Lait 86 (2006) 303–315
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DOI: 10.1051/lait:2006012

Original article

Effects of high pressure on functionality of whey protein concentrate and whey protein isolate

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Received 22 July 2005 – Accepted 29 May 2006

Abstract – The objective of this study was to evaluate the influence of high-pressure treatments on the solubility, surface hydrophobicity, foaming and emulsifying ability of whey protein concentrate (WPC) and whey protein isolate (WPI). Dispersions of WPC and WPI powders (10% (w/w)) were processed at 300 MPa and 600 MPa, for 5 and 10 min at 40 ± 2 °C. Changes in protein solubility were determined as solubility at pH 7.0 and at pH 4.6. Assessment of foaming properties was based on the foam expansion during prolonged whipping, and foam stability. Emulsifying properties were characterised by emulsion stability and emulsifying activity indices. The results show significant ($P < 0.05$) modification of solubility and surface hydrophobicity with increasing intensity and duration of applied pressure, indicating partial denaturation and aggregation of proteins. It was found that high-pressure treatments significantly ($P < 0.05$) improved the foaming behaviour of WPI, while the foaming ability of WPC was diminished. However, foams formed with high-pressure-treated WPC and WPI exhibited significantly prolonged stability ($P < 0.05$) compared with control samples. There was a significant trend of decreasing emulsifying efficiency and emulsifying stability related to the intensity of applied pressure and treatment time for both WPC and WPI.

emulsifying properties / foaming properties / high pressure / whey protein concentrate / whey protein isolate / solubility / surface hydrophobicity

摘要 – 高压处理对乳清浓缩蛋白和乳清分离蛋白的影响。本文研究了高压处理对乳清浓缩蛋白 (WPC) 和乳清分离蛋白 (WPI) 的溶解性、表面疏水性、发泡性和乳化性的影响。在 40 °C 下，乳清浓缩蛋白 (10 %, w/w) 和乳清分离蛋白 (10 %, w/w) 的分散液分别在 300 MPa 和 600 Mpa 下高压处理 5 min 和 10 min。在 pH 7.0 和 pH 4.6 测定了两种蛋白质溶解性的变化。以长时间搅打后形成的泡沫膨胀度和泡沫稳定性来评价蛋白质的发泡性能；根据测定乳液稳定性指数和乳化活性指数评价两种蛋白质的乳化特性。试验结果表明，经过高压长时间的处理后，由于蛋白质发生了部分变性和凝聚作用，使得蛋白质的溶解性和表面疏水性发生了显著的改变 ($P < 0.05$)。高压处理能够显著地改善 WPI 的发泡性 ($P < 0.05$)，而 WPC 的泡沫形成能力则降低。然而与对照样品相比，经过高压处理后的 WPC 和 WPI 形成的泡沫稳定性有显著提高 ($P < 0.05$)。随着处理压力和作用时间的增加，两种蛋白质的乳化性和乳化稳定性均呈下降的趋势。

乳化性 / 发泡性 / 高压 / 乳清浓缩蛋白 / 乳清分离蛋白 / 溶解性 / 表面疏水性

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Résumé – Effets des hautes pressions sur la fonctionnalité d'un concentré et d'un isolat de protéines de lactosérum. L'objectif de cette étude était d'évaluer l'influence des traitements haute pression sur la solubilité, l'hydrophobicité de surface, le pouvoir moussant et émulsifiant d'un concentré (WPC) et d'un isolat (WPI) de protéines de lactosérum. Les dispersions de poudres WPC ET WPI (10 % w/w) étaient traitées à 300 MPa et 600 MPa pendant 5 et 10 min, à 40 ± 2 °C. Les changements de solubilité des protéines étaient déterminés à pH 7.0 et à pH 4.6. L'évaluation des propriétés moussantes était basée sur l'expansion de la mousse au cours d'un fouettage prolongé et sur la stabilité de mousse. Les propriétés émulsifiantes étaient caractérisées par la stabilité de l'émulsion et les indices d'activité émulsifiante. Les résultats ont montré une modification significative ($P < 0,05$) de la solubilité et de l'hydrophobicité de surface avec l'application d'une pression de durée et d'intensité croissantes, indiquant une dénaturation partielle et l'agrégation des protéines. Il a été démontré que les traitements haute pression amélioraient de façon significative ($P < 0,05$) le comportement moussant de l'isolat de protéines de lactosérum, tandis que la capacité moussante du concentré de protéines était diminuée. Cependant, les mousses formées avec le WPC et le WPI traités par haute pression montraient une stabilité significativement prolongée ($P < 0,05$) par rapport au témoin. Une tendance significative à la diminution de l'efficacité émulsifiante et de la stabilité de l'émulsion, liée à l'intensité de la pression appliquée et de la durée de traitement, était observée aussi bien pour le WPC que le WPI.

propriété émulsifiante / propriété moussante / haute pression / concentré de protéines de lactosérum / isolat de protéines de lactosérum / solubilité / hydrophobicité de surface

1. INTRODUCTION

Whey protein isolate (WPI) and whey protein concentrate (WPC) are used as food ingredients due to their commercially important functional properties such as solubility, viscosity, water-holding capacity, gelation, adhesion, emulsification and foaming [45]. As foodstuffs they are applied not only because of their functional properties, but also because of their high nutritive value, reasonable cost and GRAS status [14].

Generally, the functional properties of food proteins may be classified into three main groups: (a) hydration properties, dependent upon protein-water interactions which have an important bearing on wetability, swelling, adhesion, dispersibility, solubility, viscosity, water absorption and water holding; (b) interfacial properties including surface tension, emulsification and foaming characteristics; and (c) aggregation and gelation properties, which are related to protein-protein interactions [3, 7, 19]. Improvements in functional properties may be achieved by modifying the protein structure by chemical, enzymatic or physical treatments [11, 15, 16, 18, 24, 28, 37, 43]. Functional properties of whey proteins such as emulsification, foaming and gelation are affected by their structure and mainly reflect the functionality of β-lactoglobulin as the most abundant protein, which has the ability to adsorb at the water-oil and air-water interfaces [39]. Some studies on the influence of pressure on pure β-lactoglobulin have indicated that high pressure had a notable effect on its conformational and aggregation properties, affecting its functionality [13]. However, functionality of WPC and WPI modified by high pressure still does not meet the expectations which could realise the full potential of these food ingredients in industrial application [23, 31].

The increased interest in novel technologies using mild treatments and without addition of chemicals are nowadays very much in demand. Although the effect of high pressure on food systems was first reported over 100 years ago, it is only in recent years that this technology has been seriously considered as a viable method of food processing and preservation [30]. One of the important aspects of pressure treatment is that food can be processed with minimal effect on the natural colour, flavour, taste and texture with little or no loss of vitamins [44]. High pressure has also been used as an effective technique of altering the surface

functional properties of water-soluble proteins [25, 35]. Pressure acts as a physicochemical parameter that alters the balance of intramolecular and solvent-protein interactions. Low protein concentrations and pressures up to 200 MPa usually result in reversible pressure-induced denaturation. Higher pressures (above 300 MPa) have irreversible and extensive effects on proteins, including denaturation due to unfolding of monomers, aggregation and formation of gel structures [5]. The extent of high-pressure-induced denaturation of whey proteins increases with treatment time [21, 22], treatment temperature [17] and pH [2]. The application of pressure has a disruptive effect on intramolecular hydrophobic and electrostatic interactions. As hydrogen-bonding interactions are relatively insensitive to pressure, high pressure disrupts the quaternary and tertiary structure of globular proteins with relatively little influence on their secondary structure [48].

The relevance of the present study lies in the use of a commercial WPC and WPI rather than conventionally produced proteins that are obtained by pH modification and ion exchange chromatography, and in the use of hydrostatic high-pressure treatment (up to 600 MPa) in model systems similar to commercial food systems. Selected WPC and WPI are good candidates for testing the practical utility of the application of high pressure to modify the functional properties of a complex protein system since they are in the form that the ingredients are utilised in a number of food applications. Moreover, several authors [26, 47] have observed gelation of whey proteins after pressure processing at concentrations up to 12% (w/w). In this respect, the objective of this work was to elucidate the effect of high-pressure treatment on dispersions of whey proteins at a concentration which is close to but insufficient for gelation, on the functional properties. Functionality was limited to solubility, foaming and emulsifying performance testing. We suppose that this research could encourage attempts to use these ingredients in a wide range of formulated food products to fully replace traditional additives such as milk powder or skim milk.

2. MATERIALS AND METHODS

2.1. Whey proteins

The WPC sample (Milacteal 60), obtained by ultrafiltration of casein serum, was purchased from Molkerei Strothmann (Guestersloh, Germany). The WPI sample (BiPRO) was kindly provided by Davisco Foods International (Le Sueur, MN, USA). Single batches were used without further purification. According to the manufacturers, the typical composition of this WPC on a dry basis was 61.3% protein, 4.5% ash, 26.8% lactose and 7.4% lipids, while the WPI contained (on a dry basis) 97.8% protein, 1.7% ash and 0.5% lipids.

WPI and WPC powders were each dissolved in distilled water by gentle magnetic stirring for 30 min to provide a 10% powder (w/w) dispersion and allowed to stand overnight at 4 °C before pressurisation. These extra storage periods contribute to the removal of foam bubbles and the completion of the hydration step. The pH values (at 20 °C) of the prepared dispersions were 6.47 for WPC and 6.92 for WPI, respectively.

2.2. High-pressure treatment

The pressure treatment was carried out in a LAB 50 single processor machine (SIG Simonazzi, Parma, Italy), at pressure levels of 300 and 600 MPa with holding times of 5 and 10 min. The samples (170 mL) were placed in PET bottles (45 × 135 mm) with a screw cap (internal diameter of 30 mm) and put into the high-pressure vessel of the processor through its upper opening. Care was taken not to leave any headspace between the closed screw cap and the liquid solution. Several samples in bottles were retained as controls. The vessel (400-mL capacity, 50 mm in diameter), made of the highest strength steel, was filled with a compression liquid (water-glycol mixture; 55/45, v/v) in order to isostatically transfer the high pressure. The pressure was raised to 300 MPa in 1 min 10 s, maintained at 300 MPa for 5 or 10 min, then released in 45 s. For pressurisation at 600 MPa, the pressure was raised in 1 min 55 s, maintained for 5 or 10 min, then released in 55 s.

The equipment was installed in an air-conditioned laboratory at 20 °C. Each sample was pressure-treated at a process temperature (40 ± 2 °C), taking into account the adiabatic heating which occurs during the compression phase. Compression was accompanied by an increase in temperature of 3.0–3.3 °C per 100 MPa. The temperature in the centre of the pressurisation chamber was measured using a thermocouple. Since pressurisation up to 600 MPa and starting from room temperature leads to a temperature increase of almost 20 °C, for lower pressure levels, prior to compression, samples were placed in a thermostated chamber, and after the samples reached the desired temperature hydrostatic pressure was applied (i.e. to reach 40 °C at 300 MPa the samples were kept at 31 °C). The whole pressure generation system is fully automated; a proportional valve and pressure feedback allow tuneable and very reproducible pressure cycles. Immediately after pressure treatment pressure-treated and control samples were frozen in a blast freezer at –50 °C and then freeze-dried at 10^{-2} Pa (for 48 h) [41]. Protein powder was transferred into plastic bags, hermetically closed and stored over dried silica gel until analysed.

2.3. Determination of protein solubility

Protein solubility, expressed in g of soluble nitrogen per 100 g of total nitrogen, was determined according to the modified method described by Funtenberger and coworkers [12]. After pressure processing, freeze-dried protein powders were dissolved with deionised water to obtain a protein concentration of 1% (w/w). The pH was adjusted to 4.6 or 7.0 with 0.025 mol·L^{-1} HCl or NaOH. Protein solutions were then centrifuged at 12 000× *g* for 15 min (Beckman model J-21B). Nitrogen in the supernatant was determined by Kjeldahl's method using the 6.38 conversion factor.

2.4. Surface hydrophobicity

The surface hydrophobicity of WPI and WPC was determined using 1 mL 0.1% protein solution mixed with 1 mL of 4×10^{-5} mol·L^{-1} 1-anilinonaphtalene-8-sulphonate (ANS) at ambient temperature [27]. The fluorescence intensity was recorded on a Perkin-Elmer LS 50 spectrofluorimeter (excitation at 365 nm, emission at 470 nm).

2.5. Foaming properties

For foaming properties evaluation, 10% (w/w) protein dispersions were whipped at room temperature with a mixer (MSM5220, Bosch, Germany) equipped with a wire whip beater at maximum speed settings (12 000 rpm) for 15 min. Whipping was interrupted every 5 min to determine foam expansion. Foam expansion was determined by level-filling a 100-mL plastic weighing boat with foam and weighing to ± 0.01 g. Afterwards, foam expansion was computed using the following expression:

$$\text{Foam expansion (\%)} = \frac{\text{Unwhipped dispersion wt(g)} - \text{Foam wt(g)}}{\text{Unwhipped dispersion wt(g)}} \times 100.$$

After the foam expansion was determined, the foam was returned to the bowl and whipping was resumed for an additional 5-min 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 all of the foam was determined and expressed as maximum foam stability [38].

2.6. Emulsifying properties

The emulsion stability index (ESI) and emulsifying activity index (EAI) for the protein- stabilised emulsions were determined by the turbidimetric technique [6]. For evaluation of emulsifying properties, emulsions were prepared by mixing protein dispersions (30 g·kg^{-1}) and 10 mL of sunflower oil in a ratio 1:2, for 90 s in a blender (Philips, Hamburg, Germany, model HR

2304). In the final emulsion, oil quantity was 0.67 $L \cdot L^{-1}$ and protein quantity was 0.15 $g \cdot L^{-1}$, respectively. The range of oil droplet sizes of control emulsions was established under 200× magnification using a light microscope (Universal Transmitted-Light Microscope: model Axioskop; Carl Zeiss MicroImaging, GmbH, Jena, Germany).

One millilitre of freshly prepared emulsions was pipetted out and serially diluted with 99 mL of distilled water (a hundred-fold) followed by 1 mL of the diluted emulsion into 39 mL (forty-fold) of 1 $g \cdot kg^{-1}$ SDS (to avoid flocculation), resulting in a four-thousand-fold total dilution. Absorbance of the final dispersions was measured at 500 nm (Helios-β Spectrophotometer, Pye Unicam Ltd, Cambridge, UK) and the turbidity was calculated using the following formula:

$$T = \frac{2.303 \times A}{l}$$

where T is turbidity, A is absorbance at 500 nm and l is the path length of the cuvette (m). Emulsions were placed under refrigeration at 4 °C and were gently redispersed for analysis after 24 h. The ESI and EAI were determined as follows:

$\mathrm{ESI(h)} = (A_0 / \Delta A) \times t$; where A_0 is the absorbance of the diluted emulsion immediately after formation, ΔA is the change in absorbance between 0 and 24 h (A_0–A_{24}) and t is the time interval, 24 h in this case.

$$\mathrm{EAI}(m^2 \cdot g^{-1}) = \frac{2 \times T \times \text{dilution factor}}{C \times \Phi};$$

where C is the weight of protein per unit volume of protein aqueous phase before emulsion formation, Φ is the oil volume fraction of the emulsion (0.67 in this case) and the dilution factor was 4000.

2.7. Statistical analysis

The whole study was repeated and each value represents the mean of four measurements from two independent high-pressure treatments. The effect of high-pressure treatment on the investigated functional properties was determined by two-way analysis of variance, using SPSS 10.0 for Windows software (Statistical Package for the Social Sciences, version 10.0). To test significant differences between the means Duncan's multiple range test was used. The significance level used was 5% ($\alpha = 0.05$).

Table I. Solubility (mean ± standard deviation of four measurements) of control and high-pressure-treated WPC determined at pH 4.6 and at pH 7.0.

Processing conditions	Solubility ($g \cdot 100\ g^{-1}$)	
	pH 4.6	pH 7.0
Control	89.9 ± 1.2[a]	91.1 ± 1.4[a]
300 MPa/5 min	76.2 ± 2.1[b]	86.3 ± 1.1[b]
300 MPa/10 min	72.6 ± 1.1[c]	84.7 ± 1.7[b]
600 MPa/5 min	65.1 ± 2.2[d]	81.4 ± 1.2[c]
600 MPa/10 min	65.0 ± 2.4[d]	78.9 ± 1.8[c]

Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1. Effect of high pressure on the solubility and surface hydrophobicity of WPC and WPI

The solubility at pH 7.0 or at pH 4.6 of control and samples pressurised for 5 or 10 min was determined by measuring the protein concentration in the supernatant after centrifugation, and it should be considered a reliable predictor of functionality. The value for solubility of non-pressurised samples measured at pH 7.0 was high, and it varied from 91.1 ± 1.4 $g \cdot 100\ g^{-1}$ for WPC (Tab. I) to 96.6 ± 1.5 $g \cdot 100\ g^{-1}$ for WPI (Tab. II). These high values reflect the high proportion of native whey proteins present in industrial products. The solubility of both control samples remained relatively high (in a range of 89.9 ± 1.2 $g \cdot 100\ g^{-1}$ to 95.8 ± 1.5 $g \cdot 100\ g^{-1}$), also measured at pH 4.6, which is close to the isolectric point of whey proteins. Solubility of WPC and WPI at pH 4.6 is a useful tool for estimating the degree of protein denaturation [38]. When protein solubility is measured near the

Table II. Solubility (mean ± standard deviation of four measurements) of control and high-pressure-treated WPI determined at pH 4.6 and at pH 7.0.

Processing conditions	Solubility (g·100 g^{-1})	
	pH 4.6	pH 7.0
Control	95.8 ± 1.5[a]	96.6 ± 1.5[a]
300 MPa/5 min	68.2 ± 1.8[b]	88.4 ± 2.1[b]
300 MPa/10 min	64.3 ± 2.1[c]	85.1 ± 2.4[b]
600 MPa/5 min	61.1 ± 0.9[d]	81.7 ± 0.9[c]
600 MPa/10 min	58.1 ± 2.2[d]	76.2 ± 1.2[d]

Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

Table III. Influence of high-pressure treatments on surface hydrophobicity of WPC and WPI in aqueous solution (0.1% w/w protein, 4×10^{-5} $moldm^{-3}$ ANS).

Processing conditions	Fluorescence intensity (I)*	
	WPC	WPI
Control	8.1 ± 0.2[c]	12.4 ± 0.1[c]
300 MPa/5 min	8.3 ± 0.1[c]	13.0 ± 0.2[b]
300 MPa/10 min	8.6 ± 0.1[b]	13.3 ± 0.2[b]
600 MPa/5 min	10.0 ± 0.3[a]	15.7 ± 0.4[a]
600 MPa/10 min	10.3 ± 0.3[a]	16.1 ± 0.4[a]

* Quoted values are the averages of four measurements. Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

isoelectric point of proteins, isoelectric precipitation is stimulated, leading to the removal of highly aggregated proteins.

After pressurisation, a significant reduction in protein solubility ($P < 0.05$), measured at pH 7.0 and pH 4.6, was found for all protein dispersions which were tested. The decrease was dependent on the intensity of applied pressure and the duration of pressure treatment, and consequently, for both samples, was the most pronounced when pressurisation was performed at 600 MPa for 10 min. Protein solubility of WPI, measured at pH 4.6 after processing at 600 MPa for 5 min decreased to 61.1 ± 0.9 g·100 g^{-1} and to 58.1 ± 2.2 g·100 g^{-1} after 10 min at the same pressure, respectively (Tab. II). Under the same treatment conditions, WPC samples showed solubility close to 65 g·100 g^{-1} (Tab. I). The diminished effect of high pressure on whey protein concentrate in comparison with the whey protein isolate could be explained by the smaller amount of proteins (6.13% (w/w) in WPC compared with 9.78% (w/w) in WPI counted on 10% (w/w) powder) and the presence of a significant amount of lactose. The baroprotective effects of disaccharides during pressurisation were previously reported, in similar, but not the same systems [10].

The lowest solubility values in all investigated samples may be attributed to the increased aggregation after pressure release. Since protein solubility at pH 7.0 significantly decreased ($P < 0.05$) after pressurisation, it indicates that the net charge or the surface hydrophobicity was modified by pressure processing. It seems possible that protein aggregation after pressure release, confirmed with decreased solubility measured at pH 4.6, is related to an increase in the globular protein surface hydrophobicity which resulted in the unmasking of previously hidden hydrophobic groups, as shown in Table III.

The data presented in this paper suggest that the formation of hydrophobic interactions, which are necessary for aggregate formation upon pressure release, may be extensive in the systems under investigation. Since it was revealed that the major protein component can primarily determine the functional behaviour of whey protein isolate or whey protein concentrate under the influence of high pressure [5], and taking into account that the amount of β-lactoglobulin in BiPRO is 73% (w/w) [36], we conducted a preliminary experiment on this project, with the aim of examining the susceptibility of pure β-lactoglobulin to aggregation under the same conditions of high-pressure treatment. The obtained optical density data showed strong aggregation of β-lactoglobulin even at 300 MPa [32]. According to these results we also expected an aggregation phenomenon in WPC and WPI dispersions after pressurisation at 300 MPa, which was confirmed in this work.

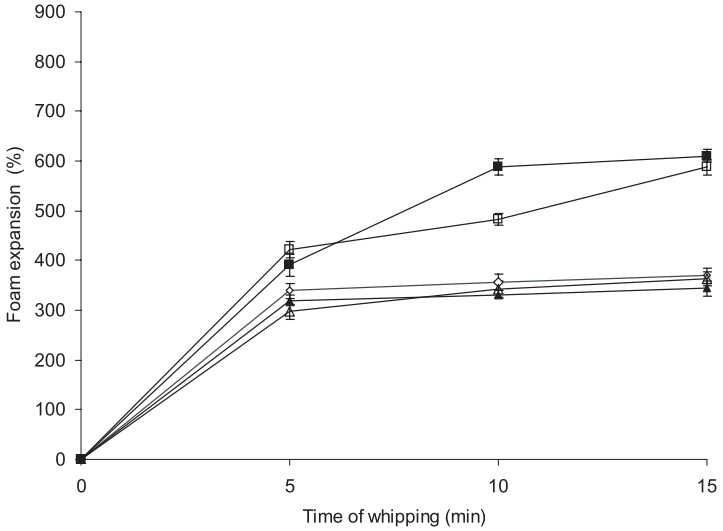

Figure 1. Foam expansion of the control WPC dispersion (10% w/w) (◇) and WPC dispersions (10% w/w) treated with high pressure under the following conditions: 300 MPa/5 min (■); 300 MPa/10 min (□); 600 MPa/5 min (▲); 600 MPa/10 min (△). Bars indicate the standard deviation of four measurements.

3.2. Effect of high-pressure treatment of WPC and WPI on their foaming ability and foaming stability

Foaming properties of WPC and WPI are commonly determined as maximum foam expansion, and foam stability. Under the same whipping conditions, control WPC (Fig. 1) exhibited reduced foam ability (foam expansion of 369%) compared with WPI (484%) due to their different composition (Fig. 2). The residual fat content in WPC has been considered as detrimental to foam formation and may even cause the foam lamella to break [29]. During whipping of all dispersions under investigation, foam volume increased with prolonged whipping time, as summarised in Figures 1 and 2. The most pronounced increase in the percentage of foam volume expansion was after the first 5 min of whipping and it slowed down during prolonged whipping. After the incorporation of a large quantity of air (40–60% volume), big bubbles occur in the first stage. Subsequently, the bubble size gets smaller and a narrower bubble size distribution is reached, while proteins form a cohesive intramolecular densely-packed film [40]. Control WPI (Tab. V) formed more stable foam, with a value for the maximum foam stability index of 290 ± 3.33 min, in comparison with the value for control WPC (210 ± 2.32 min) (Tab. IV). This is attributed to the previously discussed significant amount of β-lactoglobulin, which is capable of forming a thicker and more viscous film, thus improving foam stability [49].

After high-pressure processing of WPC, progressive loss of foaming ability was observed with the extension of treatment time. Pressure processing at 600 MPa caused a reduction in the foam volume of up to 363% due to formation of aggregates, which occurred after pressure release (Fig. 1). The role of proteins in foam film formation is in lowering interfacial tension. They concentrate at the cell interface, where they undergo partial unfolding and subsequent interaction via intermolecular bonding, which results in a cohesive film and stabilises the foam cells [1]. Aggregation

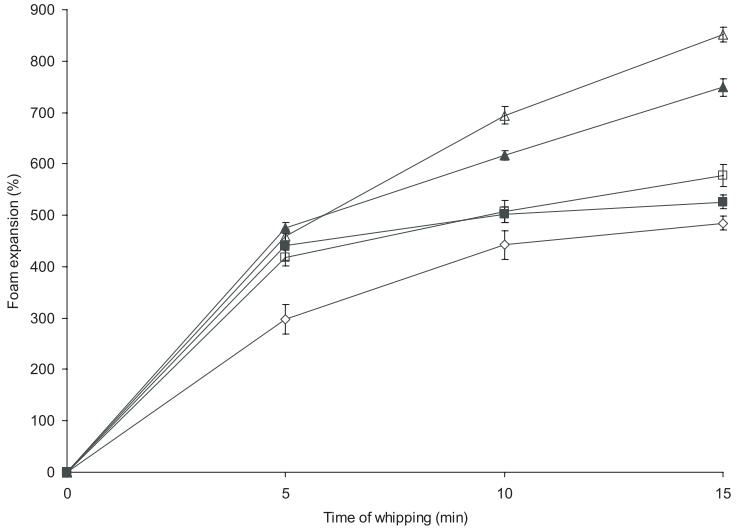

Figure 2. Foam expansion of the control WPI dispersion (10% w/w) (◇) and WPI dispersions (10% w/w) treated with high pressure under the following conditions: 300 MPa/5 min (■); 300 MPa/10 min (□); 600 MPa/5 min (▲); 600 MPa/10 min (△). Bars indicate the standard deviation of four measurements.

reduces the amount of protein available for film formation, but the films that are formed are considered to be thicker and more stable, facilitating the formation of a network structure in the protein film that results in improved rheological properties of the film, thereby increasing foam stability. It is suggested that cross-linking through S-S bridges gives good viscoelastic film formation [33]. This phenomenon was supported by the significantly increased value ($P < 0.05$) for foam stability and maximum foam stability indexes after high-pressure processing, as summarised in Table IV. High-pressure processing caused the foam stability index to increase from 1.10 ± 0.36 min (after treatment at 300 MPa / 5 min) to 6.54 ± 1.32 min (after 10 min at 600 MPa). Additionally, the maximum foam stability increased almost four-fold.

On the contrary, it was observed that increase in both hydrostatic pressure and treatment time significantly improved the foaming ability of WPI. The maximum value for foam expansion (851%) was reached in samples processed at 600 MPa for 10 min (Fig. 2). The intensity and the duration of high-pressure treatment are the most important parameters affecting the foaming behaviour of WPI. Whipping of 10% (w/w) protein dispersion ensures the

Table IV. Foam stability indicators (mean ± standard deviation of four measurements) for 10% (w/w) model dispersions prepared with control and pressure-treated WPC.

Processing conditions	Foam stability index (min)	Maximum foam stability (min)
Control	0.50 ± 0.02^{d}	210 ± 2.32^{e}
300 MPa/5 min	1.10 ± 0.36^{c}	325 ± 8.75^{d}
300 MPa/10 min	2.10 ± 0.50^{b}	475 ± 6.22^{c}
600 MPa/ 5 min	5.38 ± 1.12^{a}	728 ± 7.55^{b}
600 MPa/10 min	6.54 ± 1.32^{a}	915 ± 9.77^{a}

Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

Table V. Foam stability indicators (mean ± standard deviation of four measurements) for 10% (w/w) model dispersions prepared with control and pressure-treated WPI.

Processing conditions	Foam stability index (min)	Maximum foam stability (min)
Control	2.24 ± 0.77^{c}	290 ± 3.33^{e}
300 MPa/5 min	3.50 ± 1.33^{b}	420 ± 7.32^{d}
300 MPa/10 min	4.16 ± 1.22^{c}	789 ± 7.10^{c}
600 MPa/ 5 min	10.25 ± 2.20^{a}	701 ± 8.45^{b}
600 MPa/10 min	11.35 ± 2.12^{a}	1032 ± 9.75^{a}

Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

concentration of surface-active proteins available for the initial bubble formation. On the other hand, since previously hidden hydrophobic groups in the partially unfolded protein became exposed following pressure treatments (Tab. III) and proteins became more flexible to adsorb at a faster rate, they, in combination with an amount of surface-active proteins, are capable of counteracting the negative effects of aggregation and thus affect the foaming properties of WPI in a favourable manner. The foaming properties strongly correlate with the surface hydrophobicity [42]. Following an initial rapid drainage, macroscopic foams formed from dispersions of high-pressure-treated WPI were shown to persist over long periods of time with significant influence ($P < 0.05$) on pressure intensity and treatment duration (Tab. V). Protein aggregation imparts thickness to the film and retards the drainage of lamella liquid.

3.3. Influence of high pressure on emulsifying properties of WPC and WPI

The indices for emulsifying properties (ESI and EAI) of the emulsions produced using the control and high-pressure-treated whey protein concentrate and isolate are summarised in Tables VI and VII. There is a significant difference ($P < 0.05$) between the EAI values for WPC and WPI. The control WPI is characterised by the possibility

Table VI. Emulsifying activity index (EAI) and emulsion stability index (ESI) of emulsions prepared with control and pressure-treated WPC.

Processing conditions	EAI ($m^2 \cdot g^{-1}$)*	ESI (h)*
Control	117.81 ± 3.21^{a}	70.54 ± 1.11^{a}
300 MPa/5 min	113.45 ± 2.11^{b}	68.72 ± 0.39^{b}
300 MPa/10 min	112.45 ± 1.97^{b}	67.86 ± 0.62^{b}
600 MPa/5 min	109.07 ± 1.35^{c}	64.55 ± 2.14^{c}
600 MPa/10 min	106.42 ± 1.52^{c}	62.25 ± 2.47^{c}

* Quoted values are the averages of four measurements.
Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

Table VII. Emulsifying activity index (EAI) and emulsion stability index (ESI) of emulsions prepared with control and pressure-treated WPI .

Processing conditions	EAI ($m^2 \cdot g^{-1}$)*	ESI (h)*
Control	148.21 ± 1.56^{a}	72.18 ± 1.89^{a}
300 MPa/5 min	147.88 ± 1.71^{a}	68.14 ± 2.03^{b}
300 MPa/10 min	147.16 ± 1.65^{a}	67.88 ± 1.78^{b}
600 MPa/5 min	144.01 ± 1.34^{b}	65.11 ± 0.85^{c}
600 MPa/10 min	143.66 ± 1.18^{b}	64.34 ± 1.82^{c}

* Quoted values are the averages of four measurements.
Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

of stabilising a greater interface area per unit weight of protein compared with WPC, which can be quantified with a higher value of EAI (148.21 ± 1.56 $m^2 \cdot g^{-1}$ vs. 117.81 ± 3.21 $m^2 \cdot g^{-1}$). The presence of lactose and lipids in WPC prevent protein propagation at the interface surface, resulting in decreased values of the emulsion activity index [50]. The higher value for EAI of WPI is also related to its protein composition. WPI contains a significant amount of β-lactoglobulin (73%), which is known to be the major functional protein

and whose molecular flexibility and conformation changes at the water-oil interface enable it to act like an emulsifier [9]. Additionally, according to manufacturers, because of the ion-exchange method used in manufacturing processes, significant amounts of α-lactalbumin, BSA, immunoglobulins and lactoferrin are maintained in selected WPI. It contains less than 5% non-protein nitrogen (NPN).

In food emulsions, proteins play two major roles: on the one hand, they lower surface tension between the interfaces that are formed during the emulsification process, and on the other hand, they form a macromolecular layer surrounding the dispersed particles which structurally stabilises the emulsions by reducing the rate of coalescence, flocculation and oiling off [4, 8, 20].

The interfacial area which could be stabilised with one gram of WPI did not change significantly ($P > 0.05$) after pressurisation at 300 MPa. Hence, a significant decrease ($P < 0.05$) occurred after pressure treatment at 600 MPa for 5 and 10 min, respectively (Tab. VII.). On the contrary, a greater loss ($P < 0.05$) of the overall emulsifying efficiency of WPC was evident after pressurising even at 300 MPa (Tab. VI). It was expected that partial denaturation of proteins, with the resultant unfolding accompanied by increased surface hydrophobicity, should improve emulsifying efficiency. However, it could be seen that the proportion of proteins which could be adsorbed at the oil-water interface was insufficient to overcome the amounts of aggregated proteins that had destabilised emulsion. The complicated effects of pressure on emulsifying properties additionally could be explained by a change in conformation of the whey proteins during the emulsification process. Only a small part of the whey protein is likely to be absorbed at the water/oil interface in the coarse emulsion, because the kinetics of absorption requires a much longer time than that for emulsification.

Emulsions prepared with control WPI exhibited slightly prolonged stability in comparison with WPC (72.18 ± 1.89 h vs. 70.54 ± 1.11 h), as summarised in Tables VI and VII. This general observation is quite consistent with the measured oil droplet range (for emulsions prepared with control WPC oil droplet diameter was in the range of 0.38–0.56 μm, while for emulsions stabilised with control WPI oil droplet diameter was in the range of 0.25–0.35 μm), since emulsions with smaller oil droplet size should be more stable than those with larger droplets. Generally speaking, emulsions that are stabilised solely by protein are very stable to coalescence, provided sufficient protein is available to fully cover the droplet surface [46]. The most important single property of a protein-stabilised emulsion is the maintenance of emulsion throughout the storage period of the product. In stable emulsions the interfacial area does not change with time. However, if it occurs, coalescence causes an irreversible reduction in interfacial area, and the rates of these processes can be monitored using turbidimetry. In this experiment, the effect of high pressure on emulsifying properties of WPC and WPI was shown in terms of increased values of turbidity (during a storage period of one day) which is obvious in calculated values for emulsifying indices. According to these results (Tabs. VI and VII), it could be assumed that there was a trend toward larger droplet size with increasing pressure and treatment time. There are a number of possible reasons to account for the increase in mean droplet size: (i) the total droplet surface area that could be stabilised by the protein decreased; (ii) the rate at which the droplet surfaces were covered with protein decreased; and (iii) the frequency of droplet collisions increased due to the decrease in aqueous phase viscosity. All of these factors facilitate droplet disruption and lead to droplet coalescence, thereby leading to the formation of larger droplet sizes [34]. According to other researchers, in emulsions prepared with pressure-treated β-lactoglobulin as the emulsifier it was shown that substantially larger droplets occurred compared with those made with the native protein [13]. Consequently, in our experiment, a significant decrease ($P < 0.05$) in emulsion stability occurred in all samples after pressure treatment. The detrimental effect of high pressure was more evident in WPI (Tab. VII) due to the previously

discussed greater solubility loss accompanied by aggregation after pressurisation. Solubility, together with surface hydrophobicity, are also known as important properties for maintaining a stable emulsion [13].

It must be concluded that the positive effect of an increased surface hydrophobicity on emulsifying capacity is outweighed by the negative effect of protein aggregation, which makes less protein available for saturating the freshly formed interface with a monolayer of sterically stabilising globular protein molecules.

4. CONCLUSION

This study is part of a larger assessment of the effect of high pressure on model systems prepared with whey proteins in view of the application of high-pressure processing to the modification of functional properties of protein-rich foods.

The results presented in this paper demonstrate that pressure-induced changes in WPC and WPI greatly influence their solubility and surface hydrophobicity. Solubility decrease in combination with protein aggregation was related to the intensity of applied pressure and treatment time. These effects were much more evident in WPI model systems.

Pressurised WPI had significantly higher foam expansion, foam volume stability and foam liquid stability than the control samples due to the amount of available surface-active proteins which could ensure superior functional properties. High-pressure treatment at 600 MPa enhanced the foam formation properties of WPC, which was followed by improved foam stability due to retaining the drainage of lamella liquid. The detrimental effect of high-pressure processing on the emulsifying activity of WPI was noted after pressurisation at 600 MPa, whereas for WPC it was observed after pressurisation as low as 300 MPa. Rapid creaming, together with the loss of emulsifying stability, occurred in all emulsions prepared with high-pressure-treated whey proteins.

It should be mentioned that the phenomena taking place in the present model system do not necessarily predict the behaviour of whey proteins in commercial dairy foods, since the presence of other components (sugars, fats, other proteins, etc.) could modify the observed behaviour. Further research is underway to optimise the conditions of high-pressure treatment, in order to obtain enhanced functional properties related to protein-water interactions.

Acknowledgements: This study was funded by the Croatian Ministry of Science and Technology (Project No. 0058021). We thank Prof. Dietrich Knorr at the Berlin University of Technology, Department of Food Biotechnology and Food Process Engineering for providing access to high-pressure equipment and for fruitful advices.

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