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Invited review: Shelf-stable dairy protein beverages—Scientific and technological aspects

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ABSTRACT

Consumer focus on health and wellness is driving the growth in high-protein dairy beverages. The review discusses shelf-stable ready-to-drink beverages that are primarily dominated by sports nutrition and the "better for you" beverage categories. Both of these categories tend to have a "high in protein" claim. Because of their functionality, sensorial attributes, and protein quality, dairy protein ingredients are the ingredients of choice to meet protein claims. Due to the higher protein content of the beverages, the functionality of dairy protein ingredients plays a critical role in final product quality and stability. In the United States, Food and Drug Administration regulations classify shelf-stable foods into acid/acidified and low-acid foods. The differentiation is based on pH and water activity (a_w). In the context of shelf-stable high-protein dairy beverages, any beverage with a_w of >0.85 and with a finished equilibrium pH of >4.6 is classified as low acid. Beverages to which acids or acid foods are added and have a finished equilibrium pH of ≤4.6 and a_w >0.85 are classified as acidified food. Acid foods have a natural pH of ≤4.6. The final pH requirement of these shelf-stable products will affect the type of dairy protein used in these applications. In acidified dairy protein beverages, the go-to ingredient is whey protein. In low-acid beverages, the protein ingredients of choice are milk protein ingredients (with a casein-to-whey protein ratio of 80:20, as found in typical bovine milk) and casein-enriched ingredients. Rendering the product shelf-stable depends on whether the product is classified as acidified or low acid. Low-acid, shelf-stable beverages, in general, have 2 manufacturing options: retort and UHT processing, followed by hermetic sealing. Pasteurization is the standard processing choice for shelf-stable acidified beverages, followed by hot fill. Because of differences in pH and heat loads during the manufacture of high-protein dairy beverages, the functionality of protein ingredients will play an essential role in determining the final beverage quality. Two of the most important functional properties of dairy protein ingredients that have a role in producing these beverages are solubility and heat stability. This review elucidates the physicochemical properties of dairy protein ingredients for low- and high-acid shelf-stable dairy protein applications, analytical techniques to characterize protein ingredients, beverage processing conditions, and quality defects observed.
Key words: high-protein dairy beverage, acidity, stability, ready-to-drink

INTRODUCTION

Beverages that provide nutrition and convenience are growing in popularity among consumers who focus on health and wellness. Protein-rich diets can assist in satiety, appetite control, and maintenance of lean body mass. Globally, high-protein dairy beverages (including protein powders for ready-to-mix applications) are on the rise, with an average annual new product launch growth rate of 20.8% from 2016 to 2021 (Innova Market Insights, 2021). The protein beverage category encompasses various products that include ready-to-mix powders, ready-to-drink (**RTD**) beverages, meal replacement beverages, better-for-you beverages, and others. As the name suggests, the ready-to-mix category involves the dissolution of powders, typically in water, before consumption. The RTD protein beverages are primarily dominated by 2 product categories: sports protein-based beverages and better-for-you/meal replacement protein beverages (Innova Market Insights, 2021). Sports protein-based beverages are positioned for consumption by athletes and tend to have a high-protein claim (Innova Market Insights, 2021). The better-for-you dairy-based nutritional category is primarily consumed by seniors and retirees.

The current review focuses on shelf-stable dairy-based RTD protein beverages (sports nutrition and

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Table 1. A sample list of dairy protein-based ready-to-drink beverages available in the United States

Product name (manufacturer)	Major protein-based ingredient	Serving size	Protein per serving (g)	Protein (g) per 100 mL
Grass-Fed Iconic Protein (Iconic Beverages)	Milk protein isolate	340 mL	20	6
Premier Protein Shake (Premier Nutrition Corp.)	Milk protein concentrate, calcium caseinate, whey protein concentrate	311 g	30	10
Ensure High Protein Nutrition Shake (Abbott Nutrition)	Milk protein concentrate, soy protein isolate	237 g	16	7
Boost Balanced Nutritional Drink (Nestle)	Milk protein concentrate and less than 2% soy protein, calcium caseinate, sodium caseinate	237 g	20	8
Muscle Milk Zero Protein Shake (Cytosport Inc.)	Milk protein isolate, calcium caseinate, and sodium caseinate	330 mL	20	6
Core Power High Protein Milk Shake Chocolate (Fairlife)	Filtered low-fat grade A milk	414 mL	26	6
Chocolate Protein Plus (Bolthouse Farms)	Low-fat milk, whey protein concentrate, soy protein isolate	450 mL	30	7
Quest Protein Shake, Vanilla (Quest Nutrition)	Milk protein concentrate	325 mL	30	9
High-Performance Protein Shake (Equate)	Protein blend (milk protein concentrate, milk protein isolate, calcium caseinate, whey protein concentrate)	325 mL	30	9
Pure Protein Shake, Rich Chocolate (Pure Protein)	Protein blend (milk protein isolate, whey protein concentrate, calcium caseinate)	325 mL	30	9
Garden of Life Sport protein drink (Garden of Life)	Milk proteins isolate	325 mL	26	8
Premier Protein Clear Protein Drink (Premier Nutrition Corp.)	Whey protein isolate	500 mL	20	4
BariatricPal Whey Protein and Collagen Power Shots, Tropical Orange (Bariatric Pal)	Hydrolyzed collagen, whey protein concentrate, calcium caseinate, L-tryptophan	90 mL	25	28

better-for-you beverages). These beverages are generally formulated to meet a protein claim on the label. According to the US Food and Drug Administration (FDA) guidelines, for an on-pack "high in protein" claim, the beverage should contain a minimum of 10 g of good-quality protein [protein digestibility corrected amino acid score (**PDCAAS**) of 1] per serving (21CFR 101.54; FDA, 2016a). Similarly, the beverage should contain at least 5 g of good-quality protein (PDCAAS of 1) per serving to claim "good source of" protein on the label. Protein-containing beverages are available in various flavors to meet the needs of a wide range of consumers. An analysis of the nutrition facts panel of some RTD dairy-based protein beverages available in the US marketplace is presented in Table 1. All the products analyzed had a "high protein" claim. The primary dairy protein ingredients in these beverages were milk protein concentrates (**MPC**), milk protein isolates (**MPI**), UF milk, whey protein concentrates (**WPC**), whey protein isolates (**WPI**), and caseinates. Milk proteins (caseins and whey proteins) are the protein of choice in nutritional beverages because they contain essential AA for protein synthesis, digestibility, and health benefits. Sports protein-based RTD beverages often contain additional whey proteins to create whey-predominant mixtures because of their specific and well-documented nutritional benefits for athletes; these are generally high-acid beverages (Ali et al., 2019; Hernández Miranda et al., 2021; Melnikova and Bogdanova, 2021).

This review elucidates the physicochemical properties of dairy protein ingredients for low- and high-acid shelf-stable dairy protein applications, analytical techniques to characterize protein ingredients, beverage processing conditions, and quality defects observed.

No animals were used in this review, and ethical approval for the use of animals was thus deemed unnecessary.

MILK PROTEINS: TYPE AND IMPORTANCE

Caseins and whey proteins are the 2 major protein fractions found in bovine milk and account for 80 and 20% of total milk protein, respectively (Raikos, 2010). Casein comprises 4 primary gene products: α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN, which primarily exist in milk in a micellar form. Colloidal calcium phosphate and hydrophobic interactions are thought to hold the micelle together (Dalgleish and Corredig, 2012). Caseins are phosphorylated proteins that precipitate from milk at pH 4.6, whereas whey proteins remain soluble (Farrell et al., 2004; Dalgleish and Corredig, 2012). Caseins also have higher heat stability than whey proteins due to the lack of secondary and tertiary structures. Whey

Table 2. Heat treatments for shelf-stable high-protein dairy beverages

Heat treatment	Time–temperature combination	Bactericidal and chemical effect	Reference
UHT processing and aseptic filling (in-flow sterilization)	138°C for at least 2 s	Achieves commercial sterility of the food (by heat), equipment, and containers (by heat, chemical sterilants, or other appropriate sterilants). Denaturation of β-LG is ~70–95%.	FDA, 2016b,c
Retort (in-container sterilization)	115–120°C for 5–15 min	Achieves commercial sterility of the food and containers (heat denaturation of β-LG is near total). Maillard discoloration and cooked taste are common.	Dumpler et al., 2020
Hot filling for acid/acidified products	90–95°C for 30–90 s	Achieves commercial sterility of the food and containers by a process called "hot fill and hold." The heating temperature and holding time will depend on the pH of the product. The lower the pH, the lower the hold time at a defined temperature.	Puhan, 1979; Kumar and Sandeep, 2014

proteins are globular and start to denature when exposed to temperatures >60°C. The major whey protein in milk, β-LG, has a molecular weight of ~18.4 kDa and contains 2 disulfide bonds and a free sulfhydryl group (Walstra et al., 2005; Considine et al., 2007). α-Lactalbumin has a molecular weight of ~14.2 kDa and is the second major whey protein. It has 4 intramolecular disulfide bonds and no free sulfhydryl group. Compared with β-LG, α-LA is relatively thermostable and its calcium-binding property has a role in heat stability (O'Mahony and Fox, 2013). Caseins and whey proteins have a PDCAAS of 1 (Hoffman and Falvo, 2004); PDCAAS is an essential consideration in the United States when formulating products with a protein claim because percent daily value needs to be listed on the nutrition facts panel and PDCAAS is used for calculating % daily value. Native casein micelles, aside from being a protein source, also play a vital role in the delivery of calcium. Both casein and whey proteins are sources of many peptides with potential bioactive properties that provide health benefits.

Shelf-stable dairy protein beverages can be classified as high-acid or acidified (pH ≤4.6) and low-acid (pH > 4.6). A comparatively lower thermal load is required to render high-acid or acidified beverages shelf-stable, whereas for low-acid beverages, the thermal load required is significantly higher. Shelf-stable low-acid beverages that are aseptically processed use 2 systems to sterilize products and package. Table 2 provides the time–temperature combinations typically used in low-acid and high-acid shelf-stable beverages. In aseptic UHT products, after thermal processing, the sterile product is filled into sterile packaging using a sterile filling machine to deliver a shelf-stable product (Kumar and Sandeep, 2014). In canning, the package and food product are subjected to thermal treatment after filling. The dairy protein choice for use in low-acid and acidified beverages is critical for designing a product that is acceptable to the consumer and stable during processing and distribution. Casein-forward ingredients (ingredients with casein-to-whey protein ratio of 80:20 or higher), because of their superior stability at neutral pH, are the preferred protein ingredient in shelf-stable low-acid beverages. Shelf-stable high-acid/acidified beverages are heated to temperatures between 90 and 95°C for 30 to 90 s, followed by hot filling. Due to their superior heat stability and formation of soluble aggregates on heating at lower pH values, whey proteins are the protein of choice in high-acid/acidified clear beverages. In the following sections, low-acid and high-acid beverages will be discussed in detail with respect to ingredients, functional properties, and manufacturing process.

LOW-ACID SHELF-STABLE DAIRY PROTEIN BEVERAGES

Dairy Protein Ingredients

For low-acid RTD dairy-based beverages, casein-forward ingredients like UF milk, MPC, and MPI are the protein ingredients of choice. Liquid ingredients in the form of UF milk and powdered ingredients in the form of MPC and MPI can be used as ingredients. Caseinates, an ingredient derived from the isoelectric precipitation of milk proteins or rennet coagulation, are used as a protein source in some beverage formulations. Due to their lower heat stability, whey protein ingredients are not a preferred choice for use in low-acid applications. However, functional whey protein ingredients produced by controlled heat and mineral aggregation of whey proteins can offer colloidal stability in low-acid shelf-stable beverages (Ryan and Foegeding, 2015). Glycomacropeptide (**GMP**), a whey protein fraction obtained by the hydrolysis of κ-CN during cheese manufacture, can offer colloidal stability in low-acid beverages (Etzel, 2004). Dairy protein ingredients with protein content of 30 to 89% on a DM basis are known

Table 3. Proximate composition (min: minimum; max: maximum) of different dairy protein ingredients (ADPI, 2018)

Ingredient type[1]	Ingredient[2]	Composition (%)				
		Protein	Moisture	Fat	Lactose	Ash
MMP or MC	MMP/MC70	69.5 (min)	6.0 (max)	2.5 (max)	16.0 (max)	8.0 (max)
	MMP/MC80	79.5 (min)	6.0 (max)	3.0 (max)	10.0 (max)	8.0 (max)
	MMP/MC85	85.0 (min)	6.0 (max)	3.0 (max)	3.0 (max)	8.0 (max)
	MMP/MC90	89.5 (min)	6.0 (max)	3.0 (max)	1.0 (max)	8.0 (max)
MPC and MPI	MPC70	69.5 (min)	6.0 (max)	2.5 (max)	20.0 (max)	10.0 (max)
	MPC80	79.5 (min)	6.0 (max)	2.5 (max)	9.0 (max)	8.0 (max)
	MPC85	85[3] (min)	6.0 (max)	2.5 (max)	8.0 (max)	8.0 (max)
	MPI	89.5[3] (min)	6.0 (max)	2.5 (max)	5.0 (max)	8.0 (max)
WPC and WPI	WPC80	79.5[3] (min)	6.0 (max)	10.0 (max)	4–10.0	3–5.0
	WPI	90.0[3] (min)	6.0 (max)	1.5 (max)	0.5–1.0	2–3.0
mWPC and mWPI	mWPC80	79.5[3] (min)	6.0 (max)	2.0 (max)	13.0 (max)	5.0 (max)
	mWPI90	89.5[3] (min)	6.0 (max)	1.5 (max)	4.0 (max)	4.5 (max)

[1]MMP = microfiltered milk protein; MC = micellar casein; MPC = milk protein concentrate; MPI = milk protein isolate; WPC = whey protein concentrate; WPI = whey protein isolate; mWPC = milk whey protein concentrate; mWPI = milk whey protein isolate.

[2]Number indicates percentage protein in ingredient.

[3]On a DM basis.

as concentrates, and those with protein contents >90% of DM are known as isolates (ADPI, 2018). The composition of different dairy protein ingredients is provided in Tables 3 and 4.

Milk-Derived Ingredients Prepared Using Membrane Filtration

Membrane filtration is widely used in the dairy industry to fractionate dairy proteins. Milk protein concentrates, MPI, and micellar casein concentrates (**MCC**) are the common ingredients used as a protein source in low-acid shelf-stable dairy protein beverages (Gésan-Guiziou, 2013). In MPC and MPI, the casein-to-whey protein ratio is similar to that of the starting milk. Milk protein concentrates are commercially available in protein contents ranging from 42 to 85% (wt/wt). They are identified by a number representing the product's protein content (eg, MPC80 contains 80% protein by weight). The protein and lactose contents of MPC are inversely related. When designing products with higher protein content and lower total sugar on the nutrition facts panel, this is an important consideration. The composition of MPC powders is provided in Table 3.

Micellar casein concentrates are dairy protein ingredients manufactured from the microfiltration of skim milk. No standard of identity exists for MCC in the United States. During the manufacture of MCC, whey proteins are fractionated into the permeate during the microfiltration of skim milk, resulting in higher casein-to-whey protein ratios in the retentate. The residual whey proteins in the MCC can influence the flavor and functionality of MCC in beverage applications. When choosing MCC as an ingredient in high-protein beverage formulations, consideration must be given to the total protein content and the residual whey protein content. A simplified schematic for the preparation of different milk protein ingredients is provided in Figure 1.

Whey proteins are not typically used to produce low-acid shelf-stable beverages. Whey proteins tend to have poor solubility and heat stability at neutral pH on heating. Reconstituted WPC dispersions (2% protein) had poor UHT stability when subjected to UHT treatment (145°C for 5 s) on a benchtop system (Singh et al., 2019). Those authors also reported good UHT heat stability (defined as a run time of the UHT system without any signs of fouling for >120 min) of reconstituted protein dispersions from MPC85 and WPC80 for

Table 4. Mineral composition of different commercially available milk protein concentrates (MPC) and milk protein isolate (MPI; Sikand et al., 2011)

Type of MPC[1]	Mineral (mg/100 g)						Total
	Ca	Mg	K	Na	P	Cl	
MPC40	903–956	83–89	1,134–1,177	284–303	816–882	1,049–1,059	4,279–4,456
MPC80	1,423–1,493	69–80	217–366	45–100	375–1,109	70–286	2,614–3,339
MPI (>85%)	553–1,644	6–66	116–503	52–400	434–1,206	103–269	1,904–3,248

[1]Number following MPC indicates percentage protein.

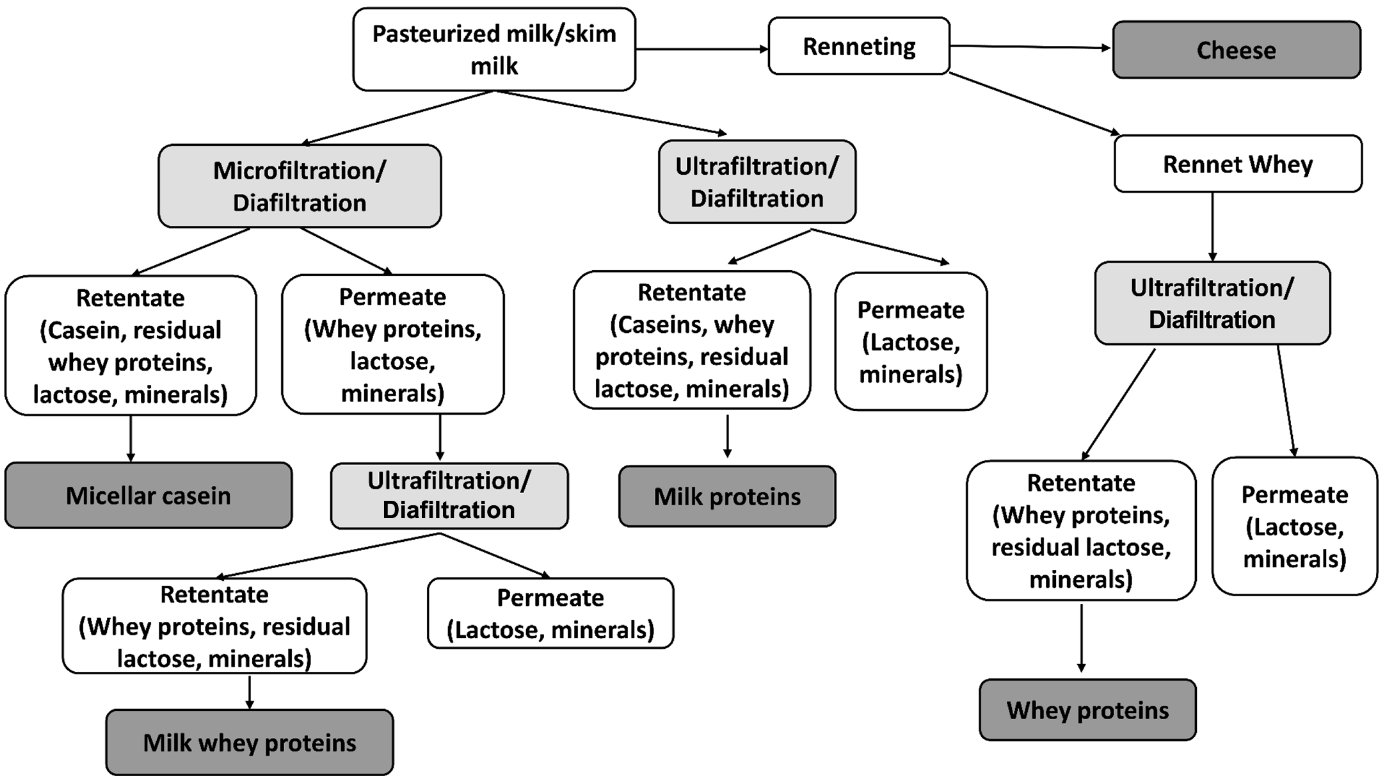

Figure 1. Schematic representation for manufacture of different milk protein ingredients used in the preparation of high-protein dairy beverages. Dark gray boxes represent ingredients used in high-protein dairy beverages, light gray boxes represent unit operations, and white boxes represent raw materials or co-products.

casein-to-whey protein ratios up to 50:50. Any further decrease in the casein-to-whey protein ratios below 50:50 decreased the run times of the UHT system below 120 min. However, soluble aggregates and complexes of whey proteins can offer colloidal stability on heating in low-acid beverages (Ryan and Foegeding, 2015).

Caseinates (Sodium Caseinate and Calcium Caseinate)

Among caseinates, sodium and calcium caseinates are used to manufacture dairy protein beverages. Sodium caseinate has a higher solubility than calcium caseinate (Carr and Golding, 2016) and therefore is the preferred ingredient of choice. Caseinates are produced by isoelectric precipitation of caseins followed by the addition of sodium hydroxide or calcium hydroxide to produce sodium and calcium caseinate, respectively. Caseinates have a pH range of 6.5 to 7.0 (Augustin et al., 2011). Calcium caseinate dispersions are turbid or milky due to their ability to form aggregates in water. In contrast, sodium caseinate solution is straw-colored, transparent, and more viscous than calcium caseinate. Caseinates are not ideal ingredients for the manufacture of high-protein beverages as they may not be perceived by consumers as clean label ingredients. Additionally, in caseinate ingredients, off-flavors like gluey, bitter, animal, tortilla, and hay flavors are well documented (Cayen and Baker, 1963; Drake et al., 2010).

Sensory Properties of Dairy Ingredients

The choice of dairy protein ingredients may influence the final beverage's sensory properties (Russell et al., 2006; Childs et al., 2008; Oltman et al., 2015). The primary drivers of dislike in high-protein low-acid dairy beverages are cooked and sulfur flavors (Lee et al., 2017). These flavors are generated because of exposure to high thermal loads (sterilization/UHT) that cause denaturation of whey proteins and exposure of sulfhydryl groups. In RTD high-protein beverages manufactured from liquid milk protein blends of MPC and MCC, sulfur/eggy flavor was higher in beverages with a higher percentage of serum protein (Vogel et al., 2021). Whey proteins primarily influence overall aroma, cooked/milky flavor, and eggy/sulfur flavor (Lee et al., 2017; Cheng et al., 2019; Jo et al., 2019; Whitt et al., 2022).

Milk protein concentrate dispersions prepared from lower protein powders have a sensory profile similar to that of skim milk (cooked/milky, sweet aromatic, cereal, and sweet). However, dispersions from MPC with protein content >70% have flavors that are characterized as tortilla, brothy, cardboard, and animal. They also have higher astringency, decreased sweet aromatic, and milky flavor (Drake et al., 2014; Smith et al., 2016a,b). Storage time and temperature also negatively affect the sensory properties of high-protein MPC powders (lowers cooked/milky and sweet aromatic flavor in dispersions). Several flavor compounds have been identified in MPC that correspond with tortilla flavor and may result from Maillard browning, lipid oxidation, or amino acid degradation (Smith et al., 2016a,b). As the protein content of MPC increases, the sweet aromatic flavor decreases. The cardboard flavor increases, presumably due to aromatic flavor compounds permeating through the UF membranes during the stages of diafiltration (**DF**) required to achieve high levels of protein purity (Park et al., 2016; Smith et al., 2016a,b).

Micellar casein concentrates have a flavor profile similar to MPC when liquid; however, on reconstituting MCC and MPC powders, the flavor intensity of tortilla/corn chips flavor is higher in MCC dispersions (Carter et al., 2018). However, MCC-based beverages tend to have a lower sulfur/cooked flavor than MPC-based beverages because of the lower whey protein concentration in the beverage. In vanilla-flavored high-protein dairy beverages subjected to UHT treatment, sulfur/eggy flavor and astringency were higher, and sweet aromatic/vanillin flavor was lower as the whey protein percent in MCC used as an ingredient increased (Vogel et al., 2021). Using UF and microfiltered skim milk (never spray dried) when available, instead of dry ingredients, may help manufacturers of high-protein beverages circumvent sensory, rehydration, and final beverage quality challenges.

Functional Properties of Dairy Protein Ingredients

Hydration/Solubility. During the high-protein dairy powder hydration process, some powder components are fast dissolving and some are slow dissolving (Mimouni et al., 2010). Whey proteins, lactose, potassium, and sodium are fast dissolving, whereas caseins, calcium, phosphorus, and magnesium are slow. If not adequately hydrated, the slow-dissolving constituents (e.g., calcium and caseins) may increase fouling during thermal processing, thereby limiting processor run times (Gandhi et al., 2017). Additionally, the proper hydration of milk proteins before thermal processing is essential to realize the full potential of the protein ingredient's functional properties (e.g., emulsification, heat stability, gelation; Mimouni et al., 2009; Bouvier et al., 2013). The poor functional properties of the protein ingredient due to insufficient hydration is thought to influence the sensory properties and shelf stability of final protein beverages (e.g., grainy/chalky texture, viscosity, and phase separation).

The batching process involves the addition of dry protein powder to water through a high-shear in-line mixer. Wettability, swelling, sinkability, dispersibility, and dissolution are the 5 critical steps in rehydrating dairy powders. The first stage, wettability, concerns the powder's ability to absorb water. With water absorption, the "swelling" stage refers to the increase in the size of powder particles. The capacity of the swelled powder to sink into the aqueous medium is known as "sinkability." The ability to disperse in water or dissolve is the fourth level. During the rehydration process, the final dissolution stage is concerned with separating and dissociation into smaller particles (Crowley et al., 2016). A schematic representation of rehydration is presented in Figure 2. This rehydration stage does not happen in any particular order; some of the stages may occur simultaneously.

The insolubility of MPC powders is a major concern during the manufacture of dairy protein beverages. The sequence of unit operations (UF followed by evaporation and drying or UF followed by nanofiltration, evaporation, and drying) used for MPC manufacture is also cited as a cause of increased insolubility of MPC powders (Cao et al., 2016). Freshly produced MPC powders (MPC32 to MPC85) have >95% solubility (Gazi and Huppertz, 2015). A negative correlation was found between solubility and MPC storage time and temperature for MPC with ≥50% protein concentration (Gazi and Huppertz, 2015). The authors also reported that the micellar fraction contributes to the insolubility of MPC powders and, with an increase in soluble casein fractions, the solubility of MPC powders increases. Various strategies exist to increase the soluble fraction of caseins in MPC powders. Mineral reduction through CO_2 injection is reported to increase the solubility of MPC80 powders (Marella et al., 2015). Ion exchange treatment of UF retentate to produce a calcium-reduced MPC powder is also reported to help with increased MPC solubility (Xu et al., 2016).

For UHT processing, the batching process for the manufacture of shelf-stable dairy protein beverages should keep pace with the UHT processor speed to maintain a continuous production run. Assuming a UHT processor speed of 20,000 kg/h (85 gallons/min), the processor will process a batch of ~30,000 kg (7,500 gallons) in ~90 min. Therefore, the entire batching operation should not exceed 90 min. That includes reconstitution of dry ingredients, adding other components

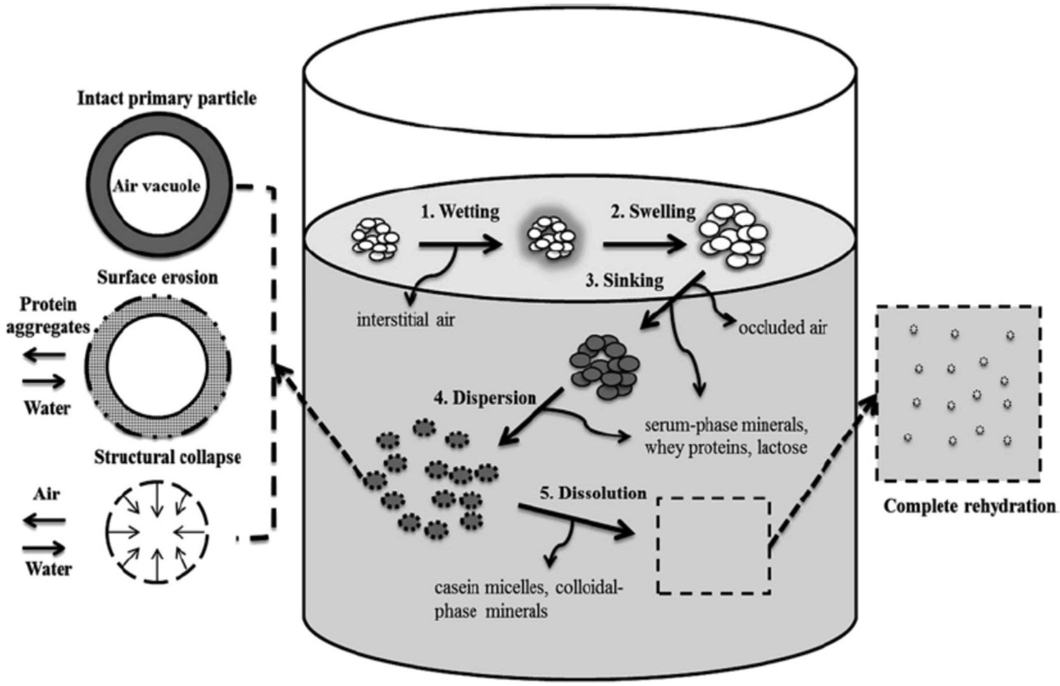

Figure 2. Schematic representation of rehydration mechanism of high-protein dairy powders (Crowley et al., 2016).

in the formula, and quality release of the batch for UHT processing. Therefore, it can be assumed that the total hydration time for dairy protein before thermal processing in a commercial manufacturing setup will be <90 min. For fresh MPC85 and above, the solubility of the powders will depend on the reconstitution temperature of the solvent (generally water) and the time for reconstitution. After 15 min, irrespective of the temperature of the water, whey proteins are nearly 100% soluble (fast dissolving). However, for caseins at 15 min of reconstitution, solubility increases as the water temperature increases: from ~50% solubility at 5°C to ~80% at 30°C (Gazi and Huppertz, 2015). After 60 min of reconstitution at 5°C, the solubility of the MPC85 powders increases to ~60%, not an ideal solubility level as reconstitution of dairy powders is not recommended at this temperature in a commercial manufacturing setup. Solubility of MPC powders is reported to increase when they are reconstituted in solvents with a higher ionic strength like milk or permeate and homogenized at approximately 13.8 MPa (138 bar; Sikand et al., 2012; Udabage et al., 2012). Increasing the ionic strength of the solvent could reduce Ca ion activity, resulting in a decrease in noncovalent interactions and consequently leading to increased solubility (Schuck et al., 2002; Hussain et al., 2011a). Stabilizing salts (to reduce Ca ion activity) and sodium chloride (to increase ionic strength) may be added first to water intended for reconstitution before dispersing the protein ingredients.

Installing a high-shear mixer and a homogenizer in the batching loop can also help address the insolubility issues encountered during the reconstitution of high-protein MPC powders. With caseinates, the solubility of sodium caseinates at neutral pH is high. As with MPC powders, increasing the ionic strength of the solvent can further increase the solubility of caseinate powders (Thomar et al., 2014). Micellar casein concentrates are novel protein ingredients that are casein-enriched using microfiltration membranes (Carter et al., 2021). They face a similar hydration challenge as MPI, and their insolubility increases with storage time and high storage temperatures (Schokker et al., 2011). Because of a lower whey protein to total protein ratio, the solubility of MCC will always be lower than that of MPI. Strategies to mitigate the hydration challenges with MPI should also improve the solubility characteristics of MCC. Manufacturers can avoid the hydration challenges of high-protein dairy ingredients by using liquid ingredients (UF milk for MPI and microfiltered milk for MCC). Additional studies on enhancing the solubility of dairy protein ingredients are described in Table 5.

Heat Stability. Low-acid shelf-stable dairy protein beverages are subjected to intense heat treatments to achieve a reasonable shelf life. Therefore, the heat stability of the protein ingredient is an essential consideration during protein ingredient selection. Heat stability is an empirical measure and is represented in terms of heat coagulation time (**HCT**), which is de-

Table 5. Different processing interventions for enhancing solubility of high-protein powders

Processing intervention	Reported results	Reference
Preacidification of milk before membrane filtration	Increased solubility of milk protein concentrates (MPC) due to decrease in calcium content from 1.84 ± 0.03 to 1.59 ± 0.03 g/100 g when glucono-delta-lactone (GDL) was added at 3.25 g/L before diafiltration step.	Eshpari et al., 2014
Ion-exchange chromatography	Replaced 30% of Ca by Na that resulted in soluble MPC.	Bhaskar et al., 2001
Addition of monovalent ions (NaCl) before drying	Positively affected solubility of MPC.	Carr et al., 2004
Addition of NaCl/KCl during the diafiltration step	Enhanced solubility of MPC.	Gualco, 2010; Mao et al., 2012, Sikand et al., 2013
Homogenization of retentate	Improved solubility of MPC.	Augustin et al., 2012; Sikand et al., 2012
High pressure (200 MPa) at 40°C before drying	Improved solubility of MPC, due to dissociation of the casein micelle in the milk stream with increased concentration of serum protein at the interface.	Udabage et al., 2012
High shear treatment such as homogenization (35/10 MPa), microfluidization (80 MPa), and ultrasonication (24 kHz, 600 W) before drying	Improved the solubility of MPC82.	Augustin et al., 2012
Extrusion-porosification	Improved rehydration properties of MPC due to nano-sized capillaries and micrometer-sized pores in particles.	Bouvier et al., 2013
The presence of NaCl, KCl, and $CaCl_2$ in rehydrating medium	Influenced the wettability of both casein and whey protein-rich powders	Hussain et al., 2011a,b; Crowley et al., 2015
Reconstitution medium is milk versus water	Higher solubility of MPC when due to higher mineral content of the milk, which promoted the re-equilibration of minerals and driving force for solubilization of colloidal calcium phosphate and casein.	Udabage et al., 2012; Crowley et al., 2015
Presence of citrate and phosphates in rehydrating medium	Increase in solubility of high-protein MPC due to calcium-binding ability of these salts	Schuck et al., 2002; Crowley et al., 2014
Heating and stirring during rehydration	Enhanced dispersion of casein-rich ingredients by breaking the solid bridges between the powder.	Mimouni et al., 2010; Forny et al., 2011
Increasing rate of stirring or impeller design	Promoted turbulence and disrupted the powders rich in interlinked casein micelles at surface.	Jeantet et al., 2010; Richard et al., 2013
Ultrasonication during rehydration (20 kHz, 450 W)	Increased solubility of MPC; however, no difference in solubility of whey protein concentrates and calcium caseinate was observed.	McCarthy et al., 2014; Chandrapala et al., 2014
Ultrasonication before drying (20 kHz, 600 W)	Improved solubility of MPC.	Yanjun et al., 2014
Agglomeration	Decreased wetting time and enhanced solubility of MPC. The agglomeration process enlarged the particles by adding a binder that linked primary particles and affected wetting phase due to easy penetration of water in large particles.	Gaiani et al., 2005, 2007

fined as the time in minutes at a defined temperature, typically at 140°C, required for the first appearance of visible clots in the milk sample (Dumpler et al., 2020). The influence of pH on HCT is the basis of classifying milk into either type A or type B milk. Type A milk has a maximum HCT at pH 6.6 and a minimum at pH 6.8 to 6.9. The heat-induced interaction of caseins and whey proteins plays a role in the maximum and minimum HCT observed as a function of pH. At pH values <6.7, heat-induced interactions of whey proteins and caseins occur on the micellar surface. Steric hindrance is attributed to increased heat stability at this pH (Dumpler et al., 2020). At pH values that show a minimum on the HCT versus pH curve, the interactions of whey protein and κ-CN occur preferentially in the serum phase. κ-Casein-depleted micelles will tend to have lower stability than micelles stabilized by steric hindrance at pH 6.7 and below. At pH values >6.9, lower calcium ion activity and greater zeta potential of the casein micelles will increase the casein micelles' heat stability. Although heat stability profiles of milk and concentrated milk are readily available in the literature, the heat stability of fractionated milk protein dispersions and the effect of added ingredients in high protein beverages are not extensively documented.

In low-acid dairy protein beverages, dispersions of dairy protein ingredients may have different heat stability profiles than skim milk (Crowley et al., 2015). Heat stability of MPC dispersions may vary depending on the protein content of MPC powders, the type of membrane filtration techniques used in the manufacture of the powder (DF, nanofiltration), the water type used for reconstitution (reverse osmosis water and treated water due to differences in ionic strength), and the protein percentage in the final formulation (Lin et al., 2018). Crowley et al. (2015) studied the HCT of MPC dispersions (3.5% wt/wt at 140°C) from MPC powders of different protein content (MPC35 to MPC90) at different heating pH values (6.3–7.3). A lower HCT was observed for MPC dispersions from MPC80 onward. A high calcium ion activity can be attributed to the decreased HCT. Sunkesula et al. (2021) observed that MPC (10% wt/wt) dispersions from MPC80 powders had an HCT (at 140°C) pH profile different from that observed in skim milk systems. The 10% protein dispersion from MPC80 had a maximum HCT at pH 6.9, followed by a decrease at pH 7.1. The authors attributed the higher HCT at pH 6.9 compared with 7.1 to the difference in Ca ion activity. At pH 6.9, the Ca ion activity of the MPC dispersion was high enough to promote whey protein–casein interactions on the micellar surface, thus providing steric hindrance to the casein micelles. At pH 7.1, the authors hypothesized that the κ-CN–whey protein interactions now occurred in the serum phase, thereby lowering the HCT. The authors also evaluated the HCT of mineral-reduced MPC powders (30% Ca reduction) and observed an increase in the HCT of MPC dispersions at pH 6.9 compared with control. It is hypothesized that a reduction in the colloidal Ca content during the MPC manufacturing process could have increased the heat stability of Ca-reduced MPC dispersions (Marella et al., 2015; Meena et al., 2018; Sunkesula et al., 2021). Calcium-reduced MPC dispersions (8% wt/wt at 140°C) tend to have a similar heat stability profile as control MPC dispersions containing sodium hexametaphosphate (Pandalaneni et al., 2018).

Meletharayil et al. (2016) showed that upon increasing lactose addition to MPC dispersions, the calcium ion activity of the dispersions increased and could contribute to decreased HCT. The addition of Ca-chelating salts like sodium hexametaphosphate help improve the heat stability of MPC protein dispersions. It is highly recommended to evaluate the heat stability of beverage formulations containing MPC powders. The pH corresponding to the maximum heat stability should be identified and used as a quality release criterion of the batch before thermal processing.

Casein-enriched ingredients such as MCC can exhibit a different heat stability profile compared with skim milk or MPC dispersions. Micellar casein concentrate dispersions, when compared with MPC dispersions at normalized protein levels, will have higher heat stability, and one of the contributing factors is the casein-to-whey protein ratio (Renhe and Corredig, 2018). Several studies have shown that in serum protein-free casein micelle (SPFCM) dispersions, heat stability increases with an increase in preheating pH of the dispersion (Singh, 2004). The addition of β-LG to SPFCM dispersions introduces a maxima and minima in the heat stability–pH curve. Therefore, depending on serum protein removal during the manufacture of MCC, MCC dispersions may have different heat stability pH profiles. In a study by de Kort et al. (2012), MCC 9% protein dispersion (from MCC85 protein powders and 95:5 casein-to-whey protein ratio), heat stability increased from pH 6.7 to 7.3 (from 2 to 55 min) mimicking profiles of SPFCM dispersions. A decrease in calcium ion activity and an increase in hydration and zeta potential of the caseins are postulated to explain the increase in heat stability. The authors also reported an increase in the heat stability of MCC dispersions at pH 6.7 and 7.0, with insignificant increases at pH 7.3 on the addition of calcium chelating salts (sodium phytate, trisodium citrate, and disodium hydrogen phosphate, Na_2HPO_4). However, the addition of sodium hexametaphosphate decreased the heat stability of MCC dispersions compared with dispersions containing sodium phytate, trisodium citrate, or Na_2HPO_4 salts. Table 6 summarizes the various interventions to enhance the heat stability of dairy protein ingredients and beverages.

Processing of Low-Acid Shelf-Stable Dairy Beverages: Batching The batching process involves the mixing of ingredients used in the manufacture of dairy protein beverages. Some protein ingredients are available both in the dry and liquid form. Milk protein concentrates, MPI, and MCC are available in both liquid and dried form. Figure 3 is a typical process flow for the manufacture of shelf-stable dairy beverages using liquid dairy ingredients (Ur-Rehman et al., 2016). The addition of dry ingredients (e.g., sugar, salts, cocoa) into the liquid protein concentrate takes place in a high-shear mixer. The batch is standardized for target measures (protein, fat, solids) followed by thermal processing and aseptic filling.

Figure 4 represents the batching process that involves dry dairy ingredients. Dried dairy protein ingredients are mixed with the aqueous phase in a high-shear mixer to which the rest of the ingredients are added (e.g., fat source, sugar, flavors, cocoa). The dried ingredients must be dispersed rapidly in the aqueous medium to prevent quality defects in the finished beverage (Mimouni et al., 2009, 2010; Fang et al., 2011).

Processing of Low-Acid Shelf-Stable Dairy Beverages: Thermal Processing. Regulations for manufacturing low-acid canned foods in the United States are specified in 21CFR 113 (FDA, 2016b). Commercial processors that manufacture low-acid canned foods need to register with the FDA the processing methods, among other details (this includes persons

Table 6. Different processing interventions for enhancing heat stability of high-protein powders

Modification	Results	Reference
Use of calcium chelators disodium uridine monophosphate, disodium hydrogen phosphate, trisodium citrate, sodium phytate, sodium hexametaphosphate on heat stability of micellar casein isolates (MCI; 9% protein dispersion)	Increased the heat stability of MCI. These chelators led to the binding of mineral ions and solubilized colloidal calcium phosphate and enhanced stability.	de Kort et al., 2012
Addition of chelating salts (citrate, phosphate)	Improved heat stability of micellar casein concentrates at 15 mEq/L.	Renhe et al., 2018
Addition of chelating salts (trisodium citrate, tripotassium citrate, and disodium hydrogen phosphate)	Enhanced heat stability and decreased fouling in heat exchangers.	Hebishy et al., 2019
Addition of hydrogen peroxide to whey	Enhanced heat stability because of oxidation of sulfhydryl groups.	Sutariya and Patel, 2017
Conjugation of whey protein concentrates (WPC) with chitosan	Higher heat stability recorded for complexes at higher pH (5.5) and medium chitosan to whey protein ratio (1:5).	Zhao and Xiao, 2017
Microparticulated WPC	Higher thermal stability due to reduced free thiol groups during microparticulation process.	Çakır-Fuller, 2015
Addition of milk protein concentrates with WPC for casein-to-whey protein ratios: 5–95:30–70	Improved thermal stability of whey protein due to chaperone-like activity of caseins.	Liyanaarachchi et al., 2015
Conjugation with polysaccharides	Improved thermal stability by formation of whey protein and polysaccharide complexes via electrostatic interaction between negatively charged polysaccharides and positively charged functional groups on the surface of the proteins.	Turgeon et al., 2007; Smulders and Somers, 2012
Complex of high methoxyl pectin and whey proteins	Improved thermal stability of whey proteins.	Wagoner and Foegeding, 2017

engaged in the manufacture of low-acid foods for consumer testing; 21CFR 113, FDA, 2016b). The beverages are rendered commercially sterile by the application of heat. Commercial sterility of the dairy protein beverage is achieved by applying heat that renders a product free of microorganisms capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution and viable microorganisms (including spores) of public health significance (21CFR 113; FDA, 2016b). The thermal process is determined by a thermal processing authority, who determines the time–temperature of the thermal treatment by considering several different factors, including ingredients in the product formula and the equipment used for the process (Dunkley and Stevenson, 1987). Commercial sterility of equipment used for aseptic processing and packaging should also be ensured by appropriate means, as mentioned in 21CFR 113 (FDA, 2016b). Ultrapasteurization is a thermal process (138°C for 2 s) that produces a product with an extended shelf life under refrigerated conditions (21CFR 131.3; FDA, 2016c).

Ultra-high temperature and retort sterilization are 2 commonly used thermal processing methods to ensure commercial sterility in low-acid dairy protein beverages. These processes must ensure an F_0 value (time in minutes of heat treatment) to ensure a minimum 12-decimal (**12D**) reduction of *Clostridium botulinum* in the beverage (Hotrum et al., 2010). The F value of a process is defined as the processing time at a particular temperature to reduce a target microbial population to a specific level. Therefore, F_0 denotes the F value at a temperature of 121.1°C (250°F) and a z-value (temperature change required for 1 log reduction in D) of 10°C (18°F; Kumar and Sandeep, 2014). In low-acid foods, a minimum thermal process equivalent to F_0 of 3 (121.1°C for 3 min) is required for the 12D reduction of *C. botulinum*. To reduce thermophilic spore-forming spoilage bacteria in low-acid beverages in tropical markets, an $F_0 > 10$ is recommended (Stannard, 1997). In the United States, processors heat the milk to at least 138°C (280°F) for 6.5 s (F_0 of 5.07 min; Dunkley and Stevenson, 1987; Toledo et al., 2018). In retort processing, heating at 120°C for 10 min equals an F_0 of 7.76. An alternative index, B* (based on a reference temperature of 135°C and z-value of 10.5°C), is also used (Deeth, 2021). A B* of 1 corresponds to 135°C for 10.1 s and equates to a 9-log reduction of thermophilic spores (Kessler and Horak, 1981). Another measure, C*, is used to outline chemical changes during UHT and retort processes; C* is equal to 1 when there is a 3% reduction in the vitamin thiamine (corresponds to 135°C for 30.5 s) (Kessler and Horak, 1981). In a typical UHT processing plant, an F_0 of 4 equals B* of < 1 and C* < 1. In a typical retort processing (120°C for 10 min.), an F_0 of 7.76 is equivalent to a B* of 2.21 and a C* of 6.55 (Deeth, 2021). Therefore, for an equivalent F_0 value, retort processes result in significantly more chemical changes than a UHT process.

Ultra-high temperature processing systems can be classified into indirect heating and direct heating systems. In indirect systems, heat is transmitted from the heating medium to the product through a thermally

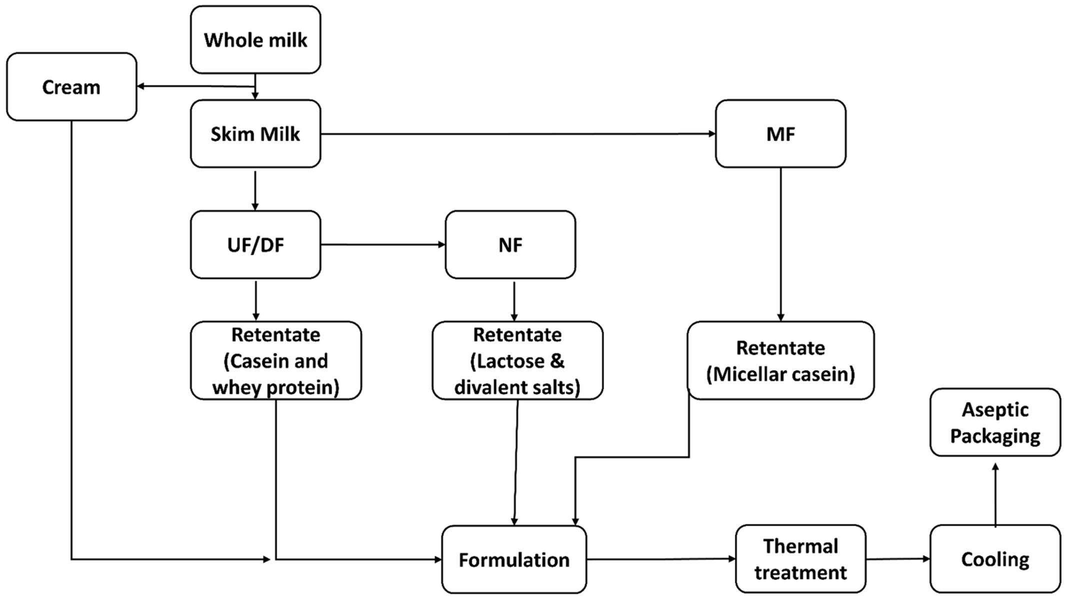

Figure 3. Process used in preparation of high-protein dairy beverages using liquid milk protein ingredients (Ur-Rehman et al., 2016). UF/DF = ultrafiltration/diafiltration; MF = microfiltration; NF = nanofiltration.

conducting but impermeable barrier such as stainless steel (tubular, plate, or scraped surface). In direct heating, instantaneous heating of the product occurs by either injecting steam into milk (injection) or milk into steam (infusion; Lewis and Heppell, 2000). Due to quicker heating and cooling rates, direct heating places a lower thermal load on the beverage, thus minimizing thermally induced chemical changes in the final product (Kelleher et al., 2018). The package is sterilized separately via chemical treatment in aseptic filling, and then filled with commercially sterile beverages in a sterile environment. The retort process (110–135°C/2–60 min) is thermally intense compared with the UHT process. Beverages for processing are filled in packages, followed by sealing, heating, and holding at the desired temperature (heating medium is steam or water). Retort can be a batch or a continuous process. Two continuous machines in the commercial settings are the hydrostatic vertical sterilizer and the horizontal sterilizer (Bylund, 1995).

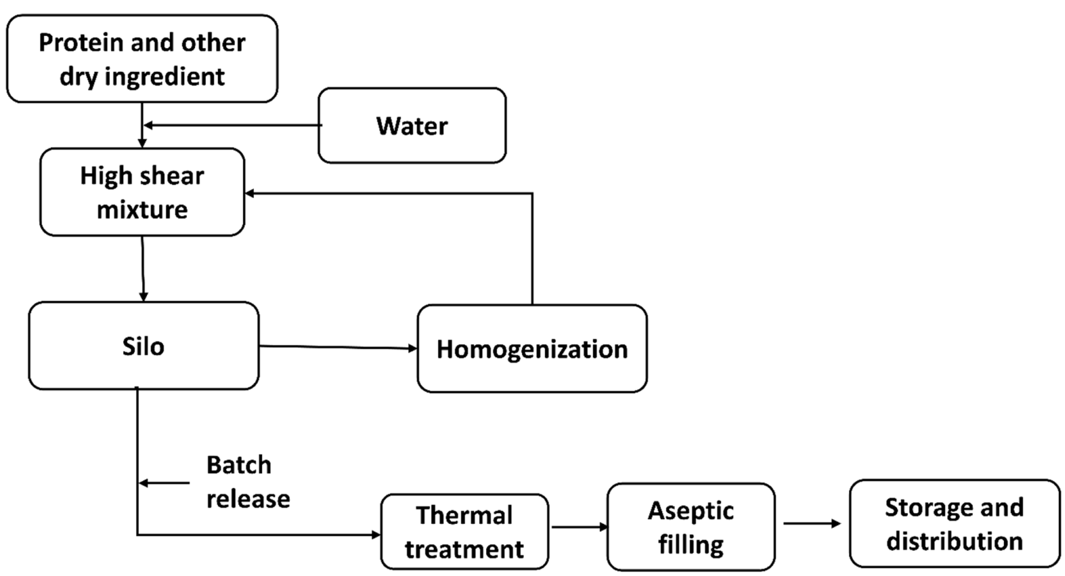

Figure 4. Process used in preparation of high-protein dairy beverages using dried milk protein ingredients.

Storage Stability and Defects of Low-Acid Shelf-Stable Dairy Beverages

One of the major quality challenges in high-protein low-acid dairy beverages is the colloidal stability of the beverages. These colloidal particles are inherently unstable and undergo phase separation and settling due to aggregation during processing and storage. Therefore, ingredient stability during processing and storage is the major area of interest in high-protein low-acid beverages.

Physical Stability of High-Protein Dairy Beverages

Physical stability of beverages is defined as the state where physical properties and appearance of beverages remain acceptable. The physical stability of protein beverages is directly related to the colloidal stability of protein (Ryan and Foegeding, 2015). Physical stability has a major influence on beverage acceptability, mouthfeel, residual perception, and overall appearance, and is represented by sedimentation and gelation. Sedimentation is explained by phase separation, where the particles fall out of the dispersed phase and settle at the bottom. Sedimentation of proteins increases with an increase in heating time and temperature, and protein aggregation is one of the reasons. During storage, aggregation occurs between protein particles due to noncovalent interactions (electrostatic, van der Waals, hydrogen, and hydrophobic bonding) that will enhance the separation rate due to an increase in particle size with time (Mahler et al., 2009). These aggregates may be soluble or insoluble. Soluble aggregates remain dispersed and insoluble aggregates lead to precipitation. Soluble aggregates are hypothesized to be responsible for chalkiness in high-protein beverage systems, although additional research is needed to validate the hypothesis. The aggregation process depends on factors such as the type and concentration of protein, temperature of processing and storage, pH, and salts (Ryan and Foegeding, 2015; Leeb et al., 2018). Increasing the heat stability of dairy protein ingredients can be an effective strategy to control the aggregation and sedimentation of dairy proteins during processing and storage.

Age gelation of high-protein beverages is another important quality defect observed. In age gelation, a 3-dimensional network of protein forms during storage. The age gelation process occurs in 4 stages (Datta and Deeth, 2001). A rapid first stage of product thinning is followed by a more extended second stage of product thinning with a slight viscosity shift. The third stage is characterized by an increase in viscosity due to gelation. The fourth stage is marked by a decrease in viscosity due to gel breakdown, resulting in syneresis. The high-protein concentration in beverages leads to accelerated age gelation (Nieuwenhuijse and van Boekel, 2003). Ionic calcium, pH, beverage composition, and plasmin activity are all factors that affect casein micelle stability during storage (Datta and Deeth, 2001; Anema, 2017). To stabilize casein micelles, chelating agents (e.g., phosphates) are added in lower concentrations (Pyne, 1958). Higher concentrations of chelating agents can chelate calcium beyond the critical limit, causing casein micelle dissociation (Augustin and Clarke, 1990; Singh, 2004). As novel dairy protein ingredients are being used in the manufacture of high-protein beverages, further studies need to evaluate the influence of these ingredients on age gelation.

Indigenous enzymes in milk such as the plasmin system can also cause age gelation in UHT milk. Filtration of milk will retain plasmin (~48 kDa), plasminogen (~88 kDa), plasminogen activator inhibitor (55 kDa), and plasmin inhibitor (~60 kDa) in the retentate (Puri et al., 2020). The influence of plasmin systems in cheese containing filtration-derived milk ingredients is well documented. Proteolysis from the plasmin system in cheese manufactured from milk containing UF milk is lower compared with that in control cheese (from milk without UF milk), which is attributed to concentration of the plasmin inhibitor system (Benfeldt, 2006). Similarly, when protein ingredients obtained by filtration are used in the manufacture of high-protein beverages, the concentration of the plasmin system is expected. However, there are limited studies on the influence of the concentrated plasmin system in high-protein dairy beverages and especially shelf-stable dairy beverages because of the high heat treatment involved that could alter the inhibitor activator balance and either enhance or slow the occurrence of age gelation. The heating of the protein beverages also affects the z-values of plasmin and plasminogen. At heating temperatures below 90°C, the z-value of plasmin and plasminogen was ~8.5°C, which increased to 80°C at temperatures above 100°C (Saint Denis et al., 2001). This could have implications for high-protein dairy beverages that contain membrane-filtered dairy ingredients and are subjected to high heat temperatures.

HIGH-ACID/ACIDIFIED SHELF-STABLE DAIRY BEVERAGES

Dairy Protein Ingredients

Membrane Filtration and Ion Exchange-Derived Ingredients. Whey protein-rich ingredients used in the preparation of high-acid beverages are fractionated from either sweet or acid whey and microfiltration of skim milk. The main components of sweet or acid whey are whey proteins (β-LG and α-LA), lactose, and minerals. However, the action of rennet during cheese production hydrolyzes κ-CN into paracaseinate and GMP, where the paracaseinate is retained with cheese curd and GMP is partitioned into cheese whey. Glycomacropeptide is concentrated during the UF of cheese whey, and 20% of the proteins in cheese whey are GMP. Depending upon the concentration of whey proteins, WPC have protein content from 34 to 80%. Whey protein can be purified by additional DF. With this further concentration, WPI can have a protein content of >90%. Native whey proteins are obtained by microfiltration of skim milk. The retentate is micellar casein, and the permeate contains whey proteins. Both UF and DF can concentrate the permeate. As filtration of skim milk is the source of native whey proteins, it is free from the residues of cheesemaking that include GMP, starter culture residues, enzymes, colorants, or bleaching agents (Kang et al., 2010; Campbell and Drake, 2013). Milk whey protein concentrate (**mWPC**) and isolate (**mWPI**) can have different β-CN levels, depending on the temperature of microfiltration of skim milk (Coppola et al., 2014). During microfiltration at lower temperatures, a greater amount of β-CN will partition into the permeate and could influence the mWPC or mWPI ingredient functionality when used in high-acid beverages. Based on the protein content, they are classified as mWPC and mWPI by the American Dairy Product Institute (ADPI, 2018). The composition of WPC, WPI, mWPC, and mWPI are given in Table 3.

Acidulants in the Manufacture of Acidified Shelf-Stable Beverages. Different acidulants can be added to beverages to decrease the pH before heating. Common acidulants added to acidified high-protein beverages are the organic citric, malic, and lactic acids and the inorganic phosphoric acid. Apart from lowering the pH of foods, these acidulants impart sourness, bitterness, astringency, and distinctive flavor profiles. Of the 4 acids listed above, phosphoric acid has the highest ionization constant (7.1×10^{-3}). The ionization constant represents the extent to which hydrogen ions are produced when added to water (Gardner, 1966). Citric acid and malic acid can be added to certain fruit-flavored beverages, and lactic acid is known for its mild acid taste (Holten et al., 1971). Phosphoric acid, aside from being an acidulant, can serve as a buffer and metal chelator. It is hypothesized that at equimolar concentration, the intensity of acid taste is correlated with the first dissociation constants of these acids (Furukawa et al., 1969). The pH values of 0.0025 M citric, malic, lactic, and phosphoric acid are 3.08, 3.1, 3.33, and 2.81, respectively. For similar sourness intensity of the above acids in water, only half the amount of phosphoric acid is needed (Rubico, 1993). Astringency, a sensory defect related to mouth drying and puckering, can be caused by different acids used. A strong negative correlation is observed between the pH and astringency of acids (Rubico and McDaniel, 1992). It is always desirable to use a blend of acidulants that provide optimum flavor, pH of the beverage, and acidity for acidification. In high-protein acidic beverages, as the load of the protein in the beverages increases, the buffering of the system will increase, leading to an increase in the concentration of acid to achieve the target pH. Therefore, acidulant blends with phosphoric acid and organic acids (citric, lactic, or malic) may provide optimum tools for pH reduction while managing sourness and acidity in the final acidified beverage.

Heat Stability of Whey Proteins and Thermal Processing of High-Acid Shelf-Stable Beverages. Whey proteins denature when exposed to heat, but the denatured whey proteins may not aggregate at the low pH that is used for the manufacture of acidified foods. For whey proteins, denaturation on heating may not always translate into precipitation (Jelen, 1989). The denaturation and subsequent precipitation (caused by aggregation) of whey proteins is influenced by several factors, the primary one being pH. For whey proteins, the decrease in clarity of heated solutions depends on the pH during heating. In whey protein solutions heated at 95°C for 5 min, the increase in turbidity or absorbance index occurred when the pH of heating increased beyond pH 3.9 (Bernal and Jelen, 1985). Therefore, for the development of high-acid shelf-stable beverages with whey protein, the beverage pH must be below 3.9, preferably 3.5 to 3.7, to produce a beverage with high clarity (Figure 5).

The denaturation temperatures of β-LG dispersions also increased as the pH of preheating decreased. In WPC dispersions, the denaturation temperature was higher than that of pure β-LG or α-LA dispersions at all pH values of preheating, although it followed a similar trend to pure β-LG dispersions (denaturation temperature increasing with decrease in pH of preheating). Although the denaturation temperatures are lower (~88°C) at pH 3.5 for WPC dispersions (Bernal and Jelen, 1985) than the processing temperatures for acidified shelf-stable beverages (~90°C for 40 s; von Bockelmann and von Bockelmann, 1998), there will be minimal precipitation of the whey proteins that will result in a loss of clarity. Removal of calcium from whey protein dispersions tends to increase the turbidity of whey proteins at lower pH values, indicating a calcium-mediated stabilizing effect against whey protein aggregation on heating at low pH (Patocka and Jelen, 1987; Patocka et al., 1987).

Acidified Whey Protein Ingredients. Whey protein isolate products that have been acidified before

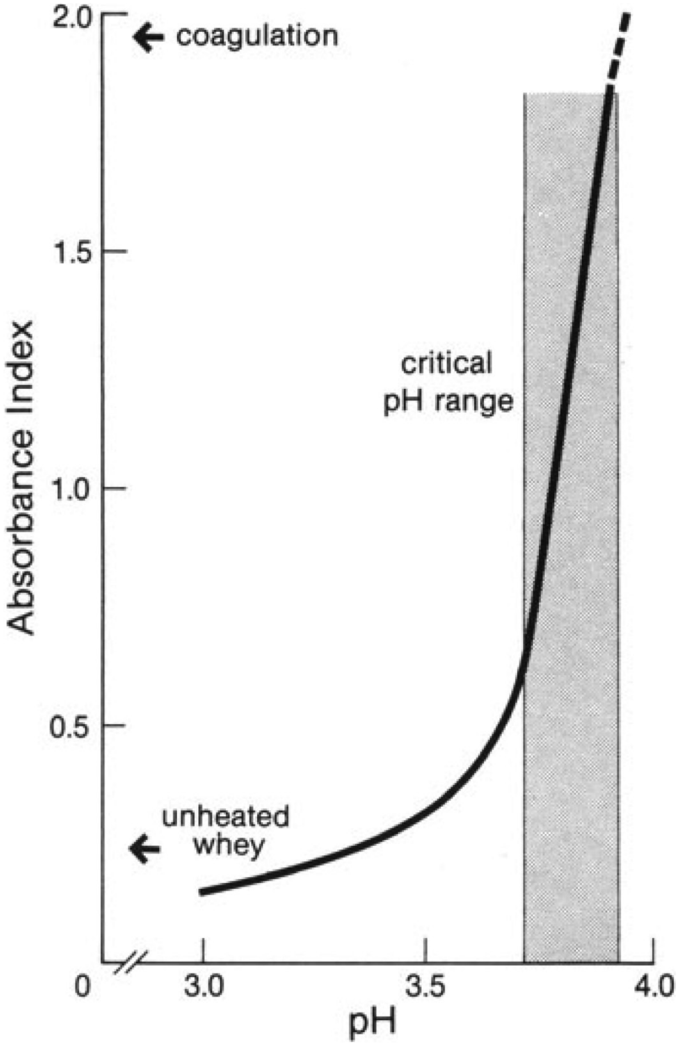

Figure 5. Effect of pH on turbidity of whey heated to 92°C for 15 min (Jelen, 1989).

drying are commercially available and ready to use in beverages. These ingredients offer a superior taste profile and improved thermal stability and translucency in beverages. Park et al. (2014) found that acidifying liquid whey to pH 3.5 before spray drying reduced off-taste due to a reduction in protein interactions with volatile compounds at low pH in liquid whey. Using WPC that has been preacidified for acidified beverage applications improves the flavor of acidic beverages.

Storage Stability and Defects of High-Acid Shelf-Stable Dairy Beverages. Unlike low-acid high-protein beverages containing colloidal proteins (casein), high-acid high-protein beverages are formulated with soluble whey proteins. These high-acid beverages mostly show soluble phase aggregates, leading to cloudiness. At pH levels <3.5, whey proteins are stable, but astringency of whey proteins is a concern in this pH range (Beecher et al., 2008). Another common defect observed in translucent whey protein shelf-stable beverages is the loss of product clarity during shelf life. Storage-induced aggregation (**SIA**) is reported as "floating threads" or "cotton-like clumps" in translucent whey protein beverages (Villumsen et al., 2015). These aggregates increase with storage time and temperature (LaClair and Etzel, 2009). Villumsen et al. (2015) found that increasing the heat treatment of high-acid whey beverages (70 g of protein per L from WPI dispersions adjusted to pH 3.0) yielded beverages with lower SIA. Electrostatic interactions between GMP (negatively charged sialic acid) and enzymatically induced peptides may be responsible for SIA. Higher heat treatment could also alter glycosylation of GMP (less glycosylation) and could contribute to lower SIA. A higher concentration of divalent ions could also help protect against SIA due to a charge screening effect, thereby reducing electrostatically induced interactions leading to SIA (Villumsen et al., 2015). However, no studies have been reported on the effect of mWPI dispersions on SIA.

Astringency in High-Protein High-Acid Beverages. In addition to the clarity of protein beverages, taste and flavor are important drivers of consumer acceptance. However, in acidified whey protein beverages, astringency is a taste concern (Beecher et al., 2008). Astringency is a sensory perception that involves the drying and perceived roughness of the tongue, loss of lubrication in the mouth and oral cavity surfaces, with the additional perception of tightening, drawing in, or puckering of the oral mucosa and muscles around the mouth (Upadhyay et al., 2016; Carter et al., 2020). Astringency can be measured by direct (e.g., descriptive analysis, consumer testing) and indirect methods (e.g., taste sensor, rate of saliva flow, and other in vitro analysis techniques). However, a well-trained descriptive panel is considered to be the gold standard in measuring astringency (Carter et al., 2020). Sano et al. (2005) compared whey protein for astringency using descriptive analysis at neutral pH and pH 3.5 and found that whey proteins are more astringent at the lower pH. Astringency was also positively correlated with the concentration of the whey proteins. Lee and Vickers (2008) studied whey protein solutions at low pH for astringency using descriptive analysis and compared these to acid solutions with similar pH and acidity. They reported that astringency is caused by high acidity in whey protein beverages and not by proteins per se. However, Carter et al. (2020), in their extensive review on astringency in whey protein beverages, proposed that astringency in whey protein bever

Table 7. Analytical techniques related to processing and storage of high-protein dairy beverages

Target study	Method used	Reference
Measurement of rehydration characteristics of milk protein ingredients	**Change in turbidity:** Turbidity is an optical phenomenon that measures loss of intensity of transmitted light due to scattering effect of particles. In turbidity meter, light in spectrophotometer (600 nm) or near-infrared region (860 nm) is used, and the reflected light (180°) is measured through a detector.	Gaiani et al., 2005, 2007, 2009; Schuck et al., 2007; Hussain et al., 2011a; Zhao and Xiao, 2017
	Change in particle size: Particle size is measured by light scattering; larger particles scatter light at small angles, and smaller particles scatter at large angles. At the beginning of rehydration, an increase in particle size is observed that decreases significantly over time. The rehydration of milk protein ingredients can also be measured using microscopy.	Gaiani et al., 2005, 2006, 2009; Hussain et al., 2011b; Mimouni et al., 2009; Richard et al., 2013
	Change in structure: Structure is measured using microscopy. Out of 5 different stages of rehydration, the swelling stage can be clearly seen in microscopy images.	HadjSadok et al., 2008; Mimouni et al., 2009; Richard et al., 2013; Burgain et al., 2016
	Solubility of protein: As a standard method after dissolving, the suspension is subjected to centrifugation, and solubility is calculated on the basis of total solids in ingredients.	Crowley et al., 2016
Measurement of heat stability of milk protein ingredients	**Heat coagulation time (HCT):** Heat stability of protein suspensions is measured as HCT determined at 140°C in an oil bath. HCT is defined as the time that elapses between placing samples in the oil bath and the first visible onset of coagulation when samples are rocked in oil bath.	Crowley et al., 2014
Measurement of physical destabilization in beverage during storage	**Change in viscosity and turbidity:** Changes to viscosity and turbidity (in high-acid translucent beverages) can be used to examine the shelf stability of beverages.	Temelli et al., 2004; LaClair and Etzel, 2009; Villumsen et al., 2015; Wagoner et al., 2015
	Sedimentation: During storage, physical instability such as phase separation and aggregation of proteins leads to sedimentation. The analysis of sediment by gel electrophoresis can be applied to elucidate mechanisms involved in destabilization.	Villumsen et al., 2015; Wagoner et al., 2015; Le et al., 2016; Anema, 2017; Gaur et al., 2018
Advanced analytical techniques	**Focused beam reflectance measurement (FBRM):** FBRM is applied to monitor rehydration behavior of milk protein ingredients. It measures the distribution of particle size and kinetics of particles in suspension.	Mitchell et al., 2020; Hauser and Amamcharla, 2016
	Electric resistance tomography (ERT): ERT is useful to observe rehydration behavior and storage-induced changes.	Babu and Amamcharla, 2021
	Proteomics analysis: Liquid chromatography (LC)-tandem MS is used to characterize storage-induced aggregates in acidic whey protein isolate drinks (7% protein). The protein profiles obtained by LC-MS were different in fresh and stored high-protein beverages due to different levels of proteins in different samples.	Le et al., 2016
	Fourier-transform infrared (FTIR): FTIR is generally applied to predict solubility and to study aging and denaturation in high-protein beverages.	Ozen et al., 2003; Kher et al., 2007; Sikand et al., 2011
	Atomic force microscopy (AFM): AFM is useful to characterize surface properties of powder and related changes in solubility of milk protein concentrates.	Murayama et al., 2021
	Nuclear magnetic resonance (NMR): NMR can be used to study casein dephosphorylation, which can determine changes in casein structure due to the presence of stabilizing salt as well as storage-induced changes in casein structure. It is also used to study rehydration of casein, caseinates, and milk powders.	Power et al., 2019
	Turbiscan: Turbiscan is useful to evaluate the physical stability of beverages. The results are expressed as the Turbiscan stability index (TSI).	Iturmendi et al., 2020
	Descriptive sensory analysis: This test is performed for evaluation of astringency, where panelists are trained to evaluate astringency using the spectrum intensity scale and tannic acid.	Drake et al., 2003; Sano et al., 2005; Beecher et al., 2008; Cheng et al., 2019; Carter et al., 2020
	Instrumental methods such as Taste Sensor (Taste Sensing System, SA402B; Intelligent Sensor Technology).	Sano et al., 2005

ages is from multiple sources including protein type, concentration, pH, acidity, and acidulant type. Several strategies exist to control astringency in whey protein beverages, and these are extensively reviewed by Carter et al. (2020).

Analytical Techniques Related to Processing and Storage of High-Protein Dairy Beverages

Analytical methods to monitor processing and storage-induced changes in high-protein beverages are shown in Table 7. The analytical methods used to observe changes during preparation and storage of high-protein beverages can be broadly categorized into 2 categories: methods to measure rehydration characteristics and heat stability of milk protein ingredients and methods to monitor physical destabilization of beverages during storage. Advanced analytical techniques like Fourier-transform infrared spectroscopy, spectrofluorometry, focused beam reflectance measurement (FBRM), and HPLC are also applied as tools to measure the physical stability of beverages during storage.

CONCLUSIONS

High-protein shelf-stable dairy beverages are categorized as high-acid/acidified (pH <4.6) and low-acid (pH >4.6) beverages. To achieve ambient temperature shelf stability, the heat treatment for low and high acid beverages will be different. The choice of dairy protein ingredient to deliver protein claims is influenced by many factors that include the final pH of the system, the heat stability of the protein ingredient, and the form factor of the beverage (cloudy or translucent). Casein-forward ingredients like MPC and MCC are the preferred choice of protein ingredients in low-acid beverages. Whey protein-forward ingredients like WPC and WPI are the ingredients of choice in high-acid beverages because of their resistance to denaturation and sedimentation at low pH values. A significant amount of research on shelf-stable dairy milk is available, but research in shelf-stable high-protein dairy beverages is still evolving. Most of the dairy protein ingredients used in the manufacture of high-protein dairy beverages are derived from membrane filtration. The physicochemical properties of these ingredients will differ from those of well-characterized dairy ingredients like skim milk powder and nonfat dry milk. The type of filtration process (UF to derive MPC and microfiltration to derive MCC) and the extent of partitioning (for protein concentration) will also influence the physicochemical properties. For whey protein-forward ingredients, the source of whey proteins (cheese whey or native whey) may influence the physicochemical properties, and so could the type of separation or partition process used in its purification and concentration. As the demand for these high-protein shelf-stable beverages is increasing, novel and rapid methods to characterize these ingredients for use in both high-acid and low-acid beverages should be developed. Additional research should focus on understanding molecular-level interactions to deliver a winning product in the marketplace.

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