MILK PROTEINS: AN OVERVIEW

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Abstract— Processing milk into dairy products leaves a large portion of milk protein, which can be used in many ways. Milk protein is composed of casein and whey protein. Casein is predominate bovine milk protein, which forms large colloidal particles and present in the form of casein micelles. Whey is liquid by products in chess manufacturing or liquid obtained from remove fat and casein from milk. Generally, protein, especially milk protein, has multiple functions in food, such as emulsification, foam formation and stability. Condensed milk protein is a high quality protein that is found naturally in the milk. These milk powders provide powerful and nutritious multifunctional nutrients for the global food and beverage industry due to their high protein content. Milk Protein also consider high quality protein and provide various nutritional benefits, which should be included into the diets. The objective of this review is to overview of milk protein and milk protein concentrates in terms of production and various application in food.

Index Terms— ***Casein, Milk Protein Concetrate, Sodium Caseinate, Whey Protein, WPI (Whey Protein Isolate), WPC (Whey Protein Concentrate)***

I. INTRODUCTION

Since 1998, India is the largest milk producer in the world. Milk production in india was approximately 187.7 million tons in 2018-19. The number of grams of milk per capita is 394 grams per day [4]. The milk is the first food of the mammals. The need for energy and nutrients of newborns for normal growth and development is supplied by milk. Milk is a complex biological liquid with large amounts of protein, minerals and lipids secreted by mammals, which can provide nutrition and provide immune protection for newborns. The main role of milk is to deliver essential amino acids and minerals. These amino acids and minerals are essential for the development of newborn mammals, and therefore for the function of muscles and other tissues [9]. Milk can be included in functional foods because it contains a lot of biologically active compounds [10]. The milk content moisture about 85-90%, followed by lactose (carbohydrate), proteins, minerals and other micronutrients, such as vitamins, enzymes, and other nitrogen-containing non-protein substances. The presence of carbohydrates in milk is called lactose, which is a disaccharide unit composed of two sugars (glucose and galactose). Galactose is responsible for the growth of the brain and nervous system. Except casein and milk fat these all component also available in whey [2],[7].
Recently, the consumption of A2 milk can prevent various diseases such as heart disease, diabetes and a few other diseases. According to the National Bureau of Animal Genetic Resources (Kamal, Haryana, India), Indian dairy breeds (i.e Gir, Tharparkar, Rathi, Red Sindhi, Sahiwal, Kankrej and Hariana) contain A2 β-casein. There is about 3.3% to 3.5% protein in milk. Milk protein is an important food for the human body because it contains all 9 essential amino acids [6, 18]. Eating milk protein can prevent chronic diseases such as diabetes, muscle loss, sarcopenia, atherosclerosis, high blood pressure, risk of cardiovascular disease, osteoporosis and other chronic diseases. Total milk protein is divided into two categories: casein and whey protein. Approximately milk protein consists of 82 parts casein and 18 parts whey protein. Whey protein is more soluble than casein, and the quality of whey protein is also high [2] , [11]. In case of protein quality, Protein Digestibility Corrected Amino Acid Score (PDCAAS) for both whey and casein are 1.00 and Biological value for whey and casein are 100 and 80 respectively [14].

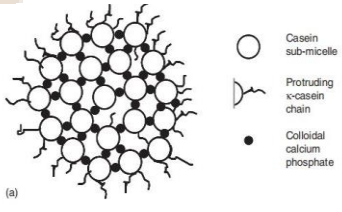
II. CASEIN

The main protein found in milk is casein, especially in cow's milk. Due to casein, milk is white and opaque appearance. This protein is combined with calcium and phosphorus as clusters of casein molecules, called micelles / calcium phospho-caseinate. Casein protein consists of different proteins, α-casein, β-casein and κ-casein [5] , [17].

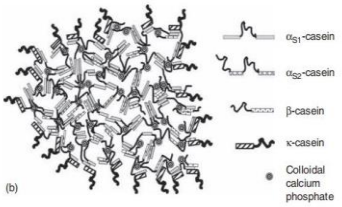
Except for κ-casein, all caseins are phosphoproteins, mainly serine and threonine. α-casein is the main casein, which contains 8-10 phosphate groups and is difficult to dissolve in water. β-casein contains 5 phosphate residues, which is more hydrophobic than the other two residues and hardly soluble in water. Kappa-casein is a glycoprotein containing negatively charged carbohydrate moieties. Since, α-casein and β-casein are highly phosphorylated, they are very sensitive to the concentration of calcium salts, that is, they will precipitate with excess Ca2+ ions. In milk, κ-casein combines with α-casein and β-casein to form micelles, so it will not precipitate in the presence of calcium ions [6] , [3].

Figure 1 The structure of casein micelle

The sub-micelle model of the casein micelle [18]



The dual binding model of the casein micelle [16]



A. *Casein Manufacturing Process*

The fat separation from milk with requires maximum efficiency. Milk is filtered and warmed to 40–45˚C, separated in a hermetic cream separator. Here, fat present in the skim milk is reduced to < 0.05%. There are two way to coagulate of casein i.e. enzymatic coagulation of casein and acid precipitation casein. In enzymatic coagulation of casein, coagulation does not affect the pH and depends on the added enzyme chymosin/rennin that cleaves glycomacropeptide (highly charged portion from the κ-casein). On the other hand, acid precipitation casein, alter the pH using hydrochloric acid and lactic acid. Both acid coagulation will give the edible casein and used one of them. In case of lactic acid casein, cool the pasteurized skim milk to 22–26 ˚C and inoculated with a 0.5% starter of mixed lactic starters and incubated for 14–16 hrs, pH decrease to 4.6 and coagulum will be produced.

Then, cooked the coagulum to 50-55˚C to form a curd, which is firmly processed for final processing. Acid and heat contribute to the syneresis of whey. For hydrochloric acid curd, the temperature of skimmed milk is maintained at about 35°C. The pH 4.1 is adjusted by adding dilute acid and continuous stirring to produce a granular curd that is easy to drain and wash. After settling, the curd coagulates and the whey quickly leaves contact with the curd. If it is in contact with whey for a long time, it will be difficult to wash away acid, salt, whey protein and lactose.

The washing of casein curd is most important step, because it is directly related to casein quality. Three separate washings of the casein curd are required, with each contact time of 15-20 minutes. For the first two washes, the final wash water temperature is set to 32-40˚C and the pH is set to 4.6 to avoid the formation of a gel-like layer on the curd particles in acidic water, then the curd is softened and dispersed in alkaline water. The third wash was performed with neutral waterBy pressing, the particles obtain a uniform size and a larger surface for drying. Uneven drying will form clumps, which will dry on the outside, and the hard outer surface will prevent the diffusion of moisture from the inside.

Place the ground curd on a tray in drier and spread it evenly. The curd on the tray is less than 0.9-1.1 kg (75 cm x 75 cm). The bottom tray has a fine screen or covered with a cloth to capture the fine particles sieved from the upper tray. The temperature of the air entering the dryer is set to 52–57˚C. Drying is done till final moisture content is 8%. Tempered casein can be kept for 24 hours to effectively cool, harden, and evenly distribute the moisture in the entire batch. The most effective tempering is to circulate the dried casein through a pneumatic conveyor. The casein is cooled before grinding, because the warm casein is plastic and will cause the roller to "burn". Use a roller mill, pin mill or hammer mill for grinding. For producing 60 mesh and 80 mesh casein, a pin mill is better than a hammer mill. After the grinding operation, it is sieved into different mesh sizes and then bagged [17].

B. *Sodium Caseinate Manufacturing Process*

Sodium hydroxide is the most commonly used base for production dry sodium caseinate. Mix it with a slurry of casein curd or powder in an aqueous suspension (2.5 M aqueous solution). The amount of sodium hydroxide required is 2% (w/w) of casein solids. Casein curds are ground using a colloid mill to reduce the particle size so that they can be dissolved to a certain extent, and then mixed with alkali through high shear. In order to obtain a spray-dried solution of sodium caseinate, the highest possible solid concentration is essential for the cheapest possible production because of the large amount of water to evaporate and the high energy cost. Concentrated solutions of sodium caseinate (> 15% solids) have a higher viscosity and require high-watt stirrers and pumps for mixing and fluid transfer. Then, since the viscosity of the sodium caseinate solution decreases as the temperature decreases, high temperatures (60-95˚C) are used throughout the dissolution stage [5].

C. *Application of casein and casein product in food*

Table 1 Different application of casein in food [5]

Food category	Use level	Casein product	Function
Ice cream	1-5%	Sodium caseinate	Enhancing viscosity, emulsion stabilizer
Cheese products	2-25%	Rennet casein, acid casein, caseinates	Fat and water holding, texture, matrix formation
Confectionery	1-25%	Caseinates (whole and hydrolyzed)	Mouthfeel
Infant foods	1-25%	Whole or hydrolyzed caseinate	Increase nutrients content
Baked products	1-25%	Casein, caseinates	Provides nutrients and moisture retaining capacity
Meat products	3-20%	Sodium caseinate	Provides nutrients, fat emulsifier, moisture retention, tenderness
Sports drinks	2-10%	Sodium caseinate	Nutrients content enhancement
Whipped toppings	5-10%	Sodium caseinate	Interfacial film formation, emulsion stability, bulking agent
Pasta and snacks	5-20%	Casein, caseinates	Provide nutrients , chewine

III. WHEY PROTEIN

The by-product whey is obtained from cheese and casein manufacturing containing serum proteins called as whey proteins[1]. It is more beneficial as it doesn't contain any antinutrients as that of soy proteins[13]. It consists of α-lactalbumin, β-lactalbumin lactoglobulin, glycomacropeptides, IgG, bovine serum albumin and lactoferrin (minor protein) in the proportions 11.3–16.5%, 37.9–49%, 15–20%, 5.0–8.0%, 3.0 –5.0% and 1.3–1.8%, respectively. Other Minor Protein present in whey: lactollin, glycoproteins, lactoperoxidase and transferrin. In addition, whey protein is rich in amino acids, less carbohydrates, and less fat. It contains cysteine which can make glutathione [11].

A. *Whey protein concentrate*

Ultrafiltration technology used to obtained WPCs. Ultrafiltration separates the molecules according to their molecular weight and pore size, concentrating whey into WPCs [10]. They can be obtained in varied protein

concentration levels of 34% (WPC34), 50% (WPC50) or 80% (WPC80). Generally, they contain less fat and cholesterol and dense amount of bioactive and lactose [11].

B. Whey protein isolate

Microfiltration and ion-exchange are used to produce whey protein isolate, these processes remove lactose and fat and increase protein concentration up to 90% or more. There are few points to consider the advantages of whey protein isolates making it effective in diets for fat loss and building muscles. It doesn't contain lactose and fat and hence can be used in lactose free, fat free food products, offering more protein per serving increasing the amino acids content[10].

C. Hydrolyzed whey protein

Hydrolysates are predigested, partly hydrolyzed whey proteins that, are a lot more easily absorbed, however their price is high. Highly hydrolyzed whey is also less allergenic than other forms of whey. Talking about sensory qualities, they have extremely bitter flavor [11].

3.4 Application of whey protein and whey protein product in food [11]:

1) Whey powder - Breads, bakery and snack items and dairy foods
2) Whey protein concentrate - The cheapest form of whey, used in protein drinks, bars, bakery and candy, dairy products and other nutritious foods
3) Whey protein isolate - Protein beverages, protein bars, protein supplementation products, nutritional food products
4) Hydrolyzed whey protein concentrate - Sports nutrition products

IV. MPC (Milk Protein Concentrates)

Milk protein concentrate (MPC) is a complete protein, composed of casein and whey protein, in the same ratio as milk. MPC is high in protein and low in lactose compared to skimmed milk powder or milk powder. Therefore, they offer concentrated protein in the final application to enhance nutritional, sensory and functional properties. Likewise, MPC is similar to nonfat dried milk (NFDM) powders, but the main difference is that MPC protein is concentrated by eliminating lactose and soluble minerals. Common MPC products are MPC 42, MPC 70, MPC 80, MPC 85, and MPI (containing 90% or more protein by weight). According to experience, as the protein content of Milk Protein Concentrates increases, the lactose level will decrease [12].

A. Milk Protein Concentrates Production:

The basic ingredient for the production of MPC is skimmed milk. Firstly, the skimmed milk is heat-treated at 70 to 75 °C for 10 to 20 seconds to deactivate unwanted microorganisms and enzymes, and then the milk is concentrated by ultrafiltration (UF). Here, the protein fraction, namely casein, residual fat, whey protein and micellar salt remain in the retentate in concentrated form, while lactose, soluble salts and non-protein nitrogen are removed with the permeate.

For high protein MPCs (such as MPC 85), a further concentration step is required, and UF only is not enough to obtain the desired protein to solid ratio in the retentate. For further removal of lactose and soluble minerals, diafiltration (DF) is used to obtain the desired protein to solid ratio. The presence of residual fat and the retention of micellar calcium phosphate limit the maximum protein content that can be achieved. After reaching an acceptable protein/solid ratio in the ultrafiltration retentate, it is evaporated and then spray dried. Since the ratio of protein to solids in the ultrafiltration retentate is significantly higher, evaporation cannot reach the solids content of MPC, which is similar to the solids content of skimmed milk. The solids content of skimmed milk in the feed of the spray dryer is 50%, while the solids content of MPC 70 is 30%. For MPC with higher protein content, its content may be lower [12].

B. Functional Properties of MPC and its application:

Milk Protein Concentrated (MPC) has developed colors / flavors in chocolate and confectionery. It forms a gel (gelation) in various products, such as cheese, yogurt, bakery products and candies. MPC has water binding, thickening, emulsification and viscosity capabilities and can be used in a variety of products such as soups and spices, meat products, baked goods, candies, chocolate, yogurt and cheese. In addition, it has the ability to foam and whipped, and is suitable for different products such as ice cream, desserts and whipped toppings. MPC acts as a heat-stable ingredient in recombined milk, soups and seasonings, and enteral and clinical nutrition [12].

V. Nutritonal Benefits

In order to evaluate the nutritional value of food protein present in various foods, bovine milk protein is regarded as a standard reference protein. Milk proteins densely comprise of branched-chain amino acids such as isoleucine, leucine, and valine and infact are much more in milk proteins than in any other food. Leucine helps minimizing muscle wasting due to increased protein breakdown. It also stimulates muscle protein synthesis. The cysteine and methionine (high content of sulfur-containing amino acids) present in whey protein are the precursors of glutathione, which can fight cancer and stimulate the immune response. The following table summerise the milk protein nutritional benefits [8].

Type of protein	Biological function	Mechanism
Bioactive peptides Lactoferricin	Inhibits cancer development	Inhibits growth of tumor and initiates programmed cell death of cancer cells
	Immunomodulation	Increase in Immunoglobins (IgM, IgG, and IgA) production
	Anti-bacterial activity	Growth inhibition of various Gram-positive and Gram-negative bacteria
Glycomacropeptide	Antiviral activity	Inhibition against human *influenza* virus and *Epstein Barr* virus
	Immunomodulation	Indirect anti-inflammatory effect in intestine by promoting host defense against microorganisms
Casomorphin peptides	Anticarcinogenic activity	Reduce the production of prostate cancer cell lines Promoting cell death in human leukemia cells (HL-60)
Lactorphin	Antihypertensive activity	Blood pressure reduction in hypertensive rats
Casein- phosphopeptides	Prevents tooth decay	Stabilizing calcium phosphate and eventually decreasing the mineral loss due to tooth decay

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