A Review: Effect of Whey Protein Under Influence of Heat and Cold

Nadia Sharif[1], Uzma Nihar[2], Iqra[2], Saba Zafar[3*]

Abstract

Background: Whey protein, a heterogeneous mixture of secreted proteins, predominantly includes β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). In mammalian milk, cow's milk contains 18% whey protein, while buffalo's milk is richer in fat and protein content. This study investigates the denaturation of whey protein in both cow's and buffalo's milk under varying thermal conditions. Methods: The denaturation of whey protein was assessed under cold storage (4–5°C) and heat treatment (70–85°C) in both cow's and buffalo's milk. The impact of these conditions on milk coagulation, bacterial growth, and changes in acidity was examined. Results: Cold storage increased milk coagulation, with viable bacterial growth and higher lactic acid levels. Heat treatment also elevated milk acidity, leading to the disappearance of β-Lg. Whey protein showed notable denaturation under both cold and heat treatments. Conclusion: Whey protein denaturation occurs under cold and heat treatments, affecting milk properties like coagulation and acidity. Beyond its role in milk stability, whey protein plays a significant role in human health, including its nutritional benefits, promotion of cell growth, fermentation processes, and disease prevention, while acting as an antioxidant.

Significance | The study demonstrated whey protein's denaturation in cow and buffalo milk, impacting coagulation, acidity, nutrition, fermentation, and health benefits.

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Introduction

Milk is a complex biological liquid that provide bioactive components and nutrients. It helps to stimulating digestive maturation, cellular growth; symbiotic microflora and development of gut-associated lymphoid tissues are successful postnatal adaptation of the newborn infant. Milk proteins, peptides, probiotic lactic acid bacteria, calcium and other minerals can significantly reduce blood pressure and milk fat contains several components having functional properties.

The importance, strength and number of bioactive compounds in milk and fermented milk products are probably greater than previously thought. They include certain vitamins, specific proteins, bioactive peptides, oligosaccharides, organic (including fatty) acids. Some of them are normal milk components; others appear during digestive or fermentation processes. Fermented dairy products and probiotic bacteria decrease the absorption of cholesterol. This protein plays an important role in the sensitization capacity of cow's milk, and its modification might be a way to reduce the allergenicity of cow's milk. It can also play a role in reducing these social and economic costs and have several putative biological effects when ingested that including an anticancer action. Milk composition of mammalian species varies widely with reference to genetic, physiological and nutritional factors and environmental conditions (Malacarne et al., 2002). This is achieved by proteins such as lysozyme, peroxidase, lactoferrin and immunoglobulins. Proteinases, such as plasmin and lipases, and serum albumin or β-lactoglobulin (β-Lg), may aid digestion

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and nutrient absorption (Creamer, 1996).

Proteins are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. There are many proteins present in different source of food such as milk, meat, egg, soy, wheat, etc. In different forms of milk contains different proteins but I study the effect of heat and cold on whey protein from cow and buffalo milk and its effects on human health.

1.1. **Whey protein**

Whey protein is the liquid remaining after milk has been curdled, filtered and strained. It is a by-product of the manufacture of cheese or casein and has several commercial uses. There are 3 types of whey protein on basis of their compositions; Whey protein concentration, Whey protein isolate and Whey protein hydrolysate. Types of whey proteins are shown in Table1.

In milk, there are two major protein types which in bovine milk are defined by acid precipitation: the caseins, which precipitate as a group at pH 4.6, and the whey proteins, which can be subdivided into the major mammary synthesized proteins and the minor, usually blood, proteins. Each of the mammary-synthesized proteins exists in several forms, known as genetic variants, which have slightly different amino acid sequences (Creamer, 1996).Whey proteins are: β-lactoglobulin (β-Lg), α-lactalbumin (α-La), lesser amounts of serum albumin, immunoglobulins, and proteose peptones. Whey proteins give 20% of total protein content in bovine milk. They are globular and are present in milk as discrete molecules with varying numbers of disulfide crosslinks. These proteins are more heat sensitive, and less sensitive to calcium than caseins. They can form disulfide linked dimers or polymers via thiol disulfide interchange e.g. with κ-casein. Composition of Whey Protein is shown in table 2.

1.1.1 ***β-lactoglobulin***

β-lactoglobulin is the major whey protein, about 54% of whey proteins is βlactoglobulin. Five genetic variants have been characterised. It is a globular protein with a molecular weight of 18,362 Dalton for variant A and 18,276 for variant B. Variant B consists of 162 amino acids. A comparison of the sequences of β-Lg in bovine, ewe's and goat milk shows, that the three proteins are highly homologous. They contain two intrachain disulfides and one sulfhydryl group.

The secondary structure of bovine β-Lg is 15% α-helix, 50% β-sheet and 15-20% reverses turn. The protein is a typical lipocalin whose structure thus contains a β-barrel with eight antiparallel β-strands, labelled A–H and a three-turn α-helix that lies parallel to three of the β 15 strands. Strands A–D form one surface of the barrel while strands E–H form the other. A significant feature in all lipocalins is the bend in strand A that allows it to interact with strand H. The three-turn-α-helix follows strand H and lies on the outer surface of the barrel between the terminal end of the A strand and the H strand. : Structure of β-lactoglobulin (Qi et al., 1997) is shown in figure 1.

The molecule contains two disulfide bonds, which are found between cisteins 106.19 and the cisteins 6.160 respectively. There is one free sulfhydril group in β-Lg, but there is no phosphorus present in this protein.

β-Lg is very acid stable. It is generally in dimer form at the isoelectric pH of 5.2 and alkaline pH range. Bovine β-Lg denatures at temperatures above 65°C at pH 6.7, typically at 70.4±0.5°C, followed by aggregation. Denaturation temperature of β-Lg depends on pH. It is most heat sensitive near pH 4.0 and most stable at pH 6.0.

Temperature affects the three-dimensional structure of β-Lg. Although β-Lg is found mainly in the dimer form in milk, monomers appear when temperature is increased up to 65°C. Critical conformational change occurs around 63°C, where there is 19% net reduction in the βsheet content. Above this temperature, unfolding of β-Lg structure leads to irreversible denaturation in the following order: D-E strand (55-60°C); C-D strand and α-helix (60-65°C); A-B, A-I and E-F strands (65- 70°C); and A-H, B-C and F-G strands (75-80°C). Thermal unfolding of β-Lg is almost complete at 80°C except for the G-H pair of disulfide-linked strands which are the most heat-resistant feature of the structure (Edwards et al., 2002; Doucet, 2004).

β-Lg was found to bind retinol and enhance its fluorescence. One molecule of retinol is bound per β-Lg monomer. Binding of retinol by β-Lg occurs in the interior of the hydrophobic 16 barrel with tryptophan19 at the bottom of the calyx interacting with the β-ionine ring of the retinol molecule (Wong et al., 1996). β-Lg is is one of those milk proteins that are responsible for milk protein intolerance or allergy in humans (Bonomi et al., 2003; Clement et al., 2002).

1.1.2 ***α-lactalbumin***

Bovine α-La is a small globular protein that is relatively stable. It constitutes 21% of whey proteins. Its genetic variant A has a molecular weight of 14,147 Da. Variant B has a molecular weight of 14,175 Da. α-La is composed of 123 amino acid residues. The molecule has an ellipsoid shape with a deep cleft dividing the protein in two parts. Four helices form one side of the cleft and two β-sheets together with a loop-like chain make up the other one. Four disulfide bonds make this protein relatively heat stable.

α-La was found to be a cofactor in lactose synthesis and the concentrations of this protein and of lactose in milk are correlated. It is a strong binder of calcium and other ions, including Zn, Mn, Cd, Cu, and Al, and changes conformation markedly on calcium binding (Wong et al., 1996). One interesting feature of α-La is that it seems to exist in three different structures: the calcium-bound, the calcium-free and the low pH or A form. Recently, this latter form has been studied intensely as it may constitute a new protein

structure. This 'molten globule' structure may be intermediate between the native and denatured forms of the protein (Creamer& MacGibbon). Figure 2 shows the structure of α-lactalbumin (1996).

1.2 ***Scope or usefulness***

Whey is biological mixture of heterogenous of secreted proteins which have wide function in nutritional, biological, food purpose and some characteristics of major whey proteins and polypeptides are summarized. Protein concentration and number per molecule of reactive amino acids such as half-cystine (cys/2) and lysine-residuesare important characteristics during heat treatments. Whey protein is a popular choice among athletes, fitness enthusiasts and people wanting to build muscles or lose weight. It is also complete source of protein and contains all the essential amino acids. Dietary whey proteins have several putative biological effects when ingested, including an anti- cancer action. This study compared the ability of several common dietary protein sources, including whey, casein, meat, and soybean, to prevent the development of colon cancer.

1.3 **Application:**

1.3.1 ***Importance in industrial and fermentation process***

The use of whey in dairy probiotics is a topic of great interest to the scientific community and the food industries. However, few studies address the effect of ohmic heating (OH) on cell metabolism and growth parameters of probiotic microorganisms. Despite of this, OH under sub-lethal conditions presents promising results regarding the enhancement of growth rate and bacteriocin activity, leading to considerable improvements in the fermentation process. Thus, this review highlights the main findings and advances on the effect of OH on probiotic metabolism, while addressing the modification of whey protein structure as potential carrier of probiotic entities, aiming at stimulating interest and encouraging the development of functional products using OH (Pereira in drugi, 2018).

1.3.2 ***Role in overweight and obesity***

A study by Bowen et al. (9) suggested that the whey and casein protein components of dairy appear to be more important for weight loss than Ca in overweight adults due to their high concentrations of branched-chain amino acids. However, there is little evidence of beneficial effects of casein beyond it being a good quality source of protein and having a likely hypotensive effect (10 – 12). In another article, it's shown that both whey and casein proteins consumed over 12 weeks significantly reduced diastolic and systolic blood pressure from baseline in overweight individuals; however, whey protein consumption also significantly reduced arterial stiffness (12). Some studies demonstrate that dairy whey proteins have a better effect on appetite control than other protein sources such as egg and casein (13 – 15). In addition, convincing evidence indicates that dairy whey proteins and their bioactive components such as lactalbumin, angiotensin-converting enzyme inhibitor and branched-chain amino acids may have an insulinotrophic effect (16 – 20), hypotriacylglycerolaemic effect (21), muscle-sparing effect (22 – 24) and cholesterol-lowering effect (25). However, most of these studies using whey proteins have been conducted in healthy individuals or animals, with limited studies in overweight/ obese individuals. Given the effect of whey proteins on appetite control, muscle sparing and lipid metabolism demonstrated previously in healthy adults, our hypothesis was that whey protein consumption would also have a beneficial effect on metabolic risk factors in overweight and obese individuals, a population highly susceptible to the metabolic syndrome. Therefore, the aim of the present study was to compare the effect of whey and casein consumption on lipids, insulin, and glucose and body composition in overweight / obese individuals.

Previous studies have demonstrated the effect of whey proteins on appetite control, muscle sparing and lipid metabolism in healthy adults, but limited data are available for the effect of whey protein consumption on metabolic risk factors in overweight and obese individuals. While in recent studies the whey protein also decreased plasma TAG, insulin and homeostasis model assessment of insulin resistance scores compared with the control. There was no effect of casein supplementation on any metabolic risk parameter compared with control supplementation. Overall, the present study demonstrated that whey protein supplementation can significantly improve metabolic risk factors associated with chronic diseases in overweight and obese individuals.

1.3.3 ***Role in cell growth:***

Whey proteins can play a role in reducing these social and economic costs. Dietary whey proteins have a number of putative biological effects when ingested, including an anticancer action. Among the many minor protein constituents of whey are several that display antimicrobial activity. Lactoferrin, present at low concentrations in whey (50 to 150 mg/L), exhibits both bacteriostatic and bactericidal activity against a range of microorganisms, including those responsible for gastroenteric infections, food poisoning, listeriosis, and mastitis. Whey contains proteins that serve as potent growth stimulants for a number of mammalian cell lines in culture. These growth factors have a dramatic impact on cell growth by promoting synthesis of DNA and protein and by inhibiting degradation of protein.

1.3.4 ***Role as antioxidant***

Flaxseed contains approximately 55% of total fatty acids of the oil aslinolenic acid and is rich in lignans, which are strong antioxidants. Diets rich in omega-3 fatty acids and antioxidants are known to have beneficial effects on human health such as a decrease in the incidence of cancer, cardiovascular diseases, hypertension, and arthritis. Flaxseed could then be an interesting natural feed to consider for changing milk composition. Cyanogenic glycosides (linustatin and neolinustatin) are present in flaxseed, but the

concentration of hydrocyanic acid is very low in milk and ruminal fluid of cows fed flaxseed products. In general, feeding up to 15% of the total dry matter as whole flaxseed has a limited effect on dry matter intake. Heat treatments such as micronization and extrusion have no effect on dry matter intake and the effect of formaldehyde treatment on feed intake is unclear. The effects of flaxseed supplementation on milk production of dairy cows in the early stage of lactation have been neutral. Diet supplementation with whole flaxseed has had no effect on milk yield and composition of dairy cows in the mid or late stages of lactation. Physical processing of flaxseed increased milk production although heat treatment did not. Results on the effect of flaxseed processing on overall milk fat concentration have been controversial, but heat and formaldehyde treatments had no effect. Flaxseed supplementation had no effect on milk fat and protein concentrations, and processing of flaxseed had little effect. The extent of change in the concentration of fatty acids in milk is generally proportional to the level of inclusion of flaxseed in the diet. In conclusion, feeding flaxseed does not affect milk production or composition in most studies, but its long-term effects on health of cows and productivity still need to be determined (Petit, 2010).

The economic output of the dairy industry is to a great extent dependent on the processing of milk into other milk-based products such as cheese. The yield and quality of cheese are dependent on both the composition and technological properties of milk. Milk parameters of the protein, lipid, and carbohydrate profiles as well as minerals were used to obtain correlations with native CN micelle size and gelation characteristics. Milk pH and protein, CN, and lactose contents were found to affect milk gelation. Smaller native CN micelles were shown to form stronger gels when poorly coagulating milk was excluded from the correlation analysis. In addition, milk pH correlated positively, whereas Mg and K correlated negatively with native CN micellar size. The milk from the elite dairy cows was shown to have good gelation characteristics. Furthermore, genetic progress in relation to CN micelle size was found for these cows as a correlated response to selection for the Swedish breeding objective if optimizing for milk gelation characteristics. The results indicate that selection for smaller native CN micelles and lower milk pH through breeding would enhance gelation properties and may thus improve the initial step in the processing of cheese (Glantz in drugi, 2010).

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1.3.5 ***Role in prevention of diseases***

Milk consumption and dairy products are important elements in healthy and balanced diet. It provides all necessary energy and nutrients to ensure development and growth. However, several controversies arise from consumption of dairy and milk products during adulthood, especially because it refers to milk from other species the epidemiologic studies confirm the nutritional importance of milk in human diet and their role in preventing chronic conditions like some forms of cancer, cardiovascular diseases, obesity and diabetes.

Dairy and milk consumption are frequently included as important elements in a healthy and balanced diet. It is the first food for mammals and provides all the necessary energy and nutrients to ensure proper growth and development, being crucial in respect to bone mass formation. However, several controversies arise from consumption of dairy and milk products during adulthood, especially because it refers to milk from other species. Despite these controversies, epidemiologic studies confirm the nutritional importance of milk in the human diet and reinforce the possible role of its consumption in preventing several chronic conditions like cardiovascular diseases (CVDs), some forms of cancer, obesity, and diabetes. Lactose malabsorption symptoms and cow milk protein allergy are generally considered to be the adverse reactions to milk consumption. The present article reviews the main aspects of milk nutritional composition and establishes several associations between its nutritious role, health promotion, and disease prevention (Pereira, 2014).

Twenty-one infants less than 6 months of age with gastrointestinal symptoms of cow milk and/or soy protein-based infant formula intolerance (diarrhea in 14, hematochezia in 16, emesis in 8, failure to thrive in 4, and colic in 10) were treated clinically with a whey protein hydrolysate formula. Six patients improved when placed directly on the formula, and 15 remained asymptomatic or improved when given the whey hydrolysate formula following initial treatment with a casein hydrolysate or elemental formula. Eighteen had supporting evidence of an allergic basis for their symptoms, including a family history of allergies in 6, a clinical challenge with the offending formula in 1, laboratory tests consistent with atopy in 11, and/or rectal biopsy with histologic allergic features in 7. The whey hydrolysate formula may be an acceptable alternative to soy or casein hydrolysate formulas in most infants with gastrointestinal symptoms of cow milk and/or formula intolerance (Merritt 1990).

Lock, (2004) studied about the increase of consumer awareness that it may have beneficial effect on health and prevention of dieases. The functional food components in milk fat include EPA, DHA and CLA. The content of these FA in milk has improved due to which that have better defined the interrelation between fermented rumen, fat milk synthesis and lipid metabolism. Milk fat is predominantly TG, and de novo FA synthesis and the uptake of circulating FA contribute nearly equal amounts (molar basis) to the FA in milk fat. Dietary EPA and DHA transfer to milk fat is very low (<4%). This is large extent, related to their extensive biohydrogenation in the rumen. It partly due to the fact that they are not transported in the plasma lipid fractions. Which serve as major mammary sources of FA uptake (TG and no esterified FA). Milk contains 20 isomers of CLA but the predominant one is cis-9, trans. The biomedical study shows that this isomer has anti-antherogenic and anti-arcinogenic activities is produce as intermediate in biohydrogenation of rumen. Vaccenic acid produced rumen biohydrogenation from both of these. Diet can affect milk fat CLA and asubstantial differences among individual cows.Thus, fat milk CLA increase with rumen. The concentration of CLA is significantly enhanced by using diet nutritional management of dairy cows.

1.3.6 ***Importance in nutritional level***

Council (1988) determined the importance to milk procedure and nutritional to consumers. Variation in milk composition occur for years however milk composition has constant marketed over the last 15 years, averaging 3.6 percent fat, 3.2 percent protein, and 4.7 percent lactose (Young et al., 1986). This is because of importance of the Holstein breed and the price of milk based on fat concentration. On components basis the milk pricing and perception by consumers that fats of animals are unhealthy. It has created new interest in how milk components can be altered to accommodate these emerging markets. The biosynthesis of milk components since changes in these reflects changes in the mammary gland synthesis or secretion of the component. Factors affecting of milk composition, such as breed, genetic variation within breed, health, environment, management practices, and diet are then reviewed.

Whey protein products are of widespread use as food ingredients because of high nutritional, biological and functional property. Whey proteins are important structural components in many foods as used in their native form, for example for their heat-induced gelation abilities. Furthermore, they also offer reliable functionalities when modified by heating processes as denatured or aggregated proteins. Heat treatment of whey proteins in a liquid state has received much attention in recent years. While dry heating of whey proteins, say heating whey proteins in the dry state, is frequently cited in the literature as a potential and efficient means to improve the functional properties of proteins, it has received very little attention. We report first on the dry heating of whey products as applied to promote glycation of whey proteins with a low denaturation, and second, to promote their denaturation and aggregation and on their consequences on the functional properties of whey proteins (Schong in Famelart, 2017).

1.3.7 ***Role in milk allergenicity***

Cow's milk allergy is quite common in the first years of human life. Protein composition plays important role in this pathology, particularly the casein and whey protein ratio. It is known that milks from different species have different sensitization capacities although their protein sources are quite similar.

General food allergies occur in about 5 to 10% of the infant and small-child populations (Bock, 1987; Sampson, 1997a). Cow's milk protein allergy (CMPA) is the most common allergy in young infants, with an incidence of 2 to 6% (Hill, 1996; Hosking et al., 2000). This atopic disease is associated with a broad spectrum of IgE-mediated reactions which are mostly expressed as immediate symptoms, such as urticaria, rhino conjunctivitis, asthma, vomiting, and diarrhea and, in the most severe cases, systemic anaphylactic shock and death (Sampson, 1997b). In contrast, cow's milk proteins (CMP) are recognized by the immune system of some newborn infants as foreign proteins, thus causing allergic reactions. The role of different CMP in the origin of CMPA has been studied. Although it is not clear which are the major allergens in cow's milk, several studies demonstrate that most children with CMPA synthesize antibodies principally against α-casein and β-LG (Restani et al., 1999; Bevilacqua et al., 2001; Ametani et al., 2003). The Casein and whey protein ratio in native cow's milk is 80:20, because a reduction of α-casein might result in a reduction of allergenicity, it would be interesting to analyze the allergenicity of modified milk with a different balance between casein and whey proteins. To clarify whether milk with a modified casein and whey protein ratio is less allergenic than native cow's milk, we used a

murine model of milk sensitization previously published (Li et al., 1999). These results suggest that the balance between caseins and whey proteins plays an important role in the sensitization capacity of cow's milk, and its modification might be a way to reduce the allergenicity of cow's milk.

Hypersensitivity to cow milk proteins is one of the main food allergies and affects mostly but not exclusively infants, while it may also persist through adulthood and can be very severe. Different clinical symptoms of milk allergy have been established. The diagnosis of milk allergy differs widely due to the multiplicity and degrees of symptoms and can be achieved by skin or blood tests. Cow milk contains more than 20 proteins (allergens), that can cause allergic reactions. Casein fractions and β-lactoglobulin are the most common cow milk allergens. Human milk is free of β-lg, like camel milk. On the contrary, β-lg is a major whey protein in cow, buffalo, sheep, goat, mare and donkey milk. Caseins in milk of the different species differ in fraction number, amino acid composition, and their peptide mappings. β-Casein is the major fraction in goat casein, which is like human casein and different from cow casein. The peptide mappings of goat α-la and β-lg are completely different from those of cow milk. Different procedures can reduce the allergenicity of cow milk proteins by heat or enzymatic treatment to some degree. Allergies to milk proteins of non-bovine mammals have also been documented due to cross-reactivity between cow milk proteins and their counterpart in other species, and even between goat and sheep caseins. Genetic polymorphisms of milk proteins play an important role in eliciting different degrees of allergic reactions. Goat milk lacking α-s1-casein, which is the main casein in cow milk, is less allergenic than goat milk with α-s2-casein, which is more typical for many goat breeds. Several studies have reported real and dramatic benefits from using goat, camel, mare or even soymilk as alternatives in cases of cow milk allergy and they can be considered hypoallergenic. However, therapeutic benefits vary with the degree of severity of the allergy and may be only around 60% of all cases, since other studies revealed allergenicity to occur also for any of those other milks (El-Agamy, 2007).

Cow's milk allergy is quite common in the first years of human life. Protein composition plays an important role in this pathology, particularly the casein/whey protein ratio. It is known that milks from different species have different sensitization capacities although their protein sources are quite similar. Thus, the objective of this work was to compare the allergenicity of native cow's milk and milk with a modified ratio of casein and whey proteins in a murine model of atopy. Twenty-four Balb/c mice were orally sensitized to native cow's milk or modified cow's milk with a casein/whey protein ratio of 40:60. During the sensitization period, the number of mice suffering from diarrhea was significantly higher in the native cow's milk-sensitized group than in the modified milk-sensitized group. Once mice were killed, plasma histamine levels were shown to be significantly higher in native cow's milk-sensitized mice. In addition, cow's milk proteins induced a higher lymphocyte sensitization in the native milk-sensitized mice, with a significant increase in the specific proliferation ratio of these cells. These results suggest that the balance between caseins and whey proteins plays an important role in the sensitization capacity of cow's milk, and its modification might be a way to reduce the allergenicity of cow's milk (Lara-Villoslada in drugi, 2005).

During the processing of marketed milk, homogenization reduces fat droplet size and alters interface composition by adsorption of casein micelles mainly, and whey proteins. The structural consequences depend on the sequence of the homogenization and heat treatments. Regarding human health, homogenized milk seems more digestible than untreated milk. Homogenization favors milk allergy and intolerance in animals, but no difference appears between homogenized and untreated milk in allergic children and lactose-intolerant or milk-hypersensitive adults. Controversies appear regarding the atherogenic or beneficial bioactivity of some casein peptides and milk fat globule membrane proteins, which might be enhanced by homogenization. In children prone to type I diabetes, early cow's milk consumption would be a risk but no link was observed in the general population and the effect of homogenization has not been studied. In the current context of obesity and allergy outbreaks, the impact of homogenization and other technological processes on the health properties of milk remains to be clarified (Michalski, 2006).

1.3.8 ***Role in food application***

Whey proteins are well known for their high nutritional value and versatile functional properties in food products. Estimates of the worldwide production of whey indicate that about 700,000 tonnes of true whey proteins are available as valuable food ingredients. Nutritional and functional characteristics of whey proteins are related to the structure and biological functions of these proteins. During recent decades, interest has grown in the nutritional efficacy of whey proteins in infant formula and in dietetic and health foods, using either native or pre-digested proteins. This paper focuses attention on the differences and similarities in composition of human and bovine milks with reference to infant formula. More desirable milk protein composition for consumption by humans is obtained by the addition of lactoferrin and more specific fractionations of proteins from bovine milk. Optimization of heating processes is important to minimize the destruction of milk components during fractionation and preservation processes. Some functional characteristics of whey proteins are discussed in relation to their properties for application in food products. Information obtained from functional characterization tests in model systems is more suitable to explain retroactively protein behaviour in complex food systems than to predict functionality (De Wit, 1998).

This chapter focuses on the structural properties of whey proteins and how these properties are influenced by environmental factors—for example, pH, temperature, and salts in manifesting characteristic functional properties, such as gelation, foaming, and emulsifying activity, which are important in food applications. Proteins are important structural components in many foods and they are also used as ingredients because of their nutritional value and physicochemical properties. The market for functional protein-rich ingredients is expanding and is currently supplied by various proteins. Proteins obtained from dairy products have traditionally been one of the main protein sources for humans. Currently, a selection of high-protein dairy powders for example, caseinates, coprecipitates and whey powders are used, yet these display a highly variable range of functional properties because of differences in composition and processing treatments. However, whey protein preparations vary immensely in functional behavior and are presently relegated to limited use as functional ingredients in the food industry. This need not be the case since conventional and new technologies permit rigorous control of production protocols—for example, careful control of heat treatments can result in the production of whey protein preparations with consistent, reliable functional properties (Kinsella in Whitehead, 1989). Figure 7 shows the Application of whey.

2 **Studies the effects of heating and freezing**

2.1 ***Heating effects***

2.1.1 ***Effect of pH and acidification on composition of buffalo and cow milk***

Composition and physico-chemical properties of buffalo and cow milks were compared at their initial pH and during acidification. As compared to cow milk, buffalo milk was richer in fat, lactose, protein and minerals such as calcium, magnesium and inorganic phosphate. Along with these differences of major components, the capacity of milk to be acidified (named buffering capacity) was higher for buffalo milk than for cow milk. The precipitation/aggregation of caseins at their isoelectric pH, solubilization of calcium and inorganic phosphate and decrease in hydration of casein as a function of decrease in pH were significant for both milks. For both species, these molecular changes were qualitatively similar but quantitatively different. These quantitative differences during acidification were related to the differences of composition between the milks (Ahmad in drugi, 2008).

2.1.2 ***Effect of coagulation and variation in composition of milk***

The objective of this study was to evaluate the effect of variations in milk protein composition on milk clotting properties and cheese yield. Milk was collected from 134 dairy cows of Swedish Red and White, Swedish Holstein and Danish Holstein-Friesian breed at 3 sampling occasions. Concentrations of αS1-, β-and κ-casein (CN), α-lactalbumin, and β-lactoglobulin (LG) A and B were determined by reversed has liquid chromatography. Cows of Swedish breeds were genotyped for genetic variants of β- and κ-CN. Model cheeses were produced from individual skimmed milk samples and the milk clotting properties were evaluated. More than 30% of the samples were poorly coagulating or noncoagulating, resulting in weak or no coagulum, respectively. Poorly and noncoagulating samples were associated with allow concentration of κ-CN and a low proportion of κ-CN in relation to total CN analyzed. Furthermore, the κ-CN concentration was higher in milk from cows with the AB genotype than the AA genotype of κ-CN. The concentrations of αS1-, β-, and κ-CN and of β-LG B were found to be significant for the cheese yield, expressed as grams of cheese per one hundred grams of milk. The ratio of CN to total protein analyzed and the β-LGB concentration positively affected Cheese yield, expressed as grams of dry cheese solids per one hundred grams of milk protein, whereas β-LG A had a negative effect. Cheesemaking properties could be improved by selecting milk with high concentrations of αS1-, β-, and κ-CN, with high κ-CN in relation to total CN and milk that contains β-LG B (Wedholm in drugi, 2006).

2.1.3 ***Effect of whey protein fraction on their composition from buffalo milk***

In the study conducted by (Buffoni in drugi, 2011) that the whey protein fractions from 120 Mediterranean water buffalo individual milks were analyzed by microchip electrophoresis (MCE), reverse-phase high-performance liquid chromatography (RP-HPLC) and mass spectrometry (ESI-MS). Validation procedures were carried out for both MCE and HPLC. The chromatographic analysis allowed the complete separation of the whey protein fractions, resulting in a well-defined peak structure; the adopted RP-HPLC and ESI-MS protocols provided identification of β-lactoglobulin (18,266 Da), α-lactoalbumin (14,236 Da) and serum albumin (66,397 Da). The calculated mean concentrations were 4.04 g/l, 2.45 g/l and 0.35 g/l, respectively.

2.1.4 ***Counting of bacteria and physicochemical properties of milk***

A study was conducted by Markiewicz et al (2013) with the objective to determine the whey protein profile somatic cell count, total bacteria count, chemical composition and some physicochemical properties of late lactation milk produced by Polish Cold-Blooded Horses mares between 141- and 210-days post-partum. The whey protein profile, expressed as a percentage of the total sum of the six whey proteins considered, was characterised by high proportions of lysozyme (11.6%), lactoferrin (14.6%) and immunoglobulin (15.8%). Whey proteins represented in greatest amounts included β-lactoglobulin (29.2%) and α-lactalbumin (25.4% of the total whey protein fraction). Mares' milk was characterised by very low numbers of somatic cells and total bacterial counts. Results presented confirm that late lactation mares' milk constitutes a valuable source of bioactive components (Markiewicz-Kęszycka in drugi, 2013).

2.1.5 ***Effect of heat treatment on molecular structure of milk proteins***

Heat treatment affects the molecular structure of milk proteins at the interfaces of oil-in-water emulsions and in aqueous media. Experimental evidence of the impact of thermal processing on milk protein structure is presented and the contribution of whey proteins and caseins at film formation during emulsification is discussed. Recent advances in understanding the effect of heat treatment in milk protein functionality at emulsion interfaces are reviewed with emphasis on the emulsifying ability of whey proteins with or without the presence of the casein fraction. The major findings regarding the destabilizing mechanisms of oil-in-water emulsions brought about by heat-induced denaturation of milk proteins are presented. This paper aims to combine recent knowledge on how thermal processing of milk proteins affects their molecular configurations in bulk and particularly at interfaces, which in turn appear to be important with respect to the physico-chemical properties of milk protein-stabilized emulsions (Raikos, 2010).

2.1.6 ***Effect of heat treatment milk protein of bovine milk***

(Zamberlin in drugi, 2010) aim of study was to investigate the effect of heat treatment of ovine milk at (60°C/5min and 90°C/5) min (control group) on the compositional and sensory properties of set yoghurt. The concentration of apparent casein and total whey protein were significantly higher while sensory properties (except consistency) were not significantly different from the yoghurts in control group ($P<0.05$). The results showed that ovine set yoghurt produced by heat treatment at low temperature possessed higher amount of preserved inherent functional and nutritional properties of milk than yoghurt produced by heat treatment at high temperature.

2.1.7 ***Effect of Heat treatment for stability of milk protein***

A subjective test for the determination of the stability of milk protein to heat was described by (Davies in White, 1966). In the test, the time required for particles of coagulated protein to become visible throughout a 2·5-ml sample of separated milk maintained at 135°C in a glass tube rocking at 8 c/min is taken as a measure of stability. The precision of the test was such that single determinations were generally adequate. Coagulation time decreased by about 12% as rocking speed was increased over the range 4–12 c/min and increased by a factor of about 3 for a decrease in heating temperature of 10°C over the range 140–105 °C; with some milks the Q10 °C value increased to 5–8 a temperature decreased. As sample volume was increased over the range 1–3 ml coagulation time increased, especially with milks whose coagulation was poor (initial clots small). This volume effect appeared to be a consequence of the accompanying decrease in the proportion of headspace oxygen to volume of milk

2.1.8 ***Effect cooling, and storing treatment of milk***

A study by (Dzurec Jr in Zall, 1985), determined that Cottage cheese yields increase as a result of heating (74°C, 10 s), cooling (3°C), and storing (7 days) milk prior to cheese making. Protein analysis of milk indicated that casein was higher and whey proteins lower in experimental heated, cooled, stored milk as compared with un-heated, fresh (<2 days old) control milk. Heating caused some proteins normally in whey to associate with casein by some undetermined mechanism. Samples of control and experimental milk subjected to centrifugation (21,000 x g/120 min) and gel electrophoresis showed that solu- ble l~-casein decreased with heat treat- ment and cold storage of milk. Pretreating raw milk with nitrogen- ethylmaleimide or ethylenetetraacetic acid prior to heat treatment and storage and subjecting these samples to electro-phoresis indicated little or no decrease of whey proteins compared with milk heated without added nitrogen-ethylmalei- mide or ethylenetetraacetic acid. Heat- treating, cooling, and storing milk seemed to cause part of the/3-casein to be trapped physically in the casein micelle. These treatments also caused portions of whey proteins to associate with casein micelles via disulfide bridging and calcium link- ages. protein changes in skim milk due to heat treatment & cold storage is shown in table 3.

2.1.9 ***Effect of heat treatment of whey protein during manufacturing of baby milk***

(Kilshaw in drugi, 1982) conducted experiments on guinea-pigs, and suggested that heat treatment applied during the manufacture of baby milk formulae reduces the immunological sensitising capacity of the cows' milk proteins. This immunological benefit must be weighed against possible damage that heat treatment may cause to the nutritional quality of the products. Severe heat treatment of skimmed milk (121°C for 20 min) destroyed all the vitamin B12, about 60% of the thiamin and vitamin B6, 70% of the ascorbic acid, and about 30% of the folate. Available lysine was reduced by 21 % and lactulose was formed (166 mg/100 ml). Despite extensive denaturation of the whey proteins the milk retained its capacity to sensitise guinea-pigs for systemic anaphylaxis when administered orally. Animals drinking heated milk also produced circulating antibodies to 3-lactoglobulin and casein, although titres were lower than for unheated milk. Unlike skimmed milk, heat-treated diafiltered whey failed to sensitise guinea-pigs orally. It caused the production of trace levels of antibodies in some of the animals, but these were specific for residual casein. We suggest that it may be possible to produce a non-sensitising baby milk without casein based on heat-denatured whey. The nutritional quality could be preserved by removing low molecular weight nutrients before heat treatment and adding back appropriate quantities later.

2.1.10 ***Effect of heat and pressure treatment on whey proteins***

(Baier in drugi, 2015) studied on the structural changes in micellar caseins and whey proteins due to high pressure--low temperature

treatments (HPLT) were investigated and compared to changes caused by high pressure treatments at room temperature. Whey protein isolate (WPI) solutions as well as micellar casein (MC) dispersions and mixtures were treated at 500 MPa (pH 7.0 and 5.8) at room temperature, -15 °C and -35 °C. Surface hydrophobicity and accessible thiol groups remained nearly unchanged after HPLT treatments whereas HP treatments at room temperature caused an unfolding of the WPI, resulting in an increase in surface hydrophobicity and exposure of the thiol groups. For HPLT treatments, distinct changes in the secondary structure (increase in the number of β-sheets) were observed while the tertiary structure remained unchanged. Large flocs, stabilized by hydrophobic interactions and hydrogen bonds, were formed in casein containing samples due to HPLT treatments. Depending on the pH and the applied HPLT treatment parameters, these interactions differed significantly from the interactions determined in native micelles

2.1.11 ***Effect of heat treatment on whey protein***

There is a great interest in the production of heats table and clear beverages containing high levels of whey proteins. A challenge of incorporating whey proteins in sports beverages is hot-fill treatment (88 °C, 2 min). The objective of this research was to analyze the effects of thermal treatment on the profile of whey proteins in a whey protein beverage (WPB). WPB were prepared mixing 5 % whey protein with 0.04 % potassium sorbate and 0.5 M phosphoric acid was used to adjust pH to 3.0 and 7.0. The protein particle size and zeta-potential were tested using a spectrophotometer. Finally, the protein profile of beverages containing whey was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Hot-fill treatment had a negative impact on the physiochemical properties of whey proteins. The formation of protein complexes produced an increase in particle size and absolute zeta potential in WPB formulations at both pH 3.0 and 7.0 (Quant in drugi, 2019). In Figure 4 Graph is showing denaturation of whey protein under time

2.1.12 ***Effect of heat treatment for stabilization of whey protein***

The effect of heating has been studied for whey protein-stabilised oil-in-water emulsions (25.0% (w/w) soybean oil, 3.0% (w/w) whey protein isolate, pH 7.0). These emulsions were heated between 55 and 95 °C as a function of time and the effect on particle size distribution, adsorbed protein amount, protein conformation and rheological properties was determined. Heating the emulsions as a function of temperature for 25 min resulted in an increase of the mean diameter (d32) and shear viscosity with a maximum at 75 °C. Heating of the emulsions at different temperatures as a function of time in all cases resulted in a curve with a maximum for d32. A maximum increase of d32 was observed after about 45 min at 75 °C and after 6–8 min at 90 °C. Similar trends were observed with viscosity measurements. Confocal scanning laser micrographs showed that after 8 min of heating at 90 °C large, loose aggregates of oil droplets were formed, while after 20 min of heating compact aggregates of two or three emulsion droplets remained. An increase of the adsorbed amount of protein was found with increasing heating temperature. Plateau values were reached after 10 min of heating at 75 °C and after 5 min of heating at 90 °C. Based on these results we concluded that in the whole process of aggregation of whey protein-stabilised emulsions an essential role is played by the non-adsorbed protein fraction, that the kinetics of the aggregation of whey protein-stabilised emulsions follow similar trends as those for heated whey protein solutions and that upon prolonged heating rearrangements take place leading to disaggregation of initially formed large, loose aggregates of emulsion droplets into smaller, more compact ones (Sliwinski in drugi, 2003).

2.1.13 ***Effect of temperature and pH on solubility of whey protein***

An integrated study was conducted on the effects of temperature and pH on the solubility of whey proteins. The solubility was determined experimentally in the range of 40–60°C for temperature and 3.5–7.8 for pH. The results showed that, both temperature and pH influenced in the protein solubility, and these properties had great interaction. Besides, for whey proteins, the solubility values were minimum at the pH value of 4.5, which is the isoelectric point of whey proteins, for all temperature values. It was also observed that at pH 4.5, the solubility decreased as the temperature increased, which indicated that the protein denaturation occurred. This behavior was also noticed in the neutrality (pH=6.8) (Pelegrine in Gasparetto, 2005).

2.1.14 ***Effect of pH, heat and ethanol on denaturation of whey protein***

(Nikolaidis in drugi, 2017) conducted a newly developed method of analysis of difference-UV spectra was successfully implemented in the study of the effect of heat, pH, ultrasonication and ethanol on the denaturation of whey protein isolate. It was found that whey proteins exhibit their highest stability against heat denaturation at pH 3.75. At very low pH values, i.e. 2.5, they exhibited considerable cold denaturation, while after heating at this pH value, the supplementary heat denaturation rate was lower compared to that at neutral pH. The highest heat denaturation rates were observed at pH values higher than neutral. High power sonication on whey proteins, previously heated at 90°C for 30min, resulted in a rather small reduction of the fraction of the heat denatured protein aggregates. Finally, when ethanol was used as a cosolvent in the concentration range 20-50%, a sharp increase in the degree of denaturation, compared to the native protein solution, was observed.

2.1.15 ***Effect of denatured whey protein interaction with other milk***

This chapter reviews the literature on the denaturation of the whey proteins in milk and their interactions with other milk proteins and A study by (Dzurec Jr in Zall, 1985), determined that Cottage cheese yields increase as a result of heating (74°C, 10 s), cooling (3°C), and

Table 1. Composition of types of whey protein

Types of whey proteins	composition	Lactose	FATS	Minerals
Whey protein concentration	70-80%	more	more	more
Whey protein isolate	90%	less	less	less
Whey protein hydrolysate	80%	absorb	absorb	absorb

Table 2. Composition of Whey Protein

S.No	PROTEIN	APPRX CONTENT G/L WHEY	TOTAL WHEY PROTEIN %
1	α-Lactoalbumin	0.6-1.7	20
2	β-lactoglobulin	2.0-4.0	55
3	Serum Albumin	0.2-0.4	5
4	Immunoglobulins	0.5-0.4	8
5	Proteose-peptone	0.2-0.4	12
6	Other(casein,glycoprotein	0.1	1

Table 3. protein changes in skim milk due to heat treatment & cold storage.

	TOTAL PROTEIN		WHEY PROTEIN	
	X%	SD[1]	X%	SD
CONTROL	3.42	0.13	0.63	0.06
Heattreated, freshed[2]	3.42	0.13	0.59	0.04
Heat-treated, stored[3]	3.42	0.13	0.55	0.05
[1]n=46				
[2]Milk heat-treated 74°:C/10 s, analysis completed within 24 h				
[3] Milk heat-treated 74°C, then stored 7 days at 3°C prior to analysis				

Table 4. Denaturation degree of whey protein for heating skim milk

Temperature Holding Time	Relative RCT (%)	Denaturation of β-lactoglobulin	Denaturation of α-Lactoalbumin
105/4	127.46 ± 13.19	0.79 ± 4.29	4.80 ± 1.21
115/4	138.92 ± 10.56	15.75 ± 11.36	9.23 ± 1.92
130/4	192.26 ± 40.79	25.29 ± 4.86	10.53 ± 5.65
145/4	420.81	39.35 ± 2.83	15.35 ± 2.97

Table 1. Denaturation of composition of whey protein

S.No	Protein	Apprx Content G/L Whey	Total Whey Protein %	Denaturation Temperature
1	α-Lactoalbumin	0.6-1.7	20	61
2	β-lactoglobulin	2.0-4.0	55	82
3	Serum Albumin	0.2-0.4	5	66
4	Immunoglobulins	0.5-0.4	8	72
5	Proteose-peptone	0.2-0.4	12	
6	Other(casein,glycoprotein	0.1	1	

Table 6. Effect of cold storage and mixing various lactating milk on the chemical composition of buffalo's and cow's milk.

S.No	Treatment	Acidity, %		Total solids, %		Fat, %		Protein, %	
		Buffalo	cow	Buffalo	Cow	Buffalo	cow	Buffalo	cow
A	Fresh morning milk.	0.16	0.15	16.7	11.6	3.3	7.4	4.84	3.14
B	Cold milk at 4 -5°C for 10 hours	0.17	0.16	16.7	11.7	3.3	7.4	4.81	3.17
C	Fresh evening milk.	0.15	0.14	16.8	11.9	4	7.4	4.94	3.27
D	Mixing milk of treatments B and C.	0.16	0.15	16.7	11.7	3.7	7.4	4.85	3,18
E	Milk of treatment D after storage at 4 -5°C for 24 hours	0.17	0.16	16.7	11.7	3.7	7.4	4.86	3.16
F	Milk of treatment A & E after cold storage 4 -5°C for evening next day.	0.18	0.16	16.6	11.6	3.7	7.2	4.77	3.17
H	Milk of treatment C & G after cold storage 4 -5°C for 48 hours	0.19	0.17	16.8	11.8	3.6	7,7	4.85	3.14

Table 2. protein changes in skim milk due to heat treatment and cold storage

	TOTAL PROTEIN		WHEY PROTEIN	
	X%	SD[1]	X%	SD
CONTROL	3.42	0.13	0.63	0.06
Heat-treated,freshed[2]	3.42	0.13	0.59	0.04
Heat-treated,stored[3]	3.42	0.13	0.55	0.05
[1]n=46				
[2]Milk heat-treated 74°:C/10 s, analysis completed within 24 h				
[3] Milk heat-treated 74°C, then stored 7 days at 3°C prior to analysis				

storing (7 days) milk prior to cheese making. Protein analysis of milk indicated that casein was higher and whey proteins lower in experimental heated, cooled, stored milk as compared with un-heated, fresh (<2 days old) control milk. Heating caused some proteins normally in whey to associate with casein by some undetermined mechanism. Samples of control and experimental milk subjected to centrifugation (21,000 x g/120 min) and gel electrophoresis showed that solu- ble l~-casein decreased with heat treat- ment and cold storage of milk. Pretreating raw milk with nitrogen- ethylmaleimide or ethylenetetraacetic acid prior to heat treatment and storage and subjecting these samples to electro-phoresis indicated little or no decrease of whey proteins compared with milk heated without added nitrogen-ethylmalei- mide or ethylenetetraacetic acid. Heat- treating, cooling, and storing milk seemed to cause part of the/3-casein to be trapped physically in the casein micelle. These treatments also caused portions of whey proteins to associate with casein micelles via disulfide bridging and calcium link- ages.

2.2.2 *Effect of cold storage and heating of camel, cow and buffalo's milk*

The effect of cold storage (4°C/48 h.) and heating (85°C/5 min.) on some properties of camel's, cow's and buffalo's milks were studied. These included acidities, pH, nitrogen distribution, rennet coagulation time (RCT), surface tension (ST), foam expansion (FE), foam volume stability (FVS), buffer intensity, the electrophoretic properties and microstructure. Cold storage of all milk samples increased acidity, and decreased pH, ST, RCT (except camel's milk) and FE (except cow's milk). No significant effect on CN, NCN and WPN was found. Heating all milk samples increased acidity, casein nitrogen (CN) and RCT (except camel's milk), decreased non-casein nitrogen (NCN), whey protein nitrogen (WPN) and nonsignificant effect on ST. Also, heating decreased only FE of buffalo's milk and increased the others, while FVS of camel's milk was not recorded (after 15 min.). In all cases, buffer intensity curves showed same peaks and were the highest at pH (7-8) and (6.5-7.5) in buffalo's and cow's milk respectively. However, camel's milk had less buffering capacity compared with either buffalo's or cow's milk. Concerning, the electrophoretic patterns, some whey proteins especially β-lactoglobulin (β-Lg) disappeared on heating, whereas cold storage slightly decreased β-casein. In general, camel's milk showed different protein patterns. There was little microstructure difference between raw and cooled (4°C/48 h.) camel's, cow's and buffalo's milk; while heat treatment increased the size of casein micelles in all samples (Hassan in drugi, 2009). Protein changes in skim milk due to heat treatment & cold storage is shown in table 7

Influence of cold storage and mixing on properties of buffalo's and cow's milk:

The effect of cold storage (4-5°C) for 48 hours and blinding different milkings to cold stored milk on some chemical composition, rheological properties and microbial quality of the buffalo's and cow's milk were studied. The results obtained indicate that no clear effects of cold storage of buffalo's and cow's milk on them TS, fat and total protein contents whereas the acidity values slightly increased. Cold storage of both kinds of milk increased the rennet coagulation time (RCT), curd tension and curd syneresis values. Also, preservation of buffalo's and cow's milk at 4-5°C increased total viable bacterial (TVBC), lactic acid, psychrophilic bacteria, proteolytic, lipolytic, colifom bacteria, sporeformers, and moulds and yeast counts. Adding evening and morning milk to cold stored buffalo's and cow's milk decreased the acidity and curd syneresis values and increased the pH, TS, fat, total protein, RCT and the curd tension values. Mixing evening and morning milk with cold stored milk lowered the above-mentioned microbial groups (Ammar in drugi, 2010).

Conclusion

Cold storage of all milk samples increased acidity, and decreased pH, ST, RCT and FE. Heat treatment decreased only FE of buffalo's milk and increased the others. Some whey proteins especially β-lactoglobulin (β-Lg) disappeared on heating, whereas cold storage slightly decreased β-casein. There was little microstructure difference between raw and cooled (4°C/48 h.) cow's and buffalo's milk; while heat treatment increased the size of casein micelles in all samples.

Author contributions

N.S. conceptualized the project and developed the methodology. U.N. and I. conducted a formal analysis and drafted the original writing and contributed to the methodology. S.Z. conducted investigations, provided resources, visualized the data and contributed to reviewing and editing the writing.

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Competing financial interests

The authors have no conflict of interest.

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