{s2}-casein	0.3
β-casein	1.0
κ-casein	0.35
γ-casein	0.12
Whey proteins	0.65
α-lactalbumin	0.12
β-lactoglobulin	0.32
Blood serum albumin	0.04
Immunoglobulins	0.08
Proteose-peptone fraction	0.1
Fat	3.7
Carbohydrate	4.8
Ash	0.7

Adapted from *Handbook of Dairy Foods and Nutrition*, Third Edition

Membrane Processes

Membrane separation technology is used in milk and whey processing to separate milk and concentrate whey proteins after cheese making and fractionate the whey proteins into specific components. Membrane technology is being utilized both in traditional dairy processing and for new and innovative applications, enabling the production of value-added dairy products.

Some of the common membrane separation technologies used in the dairy and food industry are (Figure 1):[3]

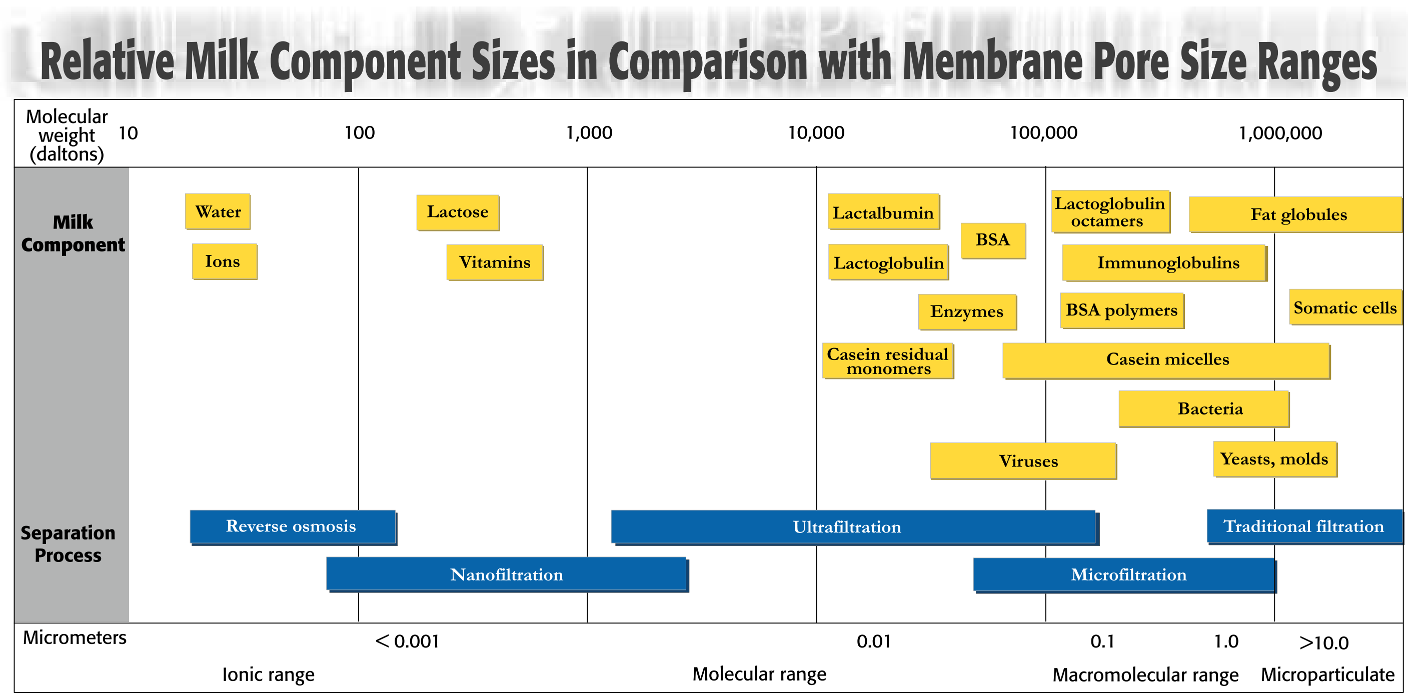
- Reverse osmosis
- Nanofiltration
- Ultrafiltration
- Microfiltration

Ultrafiltration and microfiltration technologies are the primary membrane separation technologies used by the dairy industry, and thus the focus of this report.

Ultrafiltration has been used successfully by the dairy industry to concentrate dairy proteins in both whey and milk proteins from the much smaller compounds such as lactose, vitamins and minerals (less than 1,000 daltons). Ultrafiltration of skim milk to separate milk proteins from lactose produces ingredients that are referred to as milk protein concentrates (MPCs). MPCs do not have a standard of identity, but they generally range from 42 percent to 79 percent protein. MPCs are higher in protein than nonfat dry milk but maintain the same ratio of approximately 80 percent casein and 20 percent whey protein. Ultrafiltration of cheese whey to separate whey proteins from lactose produces whey protein concentrate. Depending on the amount of lactose removed, protein concentration can range from 34 percent to 80 percent protein.

Microfiltration traditionally has been used in the dairy industry for bacteria removal, defatting of whey and micellar casein enrichment for cheese making.[4] A more recent application of microfiltration is the fractionation of caseins and whey proteins from milk. Caseins are larger in molecular weight than the majority of whey proteins and thus can be separated through the use of microfiltration. As in other membrane processes, the larger molecules that are retained in the membrane are referred to as the “retentate,” and the smaller molecules that pass through the membrane become the “permeate.” Recent research has examined different microfiltration systems to separate caseins and whey proteins from milk.[5-15] The fraction containing casein (retentate) is commonly referred to as “micellar casein,” while the fraction containing whey proteins (permeate) is referred to by many names in the literature, such as “native whey,” “serum proteins” and “ideal or virgin whey.”[16] For the purpose of this article, the whey proteins separated directly from milk will be referred to as “milk-derived whey (MDW).” Given the new technology, MDW doesn't have a standard of identity in the United States or CODEX since whey is currently defined as a result of the coagulant of milk.

Figure 1.[3]



Microfiltration systems for fractionating milk proteins have been commercially available since the 1980s.[2,4] Because the first available membranes were made of ceramic materials, the majority of the research on milk protein fractionation has been done using ceramic membranes with pore sizes from 0.1 to 0.2 µm.[4-11] Many of these studies focused on optimizing the conditions for the separation of MDW proteins from micellar casein. Early research identified optimum conditions of 50 C to 55 C (122 F to 131 F) processing temperatures and the use of a device to maintain uniform transmembrane pressure.[4] Research in the last decade, with ceramic membranes in multiple stages, has used similar conditions and successfully removed up to 95 percent of MDW protein from skim milk and produced a casein concentrate with lactose, nonprotein nitrogen and soluble minerals.[6,8-9] Micellar casein yield was most affected by the initial skim milk composition while MDW protein yield was most affected by the initial skim milk composition, followed by preheat treatment of the milk and the whey protein removal factor.[9] Researchers have reported that up to 3 percent casein remains in the MDW fraction.[8,16]

In the early 2000s, polymeric spiral-wound microfiltration membranes, similar to the materials used for ultrafiltration membranes, became available for research. Research comparing ceramic and polymeric membranes, using the same processing temperatures of 50 C (122 F), showed the ceramic membranes had a higher removal of MDW proteins (more than 95 percent) than the polymeric membranes (70 percent).[10,11] Other researchers have used colder temperatures, less than 7 C (45 F), for processing milk by membrane filtration, which allows unpasteurized milk to be filtered and reduces the possibility of whey protein denaturation and microbial growth during processing.[13] Researchers producing cold, microfiltered liquid casein concentrates for cheese making reported good separation of casein and whey protein fractions, with only 0.05 percent casein remaining in the whey protein (permeate) stream.[17] Other research has used cold filtration for more unique milk protein separations.[18]

Beta-casein present in cow's milk at 1 percent is the most surface-active of all the milk proteins and has use in infant formula production. Purification of β-casein from skim milk has been achieved using cross-flow polymeric microfiltration membranes.[19] The process uses skim milk cooled to 1 C to 2 C (34 F to 36 F), which improves the separation of β-casein and yields a pure β-casein product, a concentrated casein ingredient and an MDW protein ingredient. The β-casein has good emulsification and foaming properties, making it a suitable replacement for sodium caseinate. The remaining casein concentrate is well-designed for cheese manufacture, as it will yield a cheese with less bitterness and improved meltability because of the depletion of β-casein. The remaining milk-derived whey protein can be further concentrated and spray-dried into a whey protein concentrate or isolate. Further separation of the whey proteins β-lactoglobulin and α-lactalbumin either by use of microfiltration of skim milk or use of wide-pore ultrafiltration membranes also has been researched.[20,21]

A process that combines ultrafiltration and microfiltration of milk produces an MDW protein with increased heat stability and improved flavor. The casein concentrate is formed into a gel that is reversible and has enhanced shelf life at refrigerated temperatures. At temperatures less than 22 C (72 F), it solidifies. At temperatures above 22 C (72 F), it becomes a liquid. It can be made into 640-pound blocks and has no microbial growth after 16 weeks of storage.[22] This novel ingredient offers new alternatives to nonfat dry milk for milk standardization in cheese manufacture or other nutritional beverage applications.

Characteristics of Milk-derived Casein and Whey Protein Fractions

Composition

Manufacture of micellar casein concentrates (MCC) from milk by microfiltration produces a range of compositions depending on the amount of MDW protein removed. Further concentration and diafiltration can increase the total protein and decrease the amount of lactose in the final ingredient. MCC ingredient provides notable nutritional benefits and is an excellent source of all essential amino acids and calcium. Apart from nutritional benefit, it offers a unique functional benefit for application in retort beverages and cheese production.[23] An MCC with 95 percent of the MDW protein removed has approximately 84 percent protein, of which 80.5 percent was casein and 3.7 percent was whey protein, plus 2.7 percent fat and 3.7 percent lactose, on a dry basis.[23]

Composition of milk-derived whey protein ingredients are unique compared with those derived from cheese whey. The protein composition of cheese whey differs from MDW because it contains glycomacropeptide, which is cleaved from κ-casein through the action of chymosin in cheese making. One of the key differences between cheese whey and MDW is in their fat content. The microfiltration process retains milkfat in the retentate, which yields an MDW that is essentially free of fat, typically less than 0.3 percent, even after further concentration to 80 percent protein.[15,16] Alternatively, cheese-whey-derived whey protein concentrates (CD-WPC) with 80 percent protein will typically contain 6 percent to 7 percent fat. This difference in fat content also is a major factor in explaining their differences in functionality, flavor and appearance.[24-26]

The functional properties of MDW protein concentrates (MD-WPC) are typically better than CD-WPC. MD-WPC has greater foaming, gel strength, solubility and emulsification capacity than CD-WPC. Because of higher denaturation levels in the commercial CD-WPC, it has greater water-holding capacity and poorer solubility. The differences in functional properties were due to the differences in whey protein composition, protein denaturation and the amount of fat in the WPC.

Sensory Properties

The sensory properties of MD-WPC are notably different from commercial CD-WPC. Studies have identified similar aroma compounds in both products, many of which are lipid and protein oxidation products.[15,16] Typically, commercial CD-WPCs have much higher concentrations of these volatile compounds such as hexanal, heptanal and pentanal compared with pilot-scale MD-WPC. Commercial CD-WPC can be variable in flavor due to milk source, cheese making process, treatment of the whey and further whey processing treatments. Flavor attributes of commercial whey ingredients are discussed in detail in the *Technical Report: Sensory Properties of Whey Ingredients*.[27] One study found five compounds in MD-WPC that were more prevalent or not detected in 80 percent MD-WPC compared with a pilot-scale CD-WPC.[16] These compounds are associated with green (hexanal), smoky (2-methoxy phenol) and cucumber (E-2-nonenal) flavors. The compounds not detected in pilot-scale CD-WPC were garlic (thenylthiol) and burnt (4,5-dimethyl thiazole). Researchers suggested that the compounds present in the MD-WPC were due to the multiple filtration steps at 50 C (122 F).

Besides a cleaner flavor, MD-WPC has a very different appearance in solution compared with a CD-WPC, which is related to the fat content. The very low fat content in MD-WPC gives it a clear appearance in solution, while a CD-WPC will be very milky and cloudy (Figure 2). This clear appearance allows MD-WPC at any protein level to be used in clear beverage applications.

Figure 2.

Milk-derived Whey Protein Concentrate
(Native WPC)

Cheese-derived Whey Protein Concentrate
(Traditional WPC)

Food Applications for Milk-derived Casein and Whey Protein Fractions

Beverages

MCC and MD-WPC ingredients are available commercially but still are relatively new to the dairy ingredient world, so little application work has been conducted and published on their use.[16,28] MCC would be a good choice for neutral pH UHT or retort-processed nutritional drinks because of their heat stability.[29,30] The use of MD-WPC will produce a clear, high-acid beverage (pH 3.4) that will have similar heat stability and clarity to a commercial whey protein isolate (WPI). A raspberry-flavored, protein-enhanced water in the pH range of 3.4 to 3.5 was produced with 2 percent protein with a commercial WPI and a pilot-plant-produced MD-WPC 80 and heat-processed in an HTST at 88 C (190 F) for 30 seconds, cooled and bottled.[31] Shelf life studies on both drinks measured several factors — pH, viscosity, turbidity, protein solubility, color stability, SDS-PAGE, mass spectroscopy and sensory properties — at specific time intervals up to 39 weeks at temperatures of 20 C, 30 C and 40 C. No significant change in pH, viscosity or solubility occurred during the shelf life of either beverage. There was an increase in turbidity observed between weeks 26 and 39 at 40 C. Color stability changed at 40 C. Some acid hydrolysis occurred at 18 weeks and later. The commercial WPI beverage developed some off-flavors typically associated with high-protein beverages, while the MD-WPC 80 beverage had a clean flavor and aroma when compared with commercial WPI beverages. The flavor of both beverages was stable over time, although the flavor aroma decreased.[32] Similar results were seen in a different study with a peach-flavored, instant-type, acidified beverage formula with 6 percent protein made with an MD-WPC 80 and a CD-WPC 80.[16] The flavor and appearance of the MD-WPC 80 beverages were preferred over the CD-WPC 80 beverages.

Infant Formula

While human milk is ideal food for newborn babies, infant formulas are designed to also meet the nutritional needs of newborns and growing babies. One of the main compositional differences between human milk and cow's milk is the whey protein composition.[33] Human milk has higher levels of lactoferrin, α-lactalbumin, immunoglobulins, lysozyme; no β-lactoglobulin; and lower levels of serum albumins, whereas cow's milk is predominantly β-lactoglobulin with higher levels of serum albumin than human milk. Studies have evaluated methods to isolate α-lactalbumin and looked at the bioactivity of the ingredient for its suitability in infant formula.[34]

Another difference between human and cow's milk is the casein to whey protein ratio. The casein to whey protein ratio of human milk is approximately 40:60 (midlactation), while cow's milk is 80:20. This 40 percent casein to 60 percent whey protein ratio is the target for typical infant formulas. Combinations of milk and whey proteins are used in most dairy-based infant formulas. Cheese-derived whey proteins have been the predominant source of whey protein for infant formulas today. More recently, milk-derived WPC or WPI has been highlighted to infant formula manufacturers as a source of protein not containing glycomacropeptide, which also is not present in human milk. The consistent protein composition of milk-derived whey proteins also can be a benefit.

Cheese

One of the applications researched for MCC, early in its development, was for cheese.[35] The practice of cheese milk standardization is common and is typically done to improve yield and produce cheese with a consistent composition. Cheese makers in the United States are allowed to use ingredients such as condensed skim milk and nonfat dry milk for this purpose in standard of identity cheeses, such as Cheddar and mozzarella.[36] The ideal ingredient for standardization of cheese milk would contain predominantly casein protein, as that is the primary protein in cheese. The use of MCC currently is not allowed in standard of identity cheeses. Several studies have evaluated the use of microfiltration retentates (liquid MCC) in the manufacture of cheese.[6,37-41] These results suggest that MCC is a useful ingredient for increasing production capacity while also maintaining cheese quality.

Fractionation of milk by microfiltration creates more value-added, milk-derived ingredient opportunities that have diverse applications for use by the dairy and food industries.[42] These ingredients can provide unique functionalities along with clean flavor and excellent nutrition. The dairy industry has started to utilize these manufacturing methods and commercialize ingredients such as micellar casein. Other ingredients such as milk-derived whey protein, β-casein and individual whey proteins will soon be produced by dairy ingredient suppliers. These ingredients provide an opportunity for food and beverage companies to create new products benefiting consumers. Research efforts supported by the Dairy Research Institute will continue to explore new processing technologies and applications that offer commercial solutions to expand utilization of dairy ingredients in new places and meet consumer needs.

For more information about dairy ingredient research, visit InnovateWithDairy.com, USDairy.com/DairyResearchInstitute or USDEC.org. For assistance with new or improved products using dairy ingredients, contact Dairy Technical Support at **techsupport@innovateWithDairy.com**.

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