Possibility of nutritional enrichment of whipped dairy products with whey proteins treated with high pressure

Greta Krešić, Vesna Lelas, Zoran Herceg

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Abstract

Nutritional value in addition to diverse physico-chemical and functional properties make whey proteins highly suitable for food purpose. One of important functional demand of whey proteins is their capability to induce and stabilize aerated food products.

The aim of this work was to obtain whey protein isolate and β-lactoglobulin with improved foaming properties, what will in addition to its high nutritional value, make them more acceptable for use in whipped dairy products. For these purpose, whey protein isolate and β-lactoglobulin were treated with high hydrostatic pressure of 400, 500 and 600 MPa, for holding period of 10 minutes.

Solubility changes were expressed as nitrogen solubility. Turbidity measurements of diluted protein solutions were used to obtain information about protein aggregation in addition to scanning electron microscopic pictures. Assessment of foaming properties has been based on the foam volume and foam stability. All analysis were carried out on whey protein isolate and β-lactoglobulin before and after high pressure treatment.

The results obtained showed pressure-induced structural changes together with partial denaturation in both samples proportional to the intensity of applied pressure.

The observed effects of pressure treatments on foaming properties clearly indicate that the extent of pressure-induced changes in the physicochemical properties of whey protein isolate and β-lactoglobulin positively affected foamability and foam stability, respectively.

Key words: foaming properties, high pressure, β-lactoglobulin, whey protein isolate.

Introduction

The growing demand in the food industry for functionally superior and nutritionally excellent novel proteins provides an opportunity for increasing the utilization of whey proteins in formulated food products.

Whey proteins not only play an important role in nutrition as an exceptionally rich and balanced source of amino acids (Regester et al., 1996), but in a number of instances also appear to have specific physiological actions, *in vivo*. Many of the bioactive whey proteins, notably α-lactalbumin, β-lactoglobulin, lactoferin, lactoperoxidase, immunoglobulins, glycomacropeptide and a variety of growth factors have been implicated in a number of biological effects like anticancer activity, immunostimulatory effects, influence on digestive function, organism longevity etc. (Bounous et al., 1989; Bounous et al., 1993; Kennedy et al., 1995; Perez et al., 1992).

β-lactoglobulin is a globular protein with a monomer molecular weight of 18.4 kDa and account for about 50% of the protein in bovine whey isolate (Fox, 1989). This fraction of whey proteins is nutritionally worth because it represents a rich source of cysteine, an essential amino acid that appears to stimulate glutathione synthesis, an anticarcinogenic tripeptide produced by the liver for protection against intestinal tumors (Mcintosh et al., 1995).

As ingredients of food products, beside nutritional benefits, potential functional benefits of whey proteins include emulsification and stabilization, increased viscosity, improved appearance, taste or texture and binding of fat or water (Jost, 1993). However, according to Zhu & Damodaran (1994), functional behaviour of whey protein isolate in foams and emulsion-type food products does not meet the expectations. Their use is only limited by an intrinsic functional properties and could be increased if these properties could be enhanced to meet new needs.

Improvements in functional properties may be achieved by procedures which modify protein structure. The potential of high pressure treatment, as an alternative to physical modification of macromolecular food constituents, such as proteins, has been recognized (Hayashi, 1992; Cheftel, 1992, Balny et al., 1989). High hydrostatic pressure acts as a physicochemical parameter that alters the balance of intramolecular and solvent-protein interactions. Pressure-induced changes in protein molecules tend, in general, to change the area accessible to the solvent and, as a consequence, alter surface properties (Cheftel, 1992).

Bearing in mind the fact that whipped dairy product is a complex foam structure where the properties of each component greatly contribute to the colloidal stability (Anderson & Brooker, 1988), the aim of this work was to examine the possibility of obtain whey protein isolate and β-lactoglobulin with improved foaming properties what will make them much more suitable for usage in nutritionally enriched desserts, pastries or cakes.

Materials and Methods

Commercial whey protein isolate (BiPRO) prepared by an ion exchange process and commercial β-lactoglobulin (BioPURE) was purchased from Davisco Foods International, Le Sueur, MN, USA. The chemical compositon and pH value of investigated samples declared by the manufacturer are summarized in table 1.

Table 1: Chemical composition and pH of whey protein isolate and β-lactoglobulin

Tablica 1: Kemijski sastav i pH izolata proteina sirutke i β-laktoglobulina

Sample Uzorak	Proteins (% of total dry matter) Proteini (% izraženo na suhu tvar)	Fats (%) Masti (%)	Ash (%) Pepeo (%)	Moisture (%) Vlaga (%)	pH* pH*
Whey protein isolate Izolat proteina sirutke	94.5	0.5	1.7	5.0	7.1
β-lactoglobulin β-laktoglobulin	98.3	0.2	1.5	4.9	6.9

* 10 % (g/g) at 20 ^{0}C

The native proteins were dissolved in distilled water by gentle magnetic stirring for 30 minutes to provide a 10 % w/w dispersions. For each treatment, 170 ml of protein dispersion was hermetically filled in PET containers following characteristics: wall thickness: 0.5 mm; diameter: 45 mm; height: 135 mm; cap diameter: 30 mm. Care was taken not to leave any head space between the closing screw cap and the liquid solution.

The pressure treatment was carried out in the LAB 50 single processor machine (SIG Simonazzi, Parma, Italy). This is a laboratory system developed to test short time microbial stabilization of foods and beverages by high pressure treatment. It is scaled down version of a bigger industrial unit with the same performances so that the tested process can straightforwardly be translated to production environments.

Several samples in PET containers were retained as controls. High pressure treatments were carried out at pressure levels of 400, 500 and 600 MPa with holding period of 10 minutes. The whole pressure generation system is fully automated; a proportional valve and pressure feedback allow tuneable and very reproducible pressure cycles of a few seconds. Selection of pressure-

time profiles as well as diagnostic information and data acquisition are available at PC interface.

Protein solubility, expressed in g of soluble nitrogen per 100 g of total nitrogen was determined according to modify method described by Futenberger and co-workers (1995). After pressure processing, protein solutions (10 g/kg) were prepared with pH adjusted to 7.0 using 0.025 mol/l NaOH or HCl. Protein solutions were then centrifuged at 12,000 r.p.m. x g for 15 minutes (Beckman model J-21B). Nitrogen in the supernatant was determined by Kjeldahl´s method using 6.38 conversion factor (Chang, 1998).

The turbidity of 0.1 % (w/w) protein solution was measured as absorbance at 570 nm with a spectrophotometer (Helios-β, Pye Unicam Ltd, Cambridge, UK).

Scanning electron microscopy of samples before and after high pressure treatment was made after sample preparation technique using Edwards S-150, sputter-coater unit. Electron micrographs were taken with microscope type JOEL-JSM-5800.

For foaming properties evaluation, 10 % (w/w) protein dispersions were whipped at room temperature with a mixer (tip MSM5220, Bosch, Germany) equipped with a wire whip beater at maximum speed settings for 15 minutes. Whipping was interrupted after each 5 minutes to determine foam expansion. Foaming properties were adequately described by compilation of methods (Morr, 1985; Morr & Foegeding, 1990; Webb et al., 2002) with some moderate modifications. Foam expansion was determined by level-filling a 100 ml plastic weighing boat with foam and weighing to ± 0.01 g. Foam expansion was computed using the expression:

$$\text{Foam expansion (\%)} = [(\text{Unwhipped dispersion wt(g)} - \text{Foam wt(g)}] / [\text{Unwhipped dispersion wt(g)}] \times 100$$

After the foam expansion was determined, foam was returned to the bowl and whipping was resumed for an additional 5 minute period. Foam stability was determined by transferring 100 ml of maximum expansion foam into a filter funnel. A small plug of glass wool was placed in the top of the funnel stem to retain the foam but allow drainage of the liquid. The time required for the first drop of liquid to drain from the funnel was determined as an index of foam stability. The time for drainage of the entire foam was determined and expressed as maximum foam stability.

Results and discussion

It is now well established (Balny et al., 1989; Heremans, 1992) that changes in protein structure and functionality occur during high pressure treatment. Solubility changes, together with measurements of absorbance of diluted protein solutions could be efficient tool for assessment the degree of protein denaturation (Cheftel, 1992; Futenberger et al., 1995; Kanno et al.; 1998).

The nitrogen solubility of non-pressurised whey protein isolate and β-lactoglobulin was high (96.60 and 97.13 g/100g; for whey protein isolate and β-lactoglobulin, respectively) what is evident from data summarized in table 2. This high value reflect the high proportion of native proteins present in the industrial product what could be the indicator of non-thermal methods used for preparation and isolation of protein fraction.

Table 2: Nitrogen solubility at pH 7.0 of whey protein isolate and β-lactoglobulin

Tablica 2: Topljivost izolata proteina sirutke i β-laktoglobulina pri pH 7,0

Pressure (MPa) Tlak (MPa)	Protein solubility (Soluble N, g/100g) Topljivost proteina (Topljivi N, g/100g)	
	Whey protein isolate Izolat proteina sirutke	ß-lactoglobulin ß-laktoglobulin
0.1	96.60	97.13
400	93.91	94.92
500	92.18	93.78
600	91.24	91.52

According to investigation which Zhu & Damodaran (1994) carried out with the same commercial whey protein isolate (BiPRO), no precipitation of α-lactalbumin and β-lactoglobulin occurred at their respective isoelectric pHs clearly indicated that the water-accessible surfaces of these proteins in their native state were highly hydrophilic, which promoted protein-solvent interactions instead of protein-protein (hydrophobic) interactions even at their isolectric pH. After high pressure processing solubility of all samples decreased, and it varied from 94.92 to 91.24 g/100g. The decrease was proportional to the increase of applied pressure and it is indicator of pressure induced denaturation which took place. Pressure-induced denaturation is a complex phenomenon that depends on protein structure, pressure, temperature, pH, ionic strength and solvent composition (Masson, 1992). The solubility decreaseof high pressure treated whey protein isolate were moderately in comparison with β-lactoglobulin, because of significant amount

(approximately 22%) of α-lactalbumin which is more resistant to high pressure-induced denaturation (Nakamura et al., 1993).

According to Wong & Heremans (1988) at high protein concentration (10%) intermolecular interactions and irreversible aggregation are favoured. The decreasing solubility suggests that aggregation resulted from hydrophobic interactions and also S-S bonds and that a progressive build up of these interactions and bonds took place after pressure release (Cheftel et al., 1995). For visualisation of possible aggregation, absorbance measurements at 570 nm were performed and the changes of turbidity of diluted protein solutions as a function of pressure are shown on figure 1.

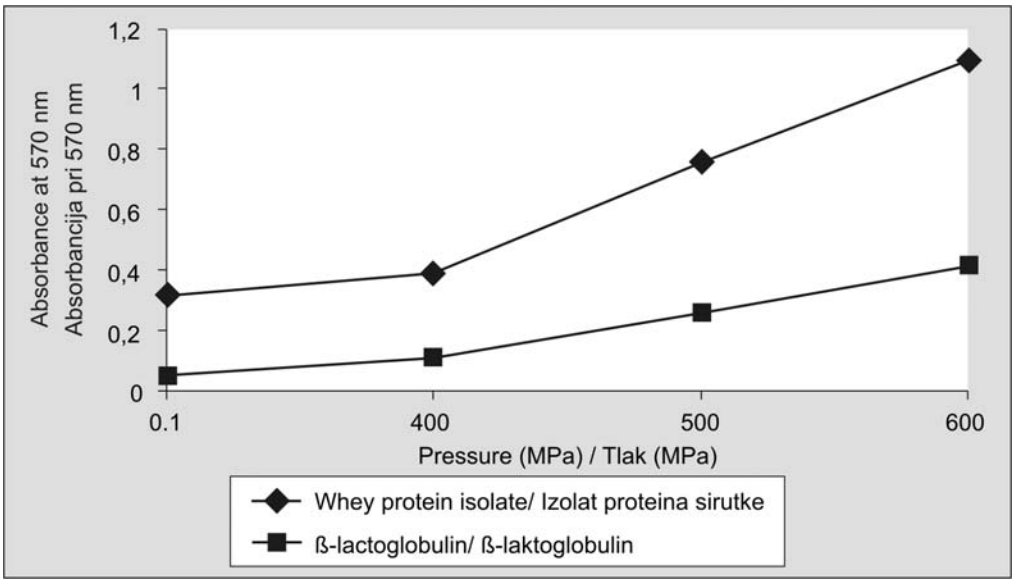

Figure 1: Effect of high pressure treatment on the turbidity of whey protein isolate and β-lactoglobulin

Slika 1: Utjecaj tretiranja visokim tlakom na mutnoću otopina izolata proteina sirutke i β-laktglobulina

The absorbance value of native β-lactoglobulin were smaller (0.045) compared with native whey protein isolate (0.316). Namely, at pH 7.0 an equilibrium exists between the monomer and dimmer forms of β-lactoglobulin. This equilibrium is shifted toward the monomer at pH 7.5-8 and toward the dimer at pH 6.0 (Monaco et al., 1987). The turbidity of all investigated solutions was greatly influenced by pressure. After pressure treatment at 600 MPa turbidity value decrease three-fold for whey protein

isolate and almost ten-fold for β-lactoglobulin. Although, Gekko (1991) has shown high value of adiabatic compressibility for β-lactoglobulin what can be indicative of relatively high stability against temperature or pressure denaturation, Dumay et al. (1994) confirmed partial unfolding and aggregation of β-lactoglobulin which have been induced by high pressure processing at 450 MPa at neutral pH.

Significant increase of absorbance measured at 570 nm confirmed that similar extensive aggregation occurred at processing conditions used in this work. It is evident that the shoulders of conformational changes are on the same level of applied pressure (500 MPa) for whey protein isolate and β-lactoglobulin, respectively. This is because of previously discussed fact that β-lactoglobulin represents the main protein constituents at whey protein isolate and thus determine the behaviour of whey protein isolate under the influence of high pressure.

Tanaka et al. (1996) have reported that high molecular weight aggregates (dimmer to hexamer) of isolated β-lactoglobulin at 450 MPa were induced by the formation of intermolecular S-S bonds, which were caused by SH-SS interchange or by other oxidation reaction.

The loss of solubility after high pressure treatment could lead as to hypothesis that the high pressure induced exposure of previously buried hydrophobic groups what enhanced the hydrophobic character of the protein surface that came in contact with the surrounding solvent, resulting in enhanced protein-protein interactions. The microstructure of native protein molecules and with the ones treated with the highest pressure (600 MPa), was observed by scanning electron microscopy and the pictures are shown in figures 2-5.

The microstructures of native and high pressure treated whey proteins are markedly different. The native whey proteins (Figures 2 and 4) are compact globules with intramolecular wrinkled structure as a result of disulfide bonds between cysteine remains which are mostly situated inside of the proteins (hydrophobic fragment of the proteins) while outer side of proteins is made of hydrophilic remains of aminoacids (Tratnik, 1998). However, after high pressure treatment (Figures 3 and 5) theirhad plateau-like structure. It is assumed that a large increase of surface hydrophobicity takes place, due to the protein unfolding, and is immediately followed by the formation of soluble aggregates. Pressure-induced protein unfolding is complex and can result in disruption of both internal hydrophobic bonds and salt bridges (Pittia et al., 1996).

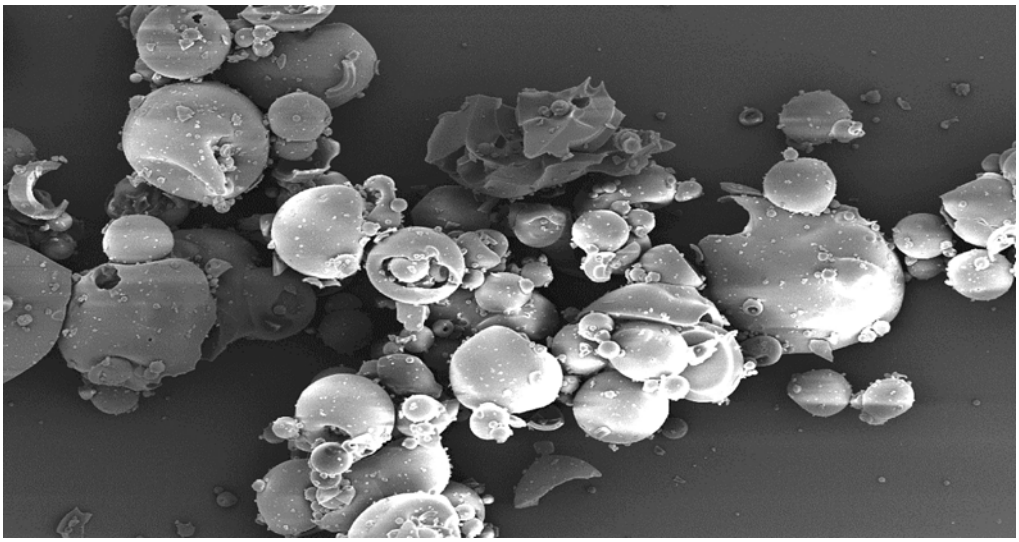

Figure 2: Scanning electron microscopic pictures of native whey protein isolate

Slika 2: Slika nativnog izolata proteina sirutke dobivena skenirajućom elektronskom mikrospijom

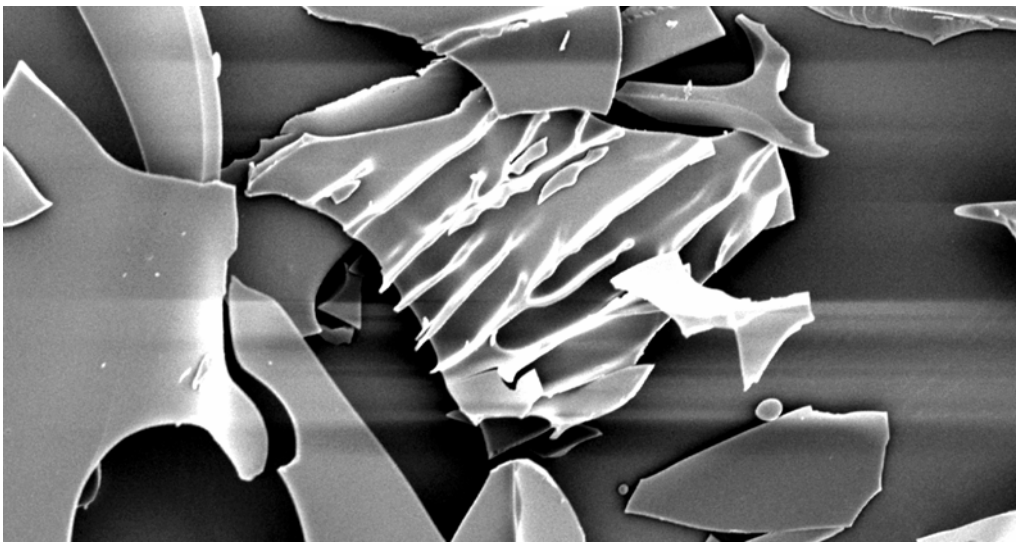

Figure 3: Scanning electron microscopic pictures of whey protein isolate treated with high pressure (600 MPa/10 minutes)

Slika 3: Slika izolata proteina sirutke tretiranog visokim tlakom, dobivena skenirajućom elektronskom mikroskopijom (600 MPa/10 minuta)

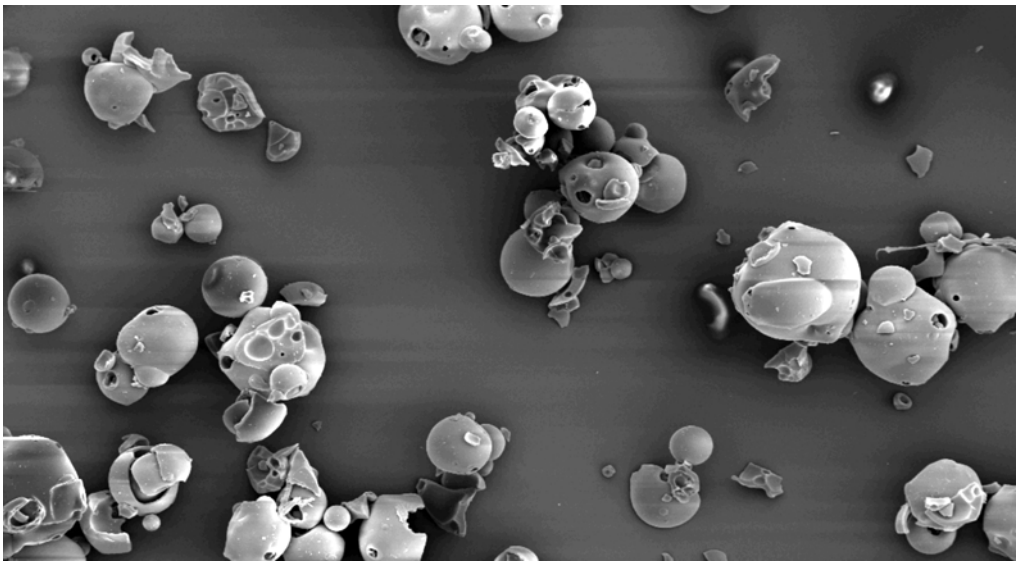

Figure 4: Scanning electron microscopic pictures of native β-lactoglobulin

Slika 4: Slika nativnog β-laktoglobulina dobivena skenirajućom elektronskom mikroskopijom

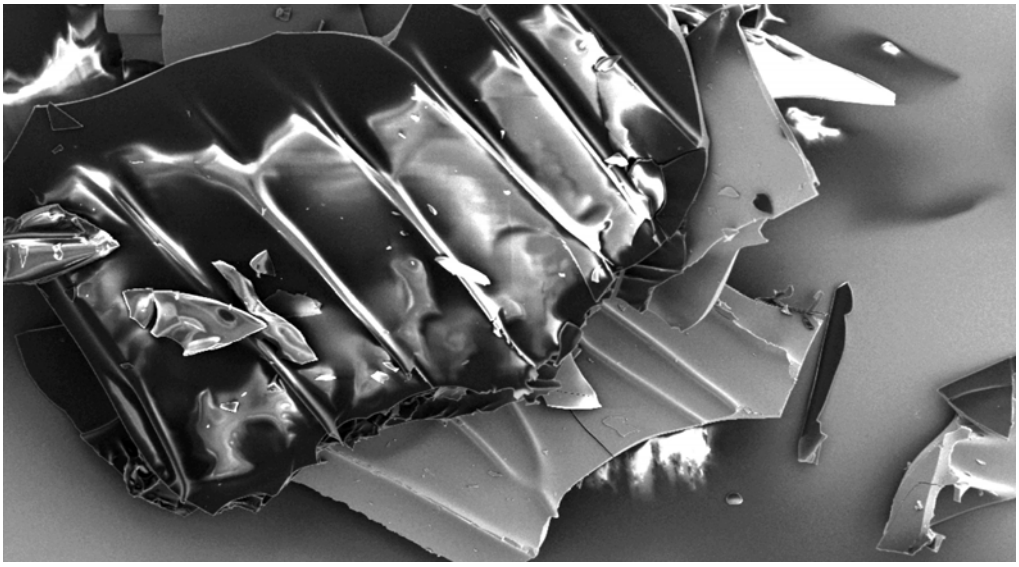

Figure 5: Scanning electron microscopic pictures of β-lactoglobulin treated with high pressure (600 MPa/10 minutes)

Slika 5: Slika β-laktoglobulina tretiranog visokim tlakom, dobivena skenirajućom elektronskom mikroskopijom (600 MPa/10 minuta)

Increase of surface hydrophobicity after high pressure treatment has also been reported by Pittia et al. (1996). Such exchanged structure of proteins can cause changes of their physico-chemical and functional properties. The difference in microstructure between untreated and high pressure treated samples seems to have been reflected in the difference of their foaming properties as shown in figures 6-8.

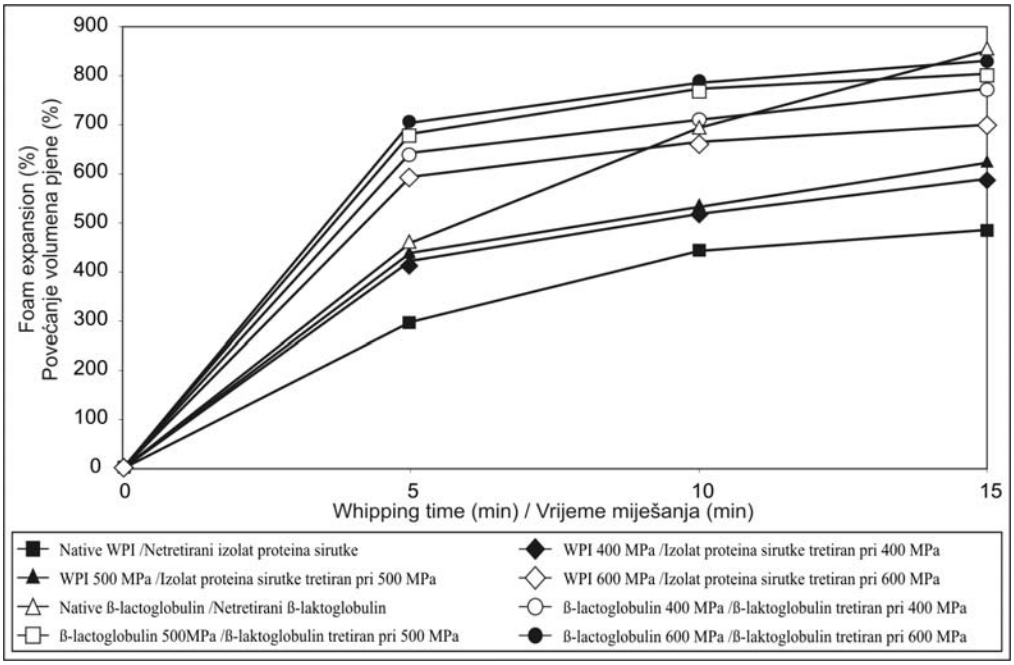

Figure 6: Foam expansion of whey protein isolate and ß-lactoglobulin
Slika 6: Povećanje volumena pjene izolata proteina sirutke i ß-laktoglobulina

During whipping, a large quantity of air (40-60% volume) is incorporated as big bubbles in the first stage. Subsequently, bubble size gets smaller and a narrower bubble size distribution is reached, while proteins forming a cohesive intramolecular densely packed film (Noda & Shiinoki, 1986). Comparison of the initial foam volumes for whey protein isolate and β-lactoglobulin suggests that β-lactoglobulin reaches the interface faster than whey protein isolate. Native ß-lactoglobulin exhibited also better foamability after maximum whipping time because of significant number of exposed apolar residues at the surface, higher protein amount, better solubility and higher molecular flexibility. It is generally believed that highly flexible random coil

proteins would exhibit better foamability than rigid and highly ordered proteins (Graham & Philips, 1979). The foamability of proteins is fundamentally related to their film-forming ability at the air-water interface. In general, proteins that rapidly adsorb at the air-water interface and readily undergo unfolding and molecular rearrangement at the interface often exhibit better foamability than those proteins that adsorb slowly and resist unfolding at the interface (Clarkson et al., 1999).

At this work we have deliberately chosen method which includes whipping protein dispersion in a mixer because it is closest to the practical production of foamed foods. As reported by Philips et al. (1990) this method can readily discern what amounts to practically important differences between proteins.

The increase in pressure was observed to improve foam volume of all samples. This may occur if pressure-application induces the protein unfolding resulting in an increase in the rate of protein adsorption. It is likely that pressure application disrupt hydrophobic interactions and ionic bonds resulting in protein molecules being more flexible to adsorb at a faster rate (Ibanoglu & Karatas, 2001). During prolonged mixing, the gas dispersion in foam generated by high agitation introduces small gas bubbles into the sample yielding a discrete dispersion of air bubbles entrapped by the stabilizing properties of the continuous protein solution.

According to Yu and Damodaran (1991), breakage of protein-stabilized foams involves two microscopic processes; namely, gravitational drainage of liquid from the lamella and disproportionation of gas bubbles due to interbubble gas diffusion.

An initial rapid drainage and macroscopic persistence of foams were monitored with foam stability index and maximum foam stability, and the obtained results are presented on figures 7 and 8.

Foam of native β-lactoglobulin is more stable against liquid drainage compared to whey protein isolate. Foam stability, the retention of air volume and water, is a reflection of film integrity, impermeability to gas, and viscoelastic and mechanical strength of the film.

Foams of high pressure treated whey protein isolate and β-lactoglobulin have shown significantly increased foam stability compared to native. This increase may be due to its increased surface hidrophobicity which, is according to Li-Chan & Nakai (1989), usually associated with an increase in foam stability, as previously hidden hydrophobic groups become exposed and available for adsorption at the air-water interface.

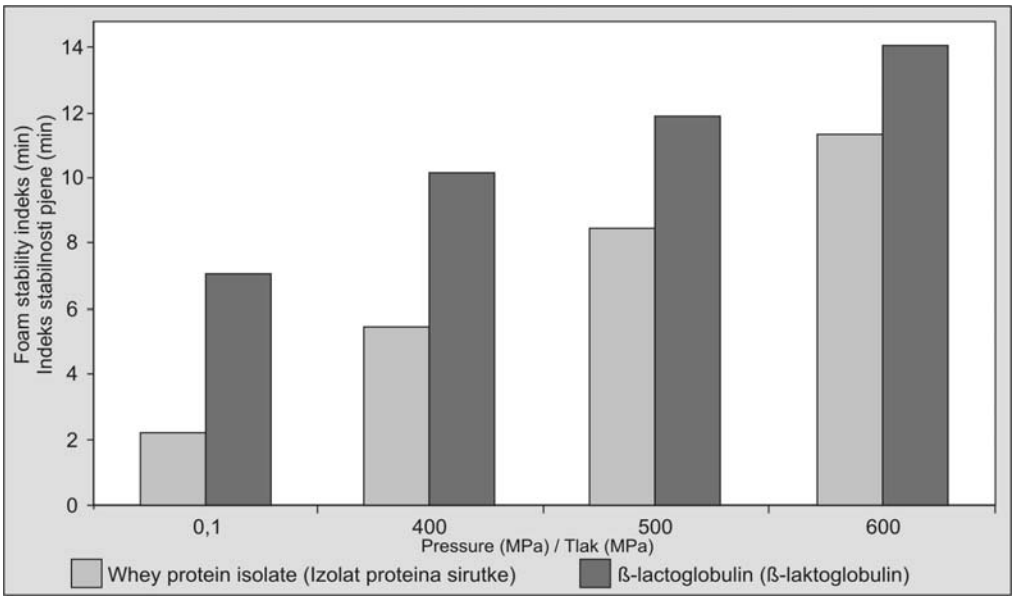

Figure 7: Foam stability index of whey protein isolate and ß-lactoglobulin

Slika 7: Indeks stabilnosti pjene izolata proteina sirutke i ß-laktoglobulina

Influence of high pressure on maximum foam stability appears to be more extensive (Figure 8). Improved maximum foam stability may be due to previously discussed and confirmed protein aggregation. Protein aggregation imparts thickness to the film and retards the drainage of lamella liquid (Zhu & Damodaran, 1994). The same authors clearly demonstrate that in denatured whey proteins, the ratio of monomeric to polymeric proteins appears to be critical to its foaming properties. The protein films formed by monomeric proteins do not appear to have the required viscoleastic properties to stabilize the foam. However, when (the late arriving) polymeric species adsorb to the preformed film, they increase the viscoelastic properties of the film and thus stabilize the foam against gravitational drainage and interbubble gas diffusion.

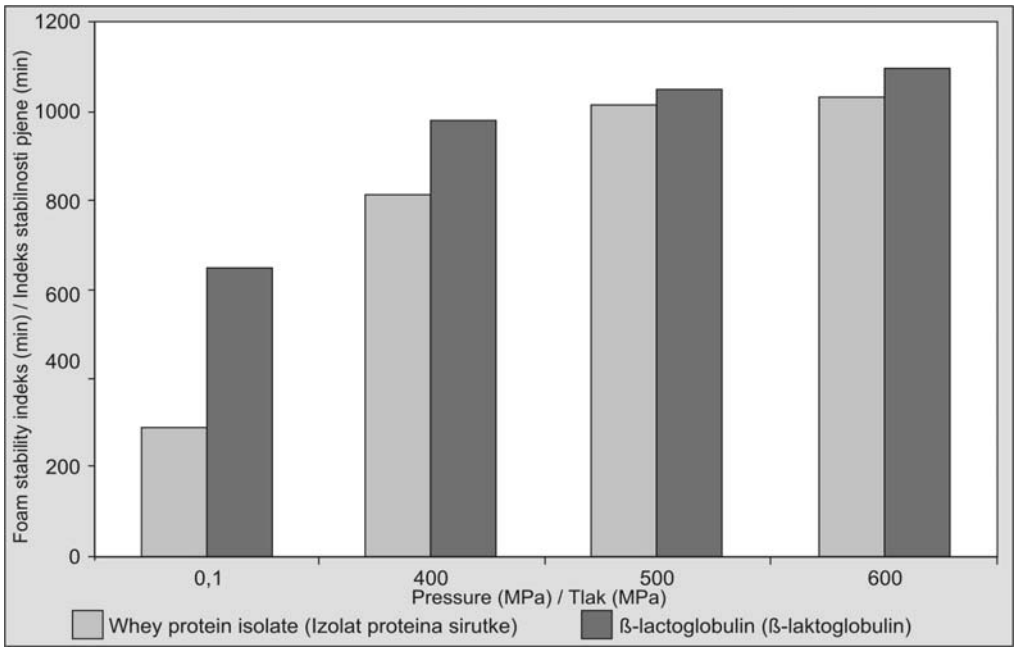

Figure 8: Maximum foam stability of whey protein isolate and ß-lactoglobulin
Slika 8: Maksimalna stabilnost pjene izolata proteina sirutke i ß-laktoglobulina

Conclusions

High pressure treatment of whey protein isolate and β-lactoglobulin induced partial denaturation resulting in the protein aggregation of different extend.

The intensity of pressure-induced modifications was proportional to the intensity of applied pressure. Under selected conditions, the influence of high pressure on the solubility and foaming properties was more evident on β-lactoglobulin, in comparison with whey protein isolate.

High pressure treated whey protein isolate and β-lactoglobulin formed foam with bigger volume and prolonged stability. The progressive increases in foam volume and foam stability of whey protein isolate and β-lactoglobulin, after high pressure treatment, may confirm a gradual alteration in the protein structure as the intensity of applied pressure increase.

Improved foaming of whey protein isolate and β-lactoglobulin, proposed in this work, together with high nutritional value could be essential and useful in a number of applications.

MOGUĆNOST OBOGAĆIVANJA PJENASTIH MLIJEČNIH PROIZVODA DODATKOM PROTEINA SIRUTKE OBRAĐENIH VISOKIM TLAKOM

Sažetak

Nutritivna vrijednost uz raznolika fizikalno-kemijska i funkcionalna svojstva čine proteine sirutke široko primjenjivim u prehrambenim proizvodima. Jedno od važnih funkcionalnih svojstava proteina sirutke je njihova sposobnost stabilizacije pjenastih proizvoda.

Cilj ovog rada je bio postići poboljšano svojstvo pjenjenja izolata proteina sirutke i β-laktoglobulina, što bi ih uz veliku nutritivnu vrijednost činilo prihvatljivijima za upotrebu u proizvodnji pjenastih mliječnih proizvoda. U tu svrhu, izolat proteina sirutke i β-laktoglobulin su obrađeni visokim hidrostatskim tlakom od 400, 500 i 600 MPa, kroz 10 minuta.

Promjene topljivosti su izražene kao topljivi dušik. Mutnoća razrijeđenih proteinskih otopina, zajedno sa skenirajućom elektronskom mikroskopijom je korištena za dobivanje uvida u agregaciju proteina. Svojstvo pjenjenja praćeno je određivanjem volumena i stabilnosti pjene. Sve analize su izvršene na izolatu proteina sirutke i β-laktoglobulinu prije i poslije tretiranja visokim tlakom.

Iz dobivenih rezultata uočljivo je da uslijed djelovanja visokog tlaka dolazi do promjene u strukturi proteina te njihove djelomične denaturacije proporcionalne jakosti primijenjenog tlaka.

Uočeni učinci tretiranja visokim tlakom jasno pokazuju da promjene fizikalno-kemijskih svojstava i strukture i proteina sirutke i β-laktoglobulina pozitivno utječu na nastanak i stabilnost pjene.

Ključne riječi: izolat proteina sirutke, ß-laktoglobulin, pjenjenje, visoki tlak

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Author's addresses - Adrese autora:
Mr.sc. Greta Krešić
Faculty of Tourism and Hospitality Management, Opatija

Dr.sc. Vesna Lelas, red.prof.
Dr.sc. Zoran Herceg, doc.
Faculty of Food Technology and Biotechnology, Croatia

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