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EFFECT OF ENVIRONMENTAL FACTORS AND PRESSURISATION ON EMULSIFYING PROPERTIES OF β-LACTOGLOBULIN PREPARATION

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Key words: β-lactoglobulin, emulsifying properties, environmental factors, high pressure, Sauters diameter (d_{vs})

A computerized imaging system was used for monitoring the emulsion droplet size distribution. The emulsifying properties of β-lactoglobulin preparation were observed to be affected by the environmental factors (protein concentration, pH, ionic strength) that determine the structure of the protein molecule. Generally, the d_{vs} value decreased with an increase in protein concentration and increased with a decrease in pH from 7.0 to 5.0 or an increase in the ionic strength from 20 to 100 mmol/L NaCl. Heterogenicity of emulsion and size of oil droplets in β-lg-stabilised emulsions increased with a pressure increase from 300 to 600 MPa and pressurisation time lengthening from 10 to 30 min at 300 MPa.

INTRODUCTION

The traditional use of whey proteins in the form of concentrates (WPC) and isolates (WPI) is not satisfying because the properties of particular proteins are not fully exposed. The need for properly purified specific whey proteins motivates intensive investigations of efficient methods for their fractionation. α-Lactalbumin (α-la) isolated with glycomacropeptide (GMP), recognized as the growth stimulator of bifidobacteria and the factor preventing the binding of viruses to intestine epithelium, and immunoglobulins (Igs) could be suitable for the production of infant formulas having the composition similar to a mother's milk, thus not causing an allergic response, and as a novel component of functional foods, so popular now. On other hand, β-lactoglobulin (β-lg) preparations could be used more widely in the food technology as the component determining the rheological properties of food products because of better functional properties than those of α-la, *i.e.* high stability in mild acid conditions and good emulsifying, foaming and gelling properties.

β-Lactoglobulin is a major protein in bovine whey, representing about 50% of the total whey protein, with an average concentration in milk and whey 3.5 g/L [Walstra *et al.*, 1999]. β-Lg is surface active and forms condensed films at interfaces. The functional properties of β-lg are strongly affected by its ability to thermal or pressure denaturation. A slight denaturation of globular molecule may have a great impact on its surface-active behaviour [Leman & Kinsella, 1989]. High pressure affects protein conformation and leads to protein denaturation, aggregation or gellation, depending on the protein system (*e.g.* type of protein, pH, ionic strength), the applied pressure and temperature, and the duration of the pressure treatment. High pressure treatment can be used to create new products or to obtain analogue products with minimal effect on flavour, colour and nutritional value and without any thermal degradation [Messens *et al.*, 1997]. Pressure denaturation of β-lg appears to be essential technique to improve the functionality of this protein for its use in the different applications fields of food manufacture.

The use of β-lg in the food production demands recognition of the relationships between the structure of the protein and its physicochemical and functional properties, and of its behaviour in the model systems. The objectives of our study were to determine the effects of some environmental factors (protein concentration, pH, ionic strength) and parameters of pressurisation (pressure, time) on the emulsifying properties of β-lg preparation.

MATERIAL AND METHODS

Material. β-Lactoglobulin was isolated from sweet whey (120 L) after rennet cheese production in a pilot scale (Chair of Dairy and Quality Management, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland), following centrifugation in a dairy centrifuge, cooling to 2°C, neutralisation to pH 7.3 with 2 mol/L NaOH and addition of 1 mol/L $CaCl_2$ solution to obtain the calcium concentration of 1.2 g/kg. The whey was then heated to 50°C and kept at this temperature for 8 min allowing deposition of phospholipoproteins [Fauquant *et al.*, 1985]. The supernatant was separated from the deposit by decanting.

Whey proteins were fractionated acc. to Caessens *et al.* [1997] method as shown in Figure 1. The whey (120 L) free

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from fat and phospholipoproteins was concentrated 24-times by ultrafiltration (10 kDa), mixed with 6% trichloroacetic acid solution at 1:1 v/v ratio, kept for 40 min at 20°C and centrifuged (7000 x G/15 min, 4°C). The supernatant was diluted 10-times, adjusted to pH 3.5 with 2.0 mol/L acetic acid and diafiltered (10 kDa) until the initial volume was obtained. The retentate 1 was ultrafiltered 6-times, Fielding the retentate 2, with a pH of approximately 4.0, wchich was vacuum evaporated at 50°C and lyophilised.

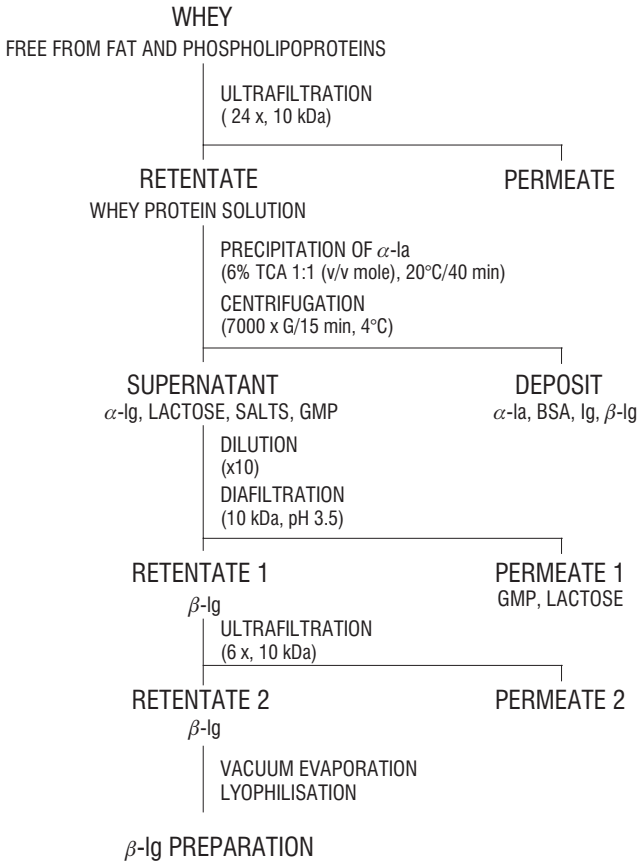

FIGURE 1. Production of β-lactoglobulin according to Caessens' *et al.* [1997] method.

Apparatus. Ultrafiltration was carried out in Alfa Laval UFS-unit with Romicon membrane from Subsidiary of Rohm and Hass Company of molecular cut-off 10 kDa. The deposit of α-lactalbumin was separated in a DuPont Sorvall OTD--Combi Ultracentrifuge with TFA-20.250 rotor. β-Lg preparation was lyophilised in a Labaconco LYPH-LOCK 6 unit.

Emulsifying properties. Soybean oil without preservatives (Olvit, Gdańsk, Poland) and buffered solution of β-lg (50 mmol/L phosphate buffer pH 5.0; 50 mmol/L citrate buffer pH 6.0: 50 mmol/L imidazole buffer pH 7.0) were mixed at the oil-water ratio 4:6 (w/w) in a lab homogeniser MPW 120 (10 000 rev/min; 5 min). The emulsion was deposited without diluting on a micro slide and observed in an optic microscope. The picture from a camera was registered in the memory of an IBM computer with Pentium 200 Processor and Matrox Magic image analysis card. On a basis of oil droplet diameters in emulsion determined with Mocha software (Jandel Scientific, Corte Madera, California, USA), the Sauter diameter (d_{vs}) was computed from the formula:

$$d_{vs} = \sum_{i=1}^{L} d_i^3 / \sum_{i=1}^{L} d_i^2 \quad (\mu m)$$

where: L – number of all measured oil droplets in emulsion, d_i – diameter of oil droplet i in emulsion (μm).

The effects of β-lg concentration (0.15; 0.5; 1.0 or 2.0%), medium acidity (pH 5.0; 6.0 or 7.0), ionic strength (20; 50 or 100 mmol/L NaCl) and pressure conditions (300; 450 or 600 MPa/10 min; 300 MPa/10; 20 or 30 min) on emulsion were examined.

The analysis was repeated thrice.

Analytical methods. β-Lg preparation was analysed using standard methods for the content of: dry matter, total nitrogen, 12% TCA soluble nitrogen (Kjeldahls method), calcium and magnesium (AAS method after wet mineralisation in a mixture of nitric and perchloric acids, 3:1 v/v), sodium and potassium (flame photometric method after wet mineralisation).

Data analysis. Statistical analysis of the results was carried out using Statsoft Statistica PL ver. 6.0 software. The significance of differences was determined at $p<0.05$.

RESULTS AND DISCUSSION

Based on microscopic observation it was shown that β-lg-stabilised emulsions were heterogenous dispersions of spheric oil droplets in water. The droplets sized 0 to 5 μm, 5 to 10 μm and 10 to 15 μm dominated the freshly made emulsions. Such tendency was observed for all analysed conditions of the experiment, irrespective of the protein concentration, medium pH and ionic strength. An increase in the β-lg concentration in dispersion caused statistically significant ($p<0.05$) decrease in Sauters diameter (d_{vs}) value for all emulsions analysed (Figure 2A). At pH 5.0 and

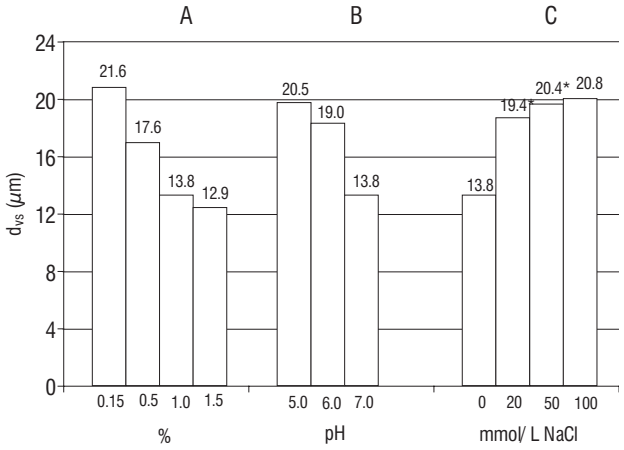

FIGURE 2. Effect of protein concentration (A), pH (B) and ionic strength (C) on d_{vs} value for β-lactoglobulin-stabilised emulsion; A: pH 7.0, o/w 4:6; B: protein concentration 1.0%, o/w 4:6; C: protein concentration 1.0%, pH 7.0, o/w 4:6; effect of protein concentration, pH and ionic strength statistically significant ($p<0.05$) except for samples with *.

6.0 the β-lg-stabilised emulsions were dominated by oil droplets with diameter of 5–10 μm (respectively, 27.5 and 32.5% total droplets), whereas at pH 7.0 the droplets sized up to 5 μm were predomianting (42.7%). A statistically significant ($p<0.05$) decrease was found in d_{vs} value with an increase in pH value from 5.0 to 7.0 (20.5 at pH 5.0, 19.0 at pH 6.0 and 13.8 μm at pH 7.0) (Figure 2B).

The study of the effect of NaCl concentration on the Sauters diameter of oil droplets in β-lg-stabilised emulsion showed the lowest d_{vs} value at 20 mmol/L NaCl (Figure 2C). Compared with control, an increase in ionic strength resulted in an increase in the Sauters diameter, but above 20 mmol/L NaCl the increases were statistically insignificant ($p<0.05$).

The following relationships resulted from the studies on the effects of protein concentration, medium pH and ionic strength on the volume surface diameter of o/w emulsions stabilised with β-lg preparations (Figures 2A, 2B, 2C): (i) as the protein concentration increased, the diameter decreased; (ii) as pH lowered, the diameter increased; (iii) as the ionic strength increased, the diameter increased. The effect of pH ranging from 5.0 to 7.0 on the d_{vs} value is evident, being consistent with the studies by other authors [Leman *et al.*, 2005; Das & Kinsella, 1989; Klemaszewski *et al.*, 1989]. Reactivity of a single thiol group and induction of exchange reactions of intra- and intermolecular disulfide bonds at pH 6.7 and above may to some extent be attributed to high emulsion stability.

An increase in pressure above 450 MPa caused that the emulsions were more heterogenous, which was expressed in an increase in d_{vs} value (Figure 3A). The d_{vs} values for emulsions stabilised with β-lg pressurised at 450 and 600 MPa were 22.8 and 23.9 μm, respectively, and were higher than d_{vs} value for control (21.6 μm) and β-lg pressurised at 300 MPa (18.5 μm). It was also shown that lengthening at the pressurisation time at 300 MPa to 20–30 min resulted in more heterogenous emulsions, thus increased the Sauters diameter (Figure 3B).

Studying the effect of β-lg pressurisation on the emulsifying properties of the protein, it was found that 10 min pressurisation at 300 MPa at pH 7.0 improved the properties. The emulsion stabilised with β-lg pressurised at the above conditions was statistically significantly ($p<0.05$) less heterogenous and had lower Sauters diameter value than the control emulsion. This agrees with our previous observations about emulsifying properties of pressurised β-lg preparations obtained after salting the protein out at pH 2.0 or following removal of α-la at mild conditions of temperature and pH [Leman & Dołgań, 2000].

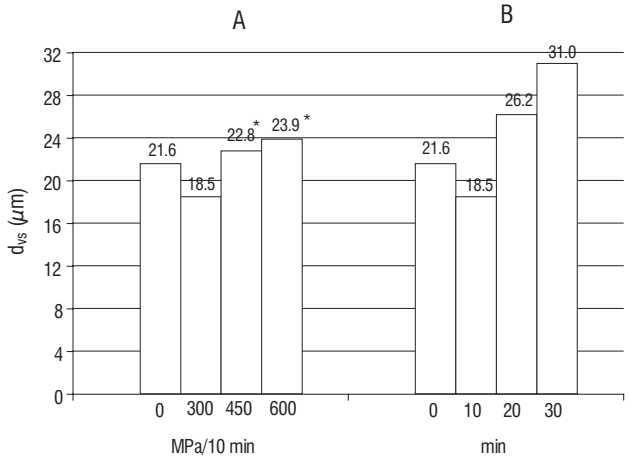

FIGURE 3. Effect of pressure (A) and pressurisation time at 300 MPa (B) of β-lactoglobulin solutions on d_{vs} value for emulsions stabilised with the pressurised protein (protein concentration 0.15%, pH 7.0, o/w 4:6); effect of pressure and pressurisation time statistically significant ($p<0.05$) except for samples with *.

Generally it was shown that emulsion heterogenicity and oil droplet sizes in β-lg stabilised emulsions increased with pressure and pressurisation time. The results obtained confirmed previous observations of other authors for highly pure β-lg. Galazka *et al.* [1997, 1995] and Dickinson and James [1998] reported on an adverse effect of β-lg and WPC pressurisation on their emulsifying properties, and Pittia *et al.* [1996] reported on an increase in d_{vs} value of oil droplets in emulsion stabilised with pressurised β-lg. These authors suggested that reversible changes in β-lg conformation occur upon high pressure due to exposure of hydrophobic groups, in consequence of which the protein hydrophobicity increases; *e.g.* Galazka *et al.* [1996] found 40% increase in surface hydrophobicity of β-lg pressurised for 20 min at 800 MPa. Nakamuras *et al.* [1993] observations about extensive β-lg aggregation induced by WPC pressurisation at 200–600 MPa confirm later Dumays *et al.* [1994] results on unfolding and extensive aggregation of β-lg as soon as after 15 min pressurisation at 450 MPa. Pressure-induced exposure of hydrophobic amino acid residues causes the formation of aggregates, thus decreases the rate of protein adsorption at the interface and the amount of protein adsorbed, and in consequence deteriorates the emulsifying properties of β-lg [Pittia *et al.*, 1996]. Funtenberger *et al.* [1997, 1995] and Yang *et al.* [2001] also inform about the formation of pressure-induced β-lg aggregates stabilised with intermolecular disulphide bonds, the size of which increases with pressurisation time. Dickinson & James [1998] found that at pH 7.0 the oil droplets in emulsion stabilised with 60 min pressurised protein increased with pressure, and that acceleration of oil droplet flocculation occurred above 400 MPa. According to Galazka *et al.* [1996], pressurised β-lg-stabilised emulsion showed a higher average diameter of droplets which increased with pressure, compared with native protein-stabilised emulsion. In their opinion, pressurisation modifies the β-lg structure, which results in the deterioration of the emulsifying ability despite increased surface hydrophobicity of the protein. Hayakawa *et al.* [1996] found that pressurisation influenced the secondary structure of β-lg, decreasing the percentage of α-helix structure from 39% in native protein to 4% in the protein pressurised at 1000 MPa for 10 min. Ledward *et al.* [1995] reported that α-helix structure decreased to 3% after 20 min pressurisation of β-lg in neutral medium. According to Yang *et al.* [2001, 2002], pressurisation of β-lg induces changes in the secondary structure leading to an increase in α-helix structures at the cost of β-structures and to formation of new disulphide bridges between free thiol group and native disulphide groups. Iametti *et al.* [1997] observed that there was a relationship between the rate of protein conformation changes and pressurisation time, founding distinct

changes at 600 MPa only after 10 min pressurisation and at 900 MPa as soon as after 2 min. The Yamauchi *et al.* [1980] studies on the influence of pH on oil droplet size in β-lg-stabilised emulsion showed the presence of the largest oil droplets in acid medium and a visible decrease in the droplet size range with pH increase.

CONCLUSIONS

The emulsifying properties of β-lg preparation were dependent on the environmental factors (protein concentration, pH, ionic strength) determining the protein molecule structure. The d_{vs} value was found to decrease with an increase in protein concentration and it increased with a decrease in pH value from 7.0 to 5.0 or an increase in ionic strength from 20 to 100 mmol/L NaCl.

Emulsion heterogenicity and oil droplet size in β-lg-stabilised emulsions increased with an increase in pressure from 300 to 600 MPa and with lengthening the pressurisation time from 10 to 30 min at 300 MPa.

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WPŁYW CZYNNIKÓW ŚRODOWISKOWYCH I PRESURYZACJI NA EMULGUJĄCE WŁAŚCIWOŚCI PREPARATU β-LAKTOGLOBULINY

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Rozkład wielkości kropli oleju w emulsji oceniono metodą komputerowej analizy obrazu. Emulgujące właściwości preparatu β-lg zależały od czynników środowiskowych (stężenie białka, pH, siła jonowa), które determinują strukturę cząsteczki białka. Generalnie, wartość d_{vs} zmniejszała się ze wzrostem stężenia białka i zwiększała się wraz z obniżeniem pH środowiska od 7,0 do 5,0 lub ze wzrostem siły jonowej od 20 do 100 mmol/L NaCl. Heterogenność emulsji oraz wielkość kropli oleju stabilizowanych preparatem β-lg zwiększały się wraz ze wzrostem ciśnienia od 300 do 600 MPa oraz z wydłużeniem czasu presuryzacji od 10 do 30 minut przy 300 MPa.