

## Whey proteins-Properties and Possibility of Application

Snežana Jovanović, Miroljub Barać, Ognjen Maćej

Review – Revijalni prikaz

UDC: 637.138/344.8

### **Abstract**

*Whey protein fractions represents 18-20% of total milk nitrogen content.  $\beta$ -lactoglobulin is the major milk serum protein, while  $\alpha$ -lactalbumin represents about 20% of total serum proteins or 2-5% of total milk nitrogen content. Whey proteins are highly susceptible to heat-induced denaturation; irreversibly denature and coagulate when exposed to high temperatures. Thermal treatment causes interaction between milk proteins, especially the interaction of  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and  $\kappa$ -casein. Complexes between them are known as coaggregates.*

*Since the early 1980s, whey proteins have grown in popularity as nutritional and functional food ingredients. Serum proteins possess favorable functional characteristics such as gelling, water binding, emulsification and foaming ability. Due to the use of membrane fractionation techniques, it is possible to produce various whey-protein based products. The most important products based on the whey proteins are whey protein concentrate (WPC), whey protein isolate (WPI) and hydrolyzed whey protein (HWP).*

*This review discusses the properties of major whey protein components and their possible use in dairy industry.*

*Key words: whey proteins, functional properties, high heat treatment.*

### **Introduction**

Whey protein products, such as whey protein concentrate and whey protein isolate are ingredients widely used in the food industry due to the excellent functional and nutritive properties. Moreover, these products represent the best way for the utilization of whey proteins.  $\beta$ -lactoglobulin ( $\beta$ -Lg),  $\alpha$ -lactalbumin ( $\alpha$ -La) are the major whey proteins (represent approximately 70% of the whey protein fraction) and the properties of whey based protein products are reflected on their composition and structure. Also, the properties of whey based protein products are heavily dependent on their processing technology. Several different treatments including heat treatments and membrane fractionation techniques, have significant influence on their properties and consequently on their possible use. The scientific literature

contains numerous references to whey protein functionality research. The objective of many of this research is to develop systematic approach that would provide better understanding of the physico-chemical properties of the individual and the total whey protein system. This information is essential for manufacturing and utilizing whey protein ingredients that will meet the food industry's functionality requirements. The purpose of this paper is to highlight the whey protein properties and their possible use in dairy industry.

### ***Whey proteins***

Whey protein fraction represents about 18-20% of total milk proteins. This fraction contains four major proteins:  $\beta$ -lactoglobulin ( $\beta$ -Lg),  $\alpha$ -lactalbumin ( $\alpha$ -La), blood serum albumin (BSA) and immunoglobulin (Ig). These proteins represent 20%, 50% and 10% of whey protein fraction, respectively. Except them, minor components of this fraction are lactoferrin, blood transferrin, lactolin and proteose-peptone fraction (PP). Due to the different amino acid composition (de Wit and Klarenbeek, 1984; Morr, 1985; Đorđević, 1987; Mullvihill and Donovan, 1987), whey proteins have different globular structures. These proteins possess high levels of secondary, tertiary and in the most cases, quaternary structures.

The major whey proteins,  $\beta$ -Lg and  $\alpha$ -La are well characterized. Native  $\beta$ -Lg is small globular protein (mw 36.6 kDa) with defined secondary and tertiary structure. Molecule of  $\beta$ -Lg is made of  $\alpha$ -helical,  $\beta$ -sheet and random coil structures present up to 10-15%, 43% and 47%, respectively (Swaisgood, 1986; Boye et al., 1996). In aqueous solution at pH 5-7 and room temperature predominantly is present as a dimer made of two identical subunits. Molecular weight of each subunit is 18.3 kDa (de Wit and Klarenbeek, 1984; Caessens et al., 1997). It is composed of 162 amino acid residues; 84 of these are essential amino acid and four cysteinil-residues.  $\beta$ -Lg contains two disulphide bonds and one sulphhydryl group (Cys<sup>121</sup>) that is buried within the native protein, but becomes exposed and active after denaturation of the protein by various agents (including heat) and can then undergo sulphhydryl-disulphide interactions with itself or other proteins (Swaisgood, 1986; Galani and Owusu Apenten, 1999; Sawyer et al., 1999; Fox, 2003). These interactions happen at pH 7.0 (de Wit and Klarenbeek, 1984; Godovac-Zimmermann and Braunitzer, 1987; Mullvihill and Donovan, 1987) and temperature range between 60-65 °C.

$\beta$ -Lg is liable to association and dissociation. The association behavior is pH-dependent (Farrell and Thompson, 1974; Dalgalarrrondo et al., 1990; Boye et al., 1996; Sawyer et al., 1999). At pH range of milk (Swaisgood, 1986; Boye et al., 1996; Caessens et al., 1997),  $\beta$ -La exists as a stable dimer (Swaisgood, 1986). Due to the high electrostatic repulsion, at pH 3.5  $\beta$ -La reversibly dissociates into monomers (Swaisgood, 1986). In opposite, several authors (Dalgalarrrondo et al., 1990; Boye et al., 1996) reported that  $\beta$ -La existed as a monomer form at lower pH (>3.5), as well as at over pH 7.5 (Demetriades et al., 1977a). According to Boye et al. (1996), in the range of pH from 3.7 to 6.5, dimer of  $\beta$ -La reversibly associates into an octamer.

$\alpha$ -lactalbumin is a small (mw 14.2 kDa) acidic (pI 4-5)  $\text{Ca}^{2+}$  binding whey protein. According to several authors (Gordon and Kalan, 1974; Whitney et al., 1976; Bernal and Jelen, 1984; Eigel et al., 1984) this protein exists in two genetic forms (A and B, m.w. 14.146, 14.174, respectively), while Fox (1989) reported one additional form (C).  $\alpha$ -lactalbumin is a single polypeptide chain, made of 123 amino acid residues; 67 of them are from essential amino acids. It contains four disulphide bonds and has no free sulphhydryl groups. Native molecule of  $\alpha$ -La consists of two domains: a large  $\alpha$ -helical domain and small  $\beta$ -sheet domain, which are connected by calcium binding loop (Permyakov and Berliner, 2000). Due to its hydrophilic properties,  $\alpha$ -La does not precipitate from milk at pI (Gordon and Kalan, 1974; Eigel, et al., 1984; Swaisgood, 1986).

### ***The influence of high temperature on whey proteins***

Whey proteins are heat-labile proteins. According to Donovan and Mullvihill (1987), heat decreases their stability in the following order: PP> $\alpha$ -La> $\beta$ -Lg>BSA>Ig. Thermal denaturation of whey proteins is two-step process. First step is unfolding, which may be reversible or irreversible and includes aggregation, which generally follows after irreversible unfolding (de Wit and Klarenbeek, 1984). Thermal treatments cause significant alteration of whey protein structures. Consequently, these treatments change their physico-chemical properties including water solubility, water holding capacity, emulsifying, foaming and gelling properties (Đorđević, 1987). Contrary to the casein, whey proteins are completely denatured after 5 minutes of heating at 90 °C. Denaturation of whey proteins starts at 65 °C, but mostly occurs during milk heating at temperature above 80 °C (Maćej, 1983).

According to Duranti et al. (1989) heating at 85 °C is critical for whey proteins denaturation. The degree of the whey protein denaturation is often determined by the degree of  $\beta$ -Lg denaturation, since it represents about 50% of total whey proteins (Morr, 1985; Đorđević, 1987).

Available literature contains numerous references that describe the effects of thermal treatments on the major whey proteins. Generally, thermal denaturation of  $\beta$ -Lg takes place in two phases, primary and secondary (Sawyer, 1968; Elfagm and Wheelock, 1978a; 1978b; Kirchmeier et al., 1985; Morr, 1985). In the first phase, dimers dissociate and four monomers interact via sulfhydryl groups and form small aggregates. Small aggregate formation occurs at temperatures above 70 °C (Sawyer, 1968, Kirchmeier et al., 1985; Morr, 1985), while the maximum velocity reaches at a range of 80-85 °C (de Rham and Chanton, 1984; Donovan and Mullvihill, 1987). In the second phase (usually named as “non-specific”), small aggregates interact via non specific interactions and form high molecular weight aggregates. According to Elfagm and Wheelock (1978a, 1978b) this phase occurs at higher temperatures than the initial phase. Factors including pH, concentration of salt, sugar and proteins have significant influence on thermal behavior of  $\beta$ -Lg. Several authors reported pH-dependent thermal denaturation of  $\beta$ -Lg (Elfagm and Wheelock 1978a, 1978b; Hillier et al., 1979; de Wit and Klarenbeek, 1984; Boye et al., 1996). This protein is most sensitive at pH 9.0; the denaturation starts at 43 °C, while the loss of secondary structures starts at 51 °C. At pH 6.0, thermal denaturation occurs at 78 °C, while at pH 5.5 starts at 76 °C (de Wit and Klarenbeek, 1984). Maximal thermal stability of  $\beta$ -Lg was registered at pH 3.0 (Boye et al., 1996).

Besides PP-fraction,  $\alpha$ -La is the most stable protein of whey fraction. Thermal stability of  $\alpha$ -La is a result of four disulfide bonds incorporated in polypeptide chain and the absence of free -SH groups. Thermal treatments at 100°C for 10-30 minutes disrupt about 12-20% of disulfide bonds (Schnack and Klostermeyer, 1980). Thermal behavior of  $\alpha$ -La is determined by several factors such as pH (de Wit and Klarenbeek, 1984), thermal treatment mode (Chaplin and Green, 1980; Bernal and Jelen, 1985) and the presence of  $\beta$ -Lg (Elfagm and Wheelock, 1978a).

***Thermally induced complex between whey proteins and casein***

During thermal treatment, milk proteins may interact and form chemical complexes. Protein complex between  $\alpha$ -La and  $\beta$ -Lg,  $\alpha$ -La and  $\kappa$ -casein, as well as complex between  $\beta$ -Lg and  $\kappa$ -casein occurs. In literature, chemical complexes between milk proteins are known as coaggregates of milk proteins (Elfagm and Wheelock, 1977; 1978a; Maćej, 1983; Maćej, 1989). Many studies have been carried out to identify the mechanism of coaggregates formation. In available literature, two theories explain the mechanism of coaggregate formation. According to the first theory, it is a two step process. In the first step denatured whey proteins ( $\alpha$ -La and  $\beta$ -Lg, mostly) aggregate at a ratio dependent on the initial individual whey concentrations. These complexes subsequently associate with the casein micelles while prolonged heated (Corredig and Dalgleish, 1996). The second theory (Mottar et al., 1989) suggests that at high temperatures  $\beta$ -Lg denatures first, than interacts with casein. The major interaction appears to involve thiol-disulfide exchange reactions between the denatured  $\beta$ -Lg and  $\kappa$ -casein at the micelle surface. Molecules of  $\beta$ -Lg radially cover the casein micelle. The formed complex pronounced hydrophobic properties. The newly-formed surface is ragged with numerous filaments that originate from the  $\beta$ -lactoglobulin. At a higher temperature (90 °C/10 min)  $\alpha$ -lactalbumin denatures and binds to the filaments of  $\beta$ -lactoglobulin, thus filling "gaps" on the micelle surface, which is converted from ragged into regular spherical form. Due to the presence of  $\alpha$ -lactalbumin, there is an increase in the complex hydrophilic properties. Higher  $\alpha$ -lactalbumin content influences more pronounced hydrophilic properties of the coaggregates at pH 4.5.

Several authors (Maćej, 1983; Maćej and Jovanović, 2000; Mottar et al., 1989; Corredig and Ddalgleish, 1996) have investigated the influence of different processing factors including thermal treatment mode, temperature and the duration of heating, pH and the whey protein concentration on coaggregates formation. The degree of complex formation is influenced by the regime of the thermal treatment. The interaction between whey proteins and casein increases with the increase of temperature from 75 to 90 °C.

According to Long et al. (1963), the largest amount of complex between  $\kappa$ -casein and whey protein is formed at 85 °C. At higher temperatures, the interactions are faster, but the amount of formed complex is minor. According

to Maćej (1989) the degree of complex formation at 87 °C/10 min. is the same as at 90, 95 °C/10 min.

The amounts of the whey proteins associated with casein micelles increase to a finite, maximum value during heating (Corredig and Dalgleish, 1999). The degree of these interactions is determined by heat transfer conditions. During HTST pasteurization  $\alpha$ -lactalbumin reacts more slowly and to the lesser extent than  $\beta$ -lactoglobulin (Corredig and Dalgleish, 1996). Heat transfer conditions during UHT sterilization (direct or indirect) also influences the rate and the degree of complex formation. It is lower during direct sterilization (DSI) than during indirect UHT sterilization.

The association of denatured whey proteins with micelles is pH dependent. The investigations performed on heated model systems and milk suggested that these association decreased as the pH increased from 6.3 to 7.3. Anema and Li (2003) registered high levels of interactions at pH 6.50-6.55, and low levels of interactions at pH 6.7.

### ***Whey protein based products***

Due to its nutritive value and beneficial functional properties, whey proteins became interesting in the late 1980s. Development of membrane fractionation techniques including ultrafiltration, reverse osmosis and microfiltration, enabled the production of wide whey protein products, such as whey powder, whey protein concentrate (WPC), whey protein isolate (WPI), whey protein hydrolysate (WPH) and pure lactoglobulin and lactalbumin (Carić, 1990; Popović-Vranješ and Vujičić, 1997).

Whey protein concentrates are products with protein content variations from 35-80%.

Figure 1 shows basic techniques involved in WPC-production. The most widely used method for the production of WPC is ultrafiltration (Fox, 2003).

Whey protein isolate is a high-quality product containing approximately 95% of proteins. The use of ion-exchange and/or whey filtration is an effective method for the WPI-preparation. Although the functionality of WPI is better than that of WPC on an equal-protein basis (due to the lower levels of lipids, lactose and salts), their production is limited due to the higher cost of production. Whey protein hydrolyzates are obtained by limited hydrolysis under strictly controlled condition; it is possible to create specific nutritive and functional properties.

*Figure 1: Techniques involved in the manufacture of whey protein concentrates (Fox, 2003)*

*Slika 1: Tehnike koje se primjenjuju u proizvodnji koncentrata proteina sirutke (Fox, 2003.)*

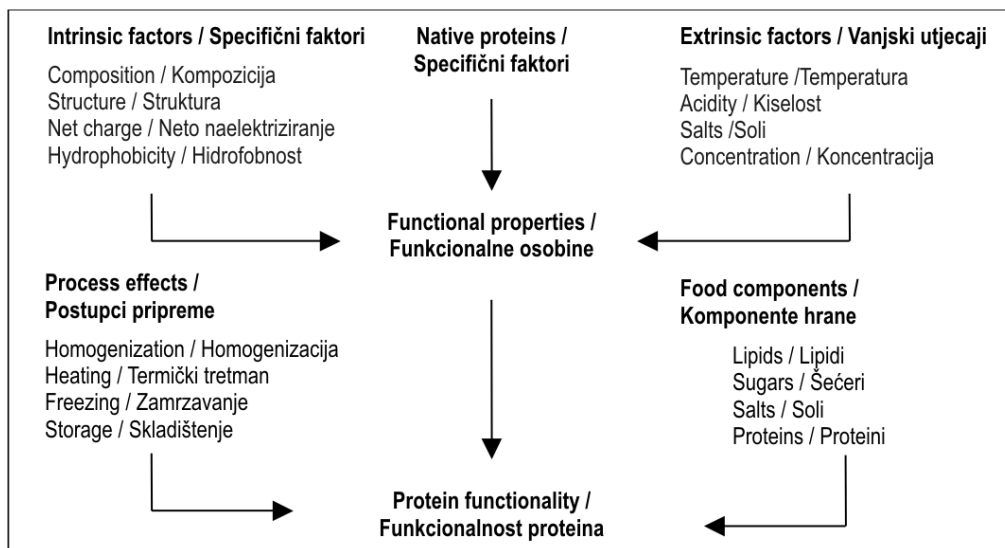
Membrane fractionation Membransko frakcioniranje	Ultrafiltration/Ultrafiltracija Reverse osmosis/Reverzna osmoza Electrodialysis/Elektrodijaliza Dialysis/Dijaliza Microfiltration/Mikrofiltracija
Precipitation or complexing with reagents Taloženje ili kompleksiranje s reagensima	Metaphosphates/Metafosfati Carboxymethyl cellulose (CMC) Karboksimetil celuloza Polyacryl acids/Poliakrilna kiselina Iron/Metal Alcohol/Alkohol
Physical and chromatographic separations Fizičke i kromatografske separacije	Gel filtration/Gel filtracija Ionska izmjena/Ion-exchange Inertni adsorbensi/Inert adsorbents Ultracentrifugation/Ultracentrifugiranje Foam concentration/Koncentracija pjene Electro-flotation/Elektroflotacija Thermal precipitation/Termička precipitacija

### ***Functional properties of whey proteins and whey protein based products***

Functional properties of the whey proteins are fundamentally related to its physical, chemical and structural/conformational properties. These include: size, shape, amino acid composition and sequence, charge and their distribution, hydrophilic / hydrophobic ratio, secondary structure content and their distribution, tertiary and quaternary arrangement of the polypeptide segments, inter- and intra- cross-links, and the rigidity/flexibility of the protein in response to external conditions. Factors such as processing conditions, the method of isolation, environmental factors (e.g., pH, temperature, ionic strength, etc.), and interaction with other food components alter the functional properties of the whey proteins (McCrae et al., 1999). Such alterations, however, are simply manifestations of alterations in the conformational and structural features of the proteins caused by these external factors. In figure 2 are shown driving forces involved in achieving functional properties and functionality of whey proteins.

Figure 2: Factors involved in determination of whey proteins functional properties and functionality (de Wit, 1998)

Slika 2: Faktori koji određuju funkcionalne osobine i funkcionalnost proteina sirutke (de Wit, 1998.)



An important aspect of whey proteins is their success as emulsifiers in food systems. Many studies have been carried out to identify the optimum conditions under which whey proteins individually and in mixtures, perform as emulsifiers (Klemaszewski et al., 1992; Demetriades et al., 1997a; 1997b; McCrae et al., 1999). Important factors determining their emulsification properties are protein concentration, pH, ionic strength, the concentration of calcium and lactose, the processing history and the storage conditions (McCrae et al., 1999).

The ability of whey proteins to form gels capable of holding water, lipids, and other components while providing textural properties is very important to the consumer acceptability of many foods such as processed meat, dairy and bakery products. Two types of whey protein gels are extensively studied; heat-induced (Mullvihill and Kinsella, 1987; Manigo, 1992; Lupano et al., 1996) and gels induced at room temperatures (Nakamura et al., 1995; Sato et al., 1995; Elofsson et al., 1998; Resch and Danbert, 2002). The second type is result of so cooled cold-set gelation. These gels are prepared by firstly heating whey protein solutions to achieve unfolding and aggregation, followed by quickly cooling to room temperature, and then producing gelation



Figure 3: Whey protein concentrate (WPC) applications depending on functional properties ([www.milkingredients.ca](http://www.milkingredients.ca))

Slika 3: Primjena koncentrata proteina sirutke ovisno o funkcionalnim osobinama ([www.milkingredients.ca](http://www.milkingredients.ca))

Functional property Funkcionalna osobina	Reacion mechanism / Način djelovanja	Foodstuff /Prehrambeni proizvod
Water binding; hydration Vezivanje vode; hidratacija	Proteins can help reduce formula costs as the proteins hold additional water Proteini utječu na smanjenje cijene proizvoda kroz vezivanje dodane vode	Meats, beverages, breads, cakes, sausages Meso, napitci, kruh, kolači, kobasice
Gelation; viscosity Želiranje; viskozitet	Protein-protein interactions produce matrix formation and setting Interakcije protein-protein uzrokuju formiranje strukture i oblika	Salad dressings, soups, setting cheeses, baked goods, gravies, meats Preljevi za salate, juhe, sirni namazi, pečena hrana, umaci, meso
Emulsification /Emulgiranje	Proteins stabilize fat emulsions Proteini stabiliziraju emulzije masti	Sausages, soups, cakes, salad dressings, infant foods Salame, juhe, keksi, preljevi za salate, dječja hrana
Foaming; whipping Obrazovanje pjene; sposobnost lupanja	Proteins form stable film. Foaming properties are the best when the whey proteins are not denaturated, and not competing with other surfactants at the air/water interface Proteini obrazuju stabilan film. Osobine pjene najpovoljnije su kada serum proteini nisu denaturirani, kada ne reagiraju s ostalim površinski aktivnim supstancama u sistemu zrak/voda	Whipped dressings, chiffon cakes, desserts Tučeni preljevi, hrskavi keksi, deserti
Browning; flavour; aroma Tamnjenje; ukus; aroma	Proteins contribute to browning by reacting with lactose and other reducing sugars present in a formulation, providing colour to heated products. WPC is bland tasting and contribute no foreign or off-flavours when used as an ingredient Proteini doprinose stvaranju tamnije boje termički tretiranog proizvoda, reakcijom s laktozom i drugim reducirajućim šećerima prisutnim u proizvodima. KPS su neutralnog ukusa i kao aditivi ne doprinose formiranju stranog ili neprihvatljivog okusa.	Confections, meats in microwave, sauces, breads, low-fat baked goods, soups, dairy products Slatkiši, meso namijenjeno za pripremu u mikrovalnoj pećnici, umaci, kruh, pečeni proizvodi sa smanjenim sadržajem masti, juhe, mliječni proizvodi

with the addition of NaCl or CaCl<sub>2</sub> (Resch and Danbert, 2002). Generally, gel formation is two step process: initial unfolding of protein structures, and

aggregation of denatured polypeptides through different interactions. Obtained gel with WPC includes several interactions, such as hydrophobic, electrostatic, disulfide interactions and hydrogen bridges. Whey protein concentrates have different gelling capacity. Generally, WPC products are able to gel at 60-90°C present in concentration of 80-120 g/L. The gelling process is influenced by temperature and duration of heating, pH, and ionic strength, concentration of salt, protein, sugar and lipids.

Foaming properties of whey proteins mainly depend on the degree of their denaturation. These properties are the best for non-denatured whey proteins. Due to a degree of denaturation, Ca-ion concentration, temperature, pH, lipid content (Popović-Vranješ and Vujičić, 1997) foaming properties of whey proteins varied.

#### ***The application of whey proteins and whey protein products acid fermented products***

Due to the wide assortment and versatility, acid-milk products become popular all over the world. The quality of these products depends on factors such as a sort of milk, physical and chemical properties of milk, protein content, heat treatments used, type and quantity of starter culture, temperature of fermentation and other. In the past ten years, many workers have studied the effect of concentrated whey; whey powder as well as whey protein concentrates addition in acid fermented-milk products preparation. The aim of these investigations was to standardize dry matter of milk used for this purpose and to obtain final products with better nutritive value, sensory and rheological properties.

Heat treatment is well known factor that produces better consistency of yoghurt. The primary aim of stronger heat treatments use is to destroy microflora of milk as well as to inactivate milk enzymes. Also, heat treatment induces the ionic status of mineral components and consequently the reduction of milk coagulation duration. As a result of coaggregates formation during strong heat treatment, gel particles have better properties and product has fine structure (Mottar et al., 1989). At the same time, heat treatment increases the hydration of casein micelles. The recommended treatments are those which cause the denaturation of 70-95% whey proteins, while 80-85% of denaturation is suggested as optimal. This degree of denaturation could be obtained during treatments at 80-85 °C for 20-30 minutes, as well as at 95 °C for 5 min. Yoghurt prepared by treatment under these conditions is more compact and has better protein network made of small casein micelles. The

perforations of matrix are 3-10  $\mu\text{m}$ , while at standard procedure these values are 5-20  $\mu\text{m}$ . This influences the water stability of yoghurt. Also, diameter of casein micelles of yoghurt prepared from heat treated milk is two times smaller (Mottar et al., 1989).

The addition of whey proteins into milk used for acid-fermented drinks caused the change of casein: whey protein ratio (CSO). This influences thermal stability of milk. Milk with low CSO (for example 20: 80 or 40: 60) coagulated during heat treatment and could not be used for this purpose. The change of CSO ratio has the influence on the hardness of acid fermented gel only in the case of usage of WPC prepared by electrodialysis, while WPC prepared by ultrafiltration does not have negative effect on gel hardness (Kalab et al., 1983). The sweet whey powder application does not have significant influence on the decrease of yoghurt hardness. The recommended concentration of whey protein for yoghurt preparation is 1-2%. Higher concentration caused undesirable odor (Swaisgood, 1986).

Huginin (1999) investigated the effect of skim milk powder and WPC products on viscosity and syneresis of acid fermented drinks. The results of these investigations are shown in Figure 4.

*Figure 4: Effect of fortification with WPC34 and skim milk powder on viscosity and syneresis (Huginin, 1999)*

*Slika 4: Efekt dodavanja KPS34 i obranog mlijeka u prahu na visokzitet i sinerezis (Huginin, 1999.).*

Sample/Uzorak	Consistency (Pas) Konzistencija	Syneresis (mL)/Sinerezis
Control /Kontrolni	$57.5 \times 10^{-3}$	22
Fortification with 2% SMP Fortifikacija s 2% OMUP	$94.5 \times 10^{-3}$	17
Fortification with 2% WPC34 Fortifikacija s 2% KPS34	$117 \times 10^{-3}$	7

Legend/Legenda

Skimmed milk powder-SMP / OMUP-obrano mlijeko u prahu

WPC – whey protein concentrates / KPS – koncentrat proteina sirutke

### ***The application of the whey proteins and whey protein products in cheese making***

Whey proteins could be inducted in casein network by several techniques of cheese making. The use and the processing method mostly depend on

cheese type and desirable texture. One of the usually used methods that reduce water phase is ultrafiltration. In production of soft, semi-hard and hard cheese, whey proteins could be added as WPC or WPI products. During preparation of Cheddar with the addition of WPC, Banks et al. (1987) obtained the increase of yield from 9.96% (cheese prepared through traditional process) to 10.31% (samples in which WPC is added after whey extracting) and to 11.21% (samples in which WPC is added during inoculation with starter culture).

Microparticulation is the second type of whey protein incorporation into casein matrix. This implies incorporation of small whey protein particles, 1-10  $\mu\text{m}$  in size. This process is conducted in the same way as in the case of the lipid particles. As a result, yield and nutritive value of cheese increases as well as sensory properties of low fat cheese (Hinrichs, 2001).

The use of stronger thermal treatment is a possible way for incorporation of whey protein into cheese structure. It is based on coaggregation of casein and whey protein. Besides positive effects, this method causes several problems. Namely, milk treated under these conditions has altered technological properties; the rennet induced coagulation is slower, as well as the ratio of extracted whey, while formed gel has less hardness (Ghosh, et al., 1996). Furthermore, cheese prepared in this way has different taste and texture than traditional cheese. Due to these facts cheese making based on coaggregates formation is complicated.

The incorporation of coaggregates during cheese making processes has been the object of several investigations. According to Lawrence and Lelièvre (1990) stronger treatments of milk used for cheese making caused the increase of water content in cheese. Especially, this is limiting factor in processing of semi-hard and hard cheese. Additionally, after strong thermal treatment it is hard to obtain compact curd. Previously, these treatments were applicable in the production of cheese without rennet and with higher water content, such as Quesco Blanco and Ricotta. Marshall (1986) has prepared Cheshire from milk treated at 97 °C for 15s with higher dry matter, protein and milk fat content. These samples had approximately for 4.5%, 6.7% and 0.7 % higher dry matters, protein and fat content, respectively, than control samples. Banks et al. (1987) prepared Cheddar cheese with coaggregates. These authors established that the increase of pH of milk to 5.8 caused better activity of hyposine and the formation of curd with better rheological properties. Also, the whey had 50% lower nitrogen content in comparison with samples obtained according to the traditional procedure. In series of experiments, Ghosh et al. (1996) prepared Camembert from pasteurized milk (72 °C/15s)

and milk treated at 80 °C for 3 min., as well as from mixture (30:70) of thermal treated milk (90 °C/6 min.) and raw milk. They obtained better yield, higher degree of dry matters and nitrogen utilization with milk treated at higher temperatures and with the mixtures of treated and raw milk. After 10-days of ripening, lower dissipation of water and soluble nitrogen for these samples was registered.

In our country, a few authors investigated possible production of coaggregates based cheese (Jovanović, 1994; 2001; Jovanović et al., 1996, 2002; 2004; 2005; Maćej et al., 1995; 2001; 2004a; 2004b; 2004c; Maćej and Jovanović, 1999). Maćej (1989; 1992; 1994) and Maćej et al. (2004a) prepared soft cheese (fresh cheese, white cheese in brine, Camembert) and registered that over 90 % of nitrogen crossed into cheese. Whey had 56% lower nitrogen content than samples prepared according to the traditional procedure. Pudja (1992a, 1992b) investigated technological parameters and biochemical alterations during ripening of semi-hard and hard cheese prepared from ultrafiltrated (UF) milk with previously formed coaggregates. These cheeses had developed taste and odor. Consequently, stronger treatments of milk could be used for cheese ripening time reduction. Jovanović (2001) modified technological process and prepared semi-hard cheese. The average yield after production was 14.52% while and 11.58% after 4 month of ripening. High percentage of nitrogen distribution and fat components from milk to cheese were registered.

Milk with formed coaggregates could be used for acid coagulated cheese making. Through this process, according to Southward (1978), the nitrogen efficiency coefficient was 92.7-95.8%, and significantly higher than in the case of pasteurized milk. The similar results were reported by Maćej (1983) and Maćej et al. (1996). According to them, through coprecipitates, 94.70 % of nitrogen was isolated. In the opposite, only 83.88% of nitrogen from pasteurized milk was retained in cheese. Jovanović (1994) prepared acid coagulated semi-hard cheese from milk with previously coaggregates formed. Milk was precipitated with citric acid, lactic acid and acetic acid. The best results were obtained with lactic acid; 87.70 % of nitrogen crossed into cheese.

### ***Instead of Conclusion***

Due to the high nutritive value, whey proteins are considered as functional food additives. During the last ten years intensive work has been carried out to investigate the effect of whey, whey protein concentrate, isolate and hydrolysate and whey powder usage on nutritive, rheological and sensory

characteristics of milk products. Furthermore, whey protein products are applicable in other industries, such as meat industry and infant food industry.

## SERUM PROTEINI – OSOBINE I MOGUĆNOST PRIMJENE

### Sažetak

Proteini mliječnog seruma predstavljaju 18-20% ukupnih dušikovitih tvari mlijeka. Dominantni serum protein je  $\beta$ -laktoglobulin, dok je  $\alpha$ -laktalbumin zastupljen s oko 20%, odnosno 2-5% od ukupnih dušikovitih tvari mlijeka. Serum proteini su osjetljivi na djelovanje topline; ireverzibilno denaturiraju i koaguliraju pri djelovanju visokih temperatura. Djelovanje visokih temperatura uzrokuje kemijske interakcije među proteinima mlijeka, a posebno između  $\alpha$ -laktalbumina,  $\beta$ -laktoglobulina i  $\kappa$ -kazeina. Kompleksi koji se ostvaruju među njima poznati su pod nazivom koagregati proteina mlijeka.

Od osamdesetih godina prošlog stoljeća, serum proteini značajni su i kao nutritivni i kao funkcionalni aditivi. Serum proteini imaju dobre funkcionalne osobine. Mogu se koristiti kao sredstva za želiranje, za vezivanje vode, emulgiranje i obrazovanje pjene. Zahvaljujući primjeni membranskih tehnika frakcioniranja, danas je moguće proizvesti različite aditive na bazi serum proteina. Najvažniji proizvodi na bazi proteina sirutke su: proteinski koncentрати, izolati i hidrolizati proteina sirutke. U ovom su radu prikazane osobine dominantnih proteina sirutke i njihova moguća primjena u industriji mlijeka.

*Ključne riječi: serum proteini, funkcionalne osobine, visoka toplinska obrada*

### References

- ANEMA, S. G., LIE, Y. (2003): Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size, *J. Dairy Res.* 70, 73-80.
- BANKS, J. M., STEWART, G., MUIR, D. D. AND WEST, I. G. (1987): Increasing the yield of Cheddar cheese by the acidification of milk containing heat denatured whey protein. *Milchwissenschaft* 42 (4), 212-215.
- BERNAL, V. and JELEN, P. (1984): Effect of calcium binding on thermal denaturation of bovine  $\alpha$ -lactalbumin. *J. Dairy Sci.* 67 (10), 2452-2454.
- BERNAL, V. and JELEN, P. (1985): Thermal stability of whey proteins-A calorimetric study. *J. Dairy Sci.* 68 (11), 2847-2852.

- BOYE, J. I., ISMAIL, A. A. and ALLI, I. (1996): Effects of physicochemical factors on the secondary structure of  $\beta$ -lactoglobulin, *J. Dairy Res.* 63 (1), 97-109.
- CARIĆ, M. (1990): Tehnologija koncentrovanih i sušenih proizvoda. Naučna knjiga, Beograd
- CAESSENS, P. W. J. R., VISSER, S., and GRUPPEN, H. (1997): Method for the isolation of bovine  $\beta$ -lactoglobulin from a cheese whey protein fraction and physicochemical characterization of the purified product. *Int. Dairy J.* 7 (4), 229-235
- CORREDIG, M. and DALGLEISH, D. G. (1996): The binding of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin to casein micelles in milk treated by different heating systems. *Milchwissenschaft* 51 (3), 123-127.
- CORREDIG, M. and DALGLEISH, D. G. (1999): The mechanisms of the heat-induced interaction of whey proteins with casein micelles in milk. *Int. Dairy J.* 9, 233-236.
- CHAPLIN, B. and GREEN, M. L. (1980): Determination of the proportion of  $\kappa$ -casein hydrolysed by rennet on coagulant of skim-milk. *J. Dairy Res.* 47 (3), 351-358.
- DALGALARRONDO, M., CHOBERT, J. M., DUFOUR, E., BERTRAND-HARB, C., DUMONT, J. P., and HAERTLE, T. (1990): Characterization of bovine  $\beta$ -lactoglobulin B tryptic peptides by reversed-phase high performance liquid chromatography. *Milchwissenschaft* 45 (4), 212-216.
- DEMETRIADES, K., COUPLAND, J. N. and MCCLEMENTS, D. J. (1997): Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. *J. Food Sci.* 62, 342-347.
- DEMETRIADES, K., COUPLAND, J. N. and MCCLEMENTS, D. J. (1997b): Physical properties of whey protein-stabilized emulsions as related to pH and NaCl, *J. Food Sci.* 62, 462-467.
- DE RHAM, O. and CHANTON, S. (1984): Role of ionic environment in insolubilization of whey protein during heat treatment of whey products. *J. Dairy Sci.* 67 (5), 939-949.
- DE WIT: Nutritional and functional characteristics of whey proteins in food products. *J. Dairy Sci.* 81 (3), 1998, 597-608.
- DE WIT, J. N. and KLARENBECK, G. (1984): Effects of various heat treatments on structure and solubility of whey proteins. *J. Dairy Sci.* 67 (11), 2701-2710.
- DONOVAN, M. and MULVIHILL, D. M. (1987): Thermal denaturation and aggregation of whey proteins. *Irish J. Food Sci. Techn.* 11, 87-100.
- DURANTI, M., PAGANI, S., IAMETTI, S. and CARPEN, A. (1989): Heat-induced changes of milk proteins:  $\alpha$ -lactalbumin rocket immunoelectrophoretic detection of *Milchwissenschaft* 44 (3), 142-144.
- ĐORĐEVIĆ, J. (1987): Mleko. Naučna knjiga, Beograd
- EIGEL, W. N., BUTLER, J. E., ERNSTROM, C. A., FARRELL, H. M. JR, HARWALKAR, V. R. JENNESS, R. and WHITNEY, R. MCL. (1984): Nomenclature of proteins of cow's milk. Fifth revision. *J. Dairy Sci.* 67 (8), 1599-1631.

- ELFAGM, A. A. and WHEELOCK, J. V. (1977): Effect of heat on  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin in bovine milk. *J. Dairy Res.* 44 (2), 367-371.
- ELFAGM, A. A. and WHEELOCK, J. V. (1978a): Interaction of bovine  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin during heating, *J. Dairy Sci.* 61 (1), 28-32.
- ELFAGM, A. A. and WHEELOCK, J. V. (1978b): Heat interaction between  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and casein in bovine milk *J. Dairy Sci.* 61 (2), 159-163.
- ELOFSSON, C., DEJMEK, P., PAULSON, M., BURLING, H. (1998): Characterization of cold/gelling whey protein concentrate, *Int. Dairy J.* 7, 601-608.
- FARRELL, H. M. JR and THOMPSON, M. P. (1974): Physical equilibria: Proteins in Fundamentals of dairy chemistry. Second edition. Ed. Webb, B. H., Johnson, A. H., Alford, J. A., The AVI Publishing company, Inc., Westport, Connecticut, 442-473.
- FOX, P.F. (1989): The milk protein system in Developments in Dairy Chemistry-4. Functional milk proteins. Ed. Fox, P. F. Elsevier Applied Science Publishers Ltd, London and New York, 1-53.
- FOX, P.F. (2003): The major constituents of milk in Dairy processing, ed. Smith, G., 5-38.
- GALANI, D. and OWUSU APENTEN, R. K. (1999): Heat-induced denaturation and aggregation of  $\beta$ -lactoglobulin: kinetics of formation of hydrophobic and disulphide-linked aggregates. *Int. J. Food Sci. and Techn.* 34, 467-476
- GHOSH, B. C., STEFFL, A. and KESSLER, H.-G. (1996): Rennetability of milk containing different heat-denatured whey protein. *Milchwissenschaft* 51 (1), 28-31.
- GODOVAC-ZIMMERMANN, J. and BRAUNITZER, G. (1987): Modern aspects of the primary structure and function of  $\beta$ -lactoglobulins. *Milchwissenschaft* 42 (5), 294-297.
- GORDON, W. G. and KALAN, E. B. (1974): Proteins of milk in Fundamentals of dairy chemistry. Second edition. Ed. Webb, B. H., Johnson, A. H., Alford, J. A., The AVI Publishing company, Inc., Westport, Connecticut, 87-124.
- HILLIER, R. M., LYSTER, R. L. J. and CHEESEMAN, G. C. (1979): Whey protein denaturation in heated milk and cheese whey. *J. Dairy Res.* 46 (1), 103-111.
- HINRICHS, J. (2001): Incorporation of whey proteins in cheese. *Int. Dairy J.* 11, 495-503.
- HUGUNIN, A. (1999): A protein for all seasons. *Dairy Ind. Internat.* 9, 19-22.
- JOVANOVIĆ, S. (1994): Uticaj pojedinih faktora i tehnoloških operacija u proizvodnji kiselinskih sireva. Magistarski rad, Univerzitet Banja Luka.
- JOVANOVIĆ, S. (2001): Uticaj obrazovanja koagregata proteina mleka na veće iskorišćenje ukupnih proteina pri proizvodnji polutvrdih sireva. Doktorska disertacija, 2001, Univerzitet u Beogradu.
- JOVANOVIĆ, S., MAĆEJ, O. i MIKULJANAC, A. (1996): Primjena koagregata proteina mlijeka u proizvodnji mekih, polutvrdih i tvrdih sireva. Zbornik radova V Međunarodnog simpozijuma "Savremeni trendovi u proizvodnji mleka". Ur. Obrenović, S., Kopaonik, 94-100.



- JOVANOVIĆ, S., MAĆEJ, O. and DENIN - DJURDJEVIĆ, J. (2002): The influence of various factors on milk clotting time. *J. Agric. Sci.*, 47 (1), 57-73.
- JOVANOVIĆ, S., MAĆEJ, O. and DENIN-ĐURĐEVIĆ, J. (2004): Influence of curd particles washing on the composition of curd made of milk in which coaggregates were formed. *J. Agric. Sci.* 49, 75-86.
- JOVANOVIĆ, S., MAĆEJ, O. and BARAC, M. (2005): The characteristics of coaggregates and coprecipitates based cheeses. *Biotech. Animal husbandry* 21, 147-173.
- KALAB, M., ALLAN-WOJTAS, P. and PHIPPS-TODD, B.E. (1983): Development of microstructure in set-style nonfat yoghurt-a review. *Food microstructure* 2, 51-66.
- KIRCHMEIER, O., KAMAL, N. M. and KLOSTERMEYER, H. (1985): Heat treatment of milk and development of SH-groups. II *Milchwissenschaft* 40 (12), 722-724.
- KLEMASZEWSKI, J. L., DAS, K. P. and KINSELLA, J. E. J. (1992): Formation and coalescence stability of emulsions stabilized by different milk proteins. *Food Sci.* 57, 366-371.
- LAWRENCE, R. C. and LELIÈVRE, J. (1990): Whey proteins in cheese. Proceedings of the XXIII International Dairy Congress "Dairying in a changing world". Volume 3, Montreal, 1880-1888.
- LONG, J. E., VAN WINKLE, Q. and GOULD, I. A. (1963): Heat-induced interaction between crude  $\kappa$ -casein and  $\beta$ -lactoglobulin. *J. Dairy Sci.* 46 (12), 1329-1334.
- LUPANO, C. E., RENZI, L. A., ROMERA, V. (1996): Gelation of Whey Protein Concentrate in Acidic Conditions: Effect of pH. *J. Agric. Food Chem.* 44, 3010-3014.
- MAĆEJ, O. (1983): Prilog proučavanju koprecipitata radi potpunijeg iskorišćavanja belančevina mleka. Magistarski rad, Univerzitet u Beogradu.
- MAĆEJ, O. (1989): Proučavanje mogućnosti izrade mekih sireva na bazi koagregata belančevina mleka. Doktorska disertacija, Univerzitet u Beogradu.
- MAĆEJ, O. (1992): Proizvodnja sira mekih sireva na bazi koagregata proteina mleka. Zbornik radova I Međunarodnog simpozijuma "Savremeni trendovi u mlekarstvu". Ur. Maćej, O., Kopaonik, 54-59.
- MAĆEJ, O. (1994): Proizvodnja kamamera na bazi koagregata proteina mleka. Zbornik radova III Međunarodni simpozijum "Savremeni trendovi u mlekarstvu". Ur. Krunić, M. i Ćurić, M., Kopaonik, 47-51.
- MAĆEJ, O. and JOVANOVIĆ, S. (1999): The application high heat treatment on milk in white brine cheese production, Book of abstracts 2<sup>nd</sup> Slovenian Congress "Milk and dairy products", Portorož, 45.
- MAĆEJ, O. D. and JOVANOVIĆ, S. T. (2000): Formation of complex between casein and whey proteins in heat-treated milk. *Acta Periodica*, 31, Part A, 83-93.
- MAĆEJ, O., MIKULJANAC, A., PUĐA, P. and JOVANOVIĆ, S. (1995): Uticaj različitih preparata sirila i organskih kiselina na ukupno iskorišćenje azotnih materija mleka Zbornik

radova IV Međunarodnog simpozijuma "Savremeni trendovi u proizvodnji mleka". Ur. Čubrilo, M., Kopaonik, 42-44.

MAĆEJ, O., JOVANOVIĆ, S., MIKULJANAC, A. and NIKETIĆ, G. (1996): Primena koprecipitata u proizvodnji kiselokoagulišućih sireva, Zbornik radova V Međunarodnog simpozijuma "Savremeni trendovi u proizvodnji mleka". Ur. Obrenović, S., Kopaonik, 67-71.

MAĆEJ O. JOVANOVIĆ, S. and DENIN, J.(2001): Characteristics of camembert-type cheese ripening produced from milk in which complex between casein and whey protein is formed. *J. Agric. Sci.*, 46 (1), 57-69.

MAĆEJ, O., JOVANOVIĆ, S., SERATLIĆ, S. and BARAĆ, M. (2004a): Fresh cheese production on the basis of milk protein coaggregates. *Biotech. Animal husbandry* 20, 119-131.

MAĆEJ, O., JOVANOVIĆ, S. and BARAĆ, M. (2004b): Influence of curd particles drying temperature on the composition of curd made of milk in which coaggregates were formed. *J. Agric. Sci.* 49, 65-73.

MAĆEJ, O., JOVANOVIĆ, S. and BARAĆ, M. (2004c): Influence of various coagulation factors on chemical composition of sera gained by ultracentrifugation from casein gel. *J. Agric. Sci.* 49, 219-232.

MANIGO, M.E. (1992): Gelation of whey protein concentrates. *Food Technology* 46, 114-117.

MARSHALL, K. R. (1986): Industrial isolation of milk proteins: Whey proteins in Developments in Dairy Chemistry-1. Ed. Fox, P. F., Elsevier Applied Science Publishers Ltd, London and New York, 339-374.

MCCRAE, C. H., LAW, A. J. R. and LEAVER, J. (1999): Emulsification properties of whey proteins in their natural environment: effect of whey protein concentration at 4 and 18% milk fat. *Food Hydrocolloids*, 389-399.

MORR, C. V. (1985): Functionality of heated milk proteins in dairy and related foods, *J. Dairy Sci.* 68 (10), 2773-2781.

MOTTAR, J., BASSIER, A., JONIAU, M. and BAERT, J. (1989): Effect of heat-induced association of whey proteins and casein micelles on yogurt texture. *J. Dairy Sci.* 72 (9), 2247-2256.

MULLVIHILL, D. M and KINSELLA, J. E. (1987): Gelation characteristics of whey proteins and  $\beta$ -lactoglobulin, *Food Technology* 41, 102-111.

MULLVIHILL, D. M and DONOVAN, M. (1987): Whey proteins and their thermal denaturation-A review. *Irish J. Food Sci. Techn.* 11 (1), 43-75.

NAKAMURA, M., SATO, K., KOISUMI, S., KAWACHI, K. and NAKAJIMA, I. (1995): Preparation and properties of salt- induced gel of whey protein. *Nippon Shokuhin Kagaku Kaishi* 42, 1-6

PERMYAKOV E. A. and BERLINER, L.J. (2000):  $\alpha$ -lactalbumin: structure and function, *FEBS letters* 473, 269-274.

POPOVIĆ-VRANJEŠ, A. and VUJIČIĆ, I. (1997): Tehnologija surutke. Poljoprivredni fakultet, Novi Sad

PUĐA, P. (1992a): Karakteristike tvrdih sireva izrađenih od mleka koncentrovanog ultrafiltracijom u zavisnosti od termičke obrade mleka. Doktorska disertacija, Univerzitet u Beogradu.

PUĐA, P. (1992b): Značaj reologije u proizvodnji sireva. Zbornik radova I Međunarodnog simpozijuma "Savremeni trendovi u mljarstvu". Ur. Mačej, O., Kopaonik, 60-65.

RESCH, M. and DANBERT, I. (2002): Rheological and physicochemical properties of derivatized whey protein concentrate powders, *Int. J. Food Properties*, 5, 419-434.

SATO, K., NAKAMURA, M., KOISUMI, S., KAWACHI, K., NAKAJIMA, I. (1995): Changes in hydrophobicity and SH content on salt-induced gel of whey protein. *Nippon Shokuhin Kagaku Kaishi* 42, 1-6.

SAWYER, W. H. (1968): Role of the sulfhydryl groups in the interaction of  $\kappa$ -casein and  $\beta$ -lactoglobulin, *J. Dairy Sci.* 51 (3), 323-329.

SAWYER, L., KONTOPIDIS, G. and WU, S.-Y. (1999):  $\beta$ -lactoglobulin- a three-dimensional perspective. *Int. J. Food Sci. Techn.* 34, 409-418.

SCHNACK, U. and KLOSTERMEYER, H. (1980): Thermal decomposition of  $\alpha$ -lactalbumin. 1. Destruction of cystine residues. *Milchwissenschaft* 35 (4), 206-208.

SWAISGOOD, H. E. (1986): Chemistry of milk protein in *Developments in Dairy Chemistry-1*. Ed. Fox, P. F., Elsevier Applied Science Publishers Ltd, London and New York, 1-60.

SOUTHWARD, C. R. (1978): La fabrication du co-précipité. Brèves communications 20<sup>th</sup> congrès international de laiterie, Paris, 914-915.

WHITNEY, R. MCL., BRUNNER, J. R., EBNER, K. E., FARRELL, H. M. JR., JOSEPHSON, R. V., MORR, C. V. and SWAISGOOD, H. E. (1976): Nomenclature of the proteins of cow's milk: fourth revision. *J. Dairy Sci.* 59 (5), 1976, 795-815.

**Author's addresses – Adrese autora:**

Doc. dr. sc. Snežana Jovanović

Prof. dr. sc. Ognjen Mačej

Doc. dr. sc. Miroljub Barać

Institute for Food Technology-Faculty of Agriculture

Institut za prehrambenu tehnologiju – Agronomski fakultet Beograd

Belgrade – Zemun

**Received – Prispjelo:** May 16, 2005

**Accepted – Prihvaćeno:** July 08, 2005