

# High pressure effect on foaming behaviour of whey protein isolate

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## Abstract

The effect of pressure-processing at 150–450 MPa for 5–25 min on foaming behaviour of whey protein isolate (WPI) have been investigated in pH range of 5.0–7.0. Assessment of foaming properties has been based on the total foam volume (FV) produced and the foam stability (FS), and the time for loss of half the initial FV. Response surface methodology has been applied to find the regression equations for predicting the FV and FS in terms of independent variables (the intensity of pressure, the treatment time and the pH). Protein solutions (1% w/v, 50 mM phosphate buffer) were pressure-processed and foams were produced from dilute solutions of WPI (0.005% w/v) by sparging with nitrogen gas at constant flow rate ( $0.2 \pm 0.01 \text{ dm}^3/\text{min}$ ) and constant temperature (20°C). It has been found that pH is significant ( $P \leq 0.01$ ) on both FV and FS; FV is influenced significantly ( $P \leq 0.05$ ) by pressure-intensity whereas the effect of pressure-treatment time on either FV or FS is not significant ( $P \leq 0.05$ ). Foaming properties of WPI were reduced as the pH approached to the isoelectric point of proteins due to enhanced aggregation. Pressurisation at high-protein concentration (2% w/v) or in high-buffer molarity (100 mM) has been observed to diminish the foaming properties of WPI. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** High pressure; Whey protein isolate; Foamability; Foam stability; pH

## 1. Introduction

Proteins are used in a variety of foods and comprise a major class of functional ingredients to enhance foaming, emulsifying, gelling and water binding properties (Phillips, Hawks, & German, 1995). The presence of adsorbed protein at the fluid interface between the aqueous and gaseous phases stabilises the structure of typical food foam at least in part. There is a growing interest for the use of whey proteins as a food ingredient (Zhu & Damodaran, 1994). If functional properties of whey proteins are enhanced, then they could provide better foam with improved stability. This facilitates imparting the desirable features to a wide range of food products including appearance, texture and consistency. Also, it may provide the requirements for new products, increase the quality of existing food foams by imparting body and smoothness and facilitates the dispersion of flavours in food foams. Enhancement of foaming properties can reduce the cost of production since less protein can provide the required features. Improvements

in functional properties may be achieved by modifying the protein structure by chemical, enzymatic methods or physical treatments (Kinsella & Whitehead, 1988; Kato, Osako, Matsudomi, & Kobayashi, 1983; Arai & Watanabe, 1988). Application of high pressure as a physical method is preferential accompanying with food safety and retention of food quality (Knorr et al., 1992). High pressure has been reported to be used in food systems such as inactivation of microorganisms (Karataş & Ahi, 1992; Erkmén & Karataş, 1997), modification of textures (Hayakawa & Kajiwara, 1992) as well as modification of biopolymers including enzyme inactivation and activation, protein denaturation and gelation or susceptibility to enzyme action (Masson, 1992; Dufour, Herve, & Haertle, 1992; Cheftel, 1992; Denda & Hayashi, 1992). Several studies have demonstrated alterations in the structure and/or functionality of proteins after high-pressure processing up to 1000 MPa (Weber & Drickamer, 1983; Pittia, Wilde, Husband, & Clark, 1996; Funtenberger, Dumay, & Cheftel, 1995).

Pressure-induced protein unfolding is a complex process. High pressure mainly ensues the formation of hydrogen bonds, and the disruption of hydrophobic bonds (Heremans, 1982). The extent of pressure-induced

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Notation		$r^2$	regression constant
FV	foam volume (cm <sup>3</sup> )	$X$	pressure (MPa)
FS	foam stability (s)	$Y$	treatment time (min)
		$Z$	pH

changes in proteins depends on the native protein structure and the applied pressure as well as extrinsic factors such as temperature, pH, solvent, and ionic strength (Iametti et al., 1997). The change in conformation at <200 MPa usually becomes reversible at low concentrations. Nonreversible denaturations leading to aggregation and gel formation have been observed at >200 MPa (Silva, Villas-Boas, Bonafe, & Meirelles, 1989). Whey protein concentrate (WPC) was found to produce pressure-set gels in the pressure range of 200–400 MPa at high-protein concentrations (Van Camp & Huyghebaert, 1995). Protein–protein interactions are favoured near the isoelectric point of whey proteins and, neutral or alkaline pHs stimulate the formation of intermolecular disulphide bonds. Dufour, Hui Bon Hoa, and Haertle (1994) indicated the extensive and irreversible denaturation of  $\beta$ -lactoglobulin between 150 and 300 MPa at pH 7, which enhances the reactivity of the SH group. Thus, the formation of intermolecular S–S bonds contributes to the irreversibility of unfolding (Funtenberger, Dumay, & Cheftel, 1997). In addition, the thiol group is capable of inducing thiol  $\leftrightarrow$  disulphide exchange between  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin (Jegouic, Grinberg, Guingant, & Haertle, 1997). The aggregation and gel formation of WPC  $\beta$ -lactoglobulin, which is the major protein component, were observed as indications to determine the functionality under high-pressure (Van Camp, Messens, Clement, & Huyghebaert, 1997). The highest solubility of whey proteins was observed at 400 MPa for 30 min and at pH 6 and the lowest solubility was encountered at pH 5 at the same pressure. Galazka, Ledward, Dickinson, and Langley (1995) concluded that substantial changes in emulsifying behaviour of WPC at pH 7.0 after high pressure processing were similar to those obtained for  $\beta$ -lactoglobulin. Improvements of surface properties and functionality could lead to the modification of protein structure by pressure-treatment. Changes in emulsification of ovalbumin (Denda & Hayashi, 1992), foaming of egg white (Knorr et al., 1992), surface properties of  $\beta$ -lactoglobulin (Pittia et al., 1996) after pressurisation have been reported.

The present paper represents the investigation of the foaming behaviour of whey protein isolate (WPI) as changed by the intensity of pressure applied and the treatment time using response surface methodology. Foaming properties of WPI were evaluated as foamability and the foam stability (FS) in the pH range of 7–5.

## 2. Materials and methods

WPI (99% protein) was obtained from Davisco Foods International (Le Sueur, MN, USA). Buffer salts were purchased from BDH Chemicals (Poole, UK).

### 2.1. High-pressure treatment

Experiments were carried out in 50 mM potassium phosphate buffer in the pH range of 5.0–7.0. WPI solution (1% w/v) was prepared by dissolving the powder in the buffer whose pH was adjusted according to Table 1. Protein solution was hermetically sealed in polyethylene bags (12.0  $\times$  6.5 cm, Gezgin Plastik, Gaziantep, Turkey) as 20 ml per bag for pressure-application. The pressure-treatment was carried out in water in a cylindrical hydrostatic pressure unit having an internal capacity of 150 ml, as previously described (Karataş & Ahi, 1992). The samples were pressurised according to the combinations given in Table 1. The pressure chamber was not temperature-controlled. The initial temperature of the water was 20°C before treatment. Maximum temperature reached was 25–26°C up to 450 MPa in high-pressure application. The required pressure was maintained at a rate of 0.3 min/100 MPa and released in 0.7 min after holding time. Immediately after pressure-release the temperature of the sample was reduced to 20°C and foaming experiments were performed. Pressure-treatment (300 MPa for 15 min) was applied to 2% (w/v) WPI solution (in 50 mM phosphate buffer) and to 1% (w/v) WPI solution (in 100 mM phosphate buffer) to investigate the effects of protein concentration and the buffer strength during pressurisation. In pressure applications, two different samples were treated separately in subsequent operations in each time.

Table 1  
Independent variables and experimental design levels used in foaming experiments<sup>a</sup>

	Standardized levels				
	$-\alpha$	-1	0	+1	$+\alpha$
Pressure (MPa)	150	211	300	389	450
Time (min)	5	9	15	21	25
pH	5	5.41	6	6.59	7

<sup>a</sup>  $\alpha = 1.682$ , the coded axial distance from the centre point of the design.

## 2.2. Foam formation and stability

Pressure-treated protein solutions were diluted to 0.005% (w/v). The samples were foamed in a glass-sintered column of 250 mm in length and 35 mm in internal diameter. The column has a sintered disc of 40–100  $\mu\text{m}$  located throughout the whole cross-section at the lower end and an extension for nitrogen inlet below sinter. The temperature was kept at 20°C throughout the experiment by means of a water jacket around the column. Nitrogen gas was fed into the column after passing through a copper coil placed in a thermostat tank and a flowmeter. Five millilitres of sample solution was sparged at a constant flow rate ( $0.2 \pm 0.01 \text{ dm}^3/\text{min}$ ) for 15 s in each time. Immediately after turning off the gas, the foam height was read from the calibrated markings on the column and the foam volume (FV) was calculated. The time for the collapse of the foam to half of its initial value is measured to express the FS. Foaming experiments were done at least in triplicates.

## 2.3. Experimental design and statistical analysis

A three-factor composite rotatable design was chosen to study the contribution of the three independent variables: pH, pressure and treatment time. Ranges of the selected independent variables are shown in Table 1 (pH: 5.0–7.0; pressure: 150–450 MPa; treatment time: 5–25 s). Composite rotatable designs have been used in food research (Mullen & Ennis, 1979) due to the smaller number of experiments needed while maintaining statistical effectiveness. Response surface methodology was applied to the data using a commercial statistical package, Design-Expert version 5.0 (Stat-Ease, Minneapolis, MN, USA). Polynomial equations were fitted to the data to obtain regression equations. The model with a higher regression coefficient ( $r^2$ ) was then chosen to represent the actual data points. Statistical significance of the terms in the regression equations were examined. Response surface plots were generated using the same software. The whole study was replicated.

## 3. Results and discussion

High pressure and treatment time are two important parameters affecting the foaming behaviour of food proteins (Halling, 1981). In the present study, the influence of the intensity of pressure and application time on the FS and FV of WPI was investigated using response surface methodology in the pH range of 5.0–7.0. The regression equations giving the relationship between responses (FV and FS) and the parameters studied (pressure, treatment time and pH) are given in Table 2. The regression equation coefficients can be used in determining the significance of the terms relative to each

Table 2

Regression equation coefficients for FV ( $\text{cm}^3$ ) and FS (s) of WPI in terms of actual variables<sup>a</sup>

	FV ( $r^2 = 0.83$ )	FS ( $r^2 = 0.88$ )
Constant	+48.91	+99.75
$X$	-1.08**	+0.24
$Y$	-13.97	-3.72
$Z$	+78.14*	-41.55*
$X^2$	$-2.62 \times 10^{-4}$	$-3.4 \times 10^{-4**}$
$Y^2$	+0.12	-0.034
$Z^2$	-10.5	+3.60
$XY$	-0.014	$-1.18 \times 10^{-4}$
$XZ$	+0.22	$-5.89 \times 10^{-3}$
$YZ$	+2.27	+0.8**

<sup>a</sup>  $X$ : pressure (MPa);  $Y$ : treatment time (min);  $Z$ : pH.

\*  $P \leq 0.01$  (significant at 99% confidence interval).

\*\*  $P \leq 0.05$  (significant at 95% confidence interval).

other. Results show that pH was significant ( $P \leq 0.01$ ) on both FV and FS. Pressure was significant ( $P \leq 0.05$ ) on the FV but not on the FS. Pressure-treatment time was not significant ( $P \leq 0.05$ ) for either FV or FS. Interaction of the pressure-treatment time with pH and the square of pressure were significant ( $P \leq 0.05$ ) for the FS. In the generation of response surface plots the fitted data was used rather than the actual data points. The regression coefficient ( $r^2$ ) indicate the correlation of the actual data to the fitted data (Table 2).

High pressure induces extensive unfolding of protein chains, permitting subsequent intermolecular interactions upon pressure-release (Cheftel, 1992). Fig. 1 represents the variation of foaming ability of WPI over the pressure range of 150–450 MPa, and the pressure-treatment period of 5–25 min. The increase in both

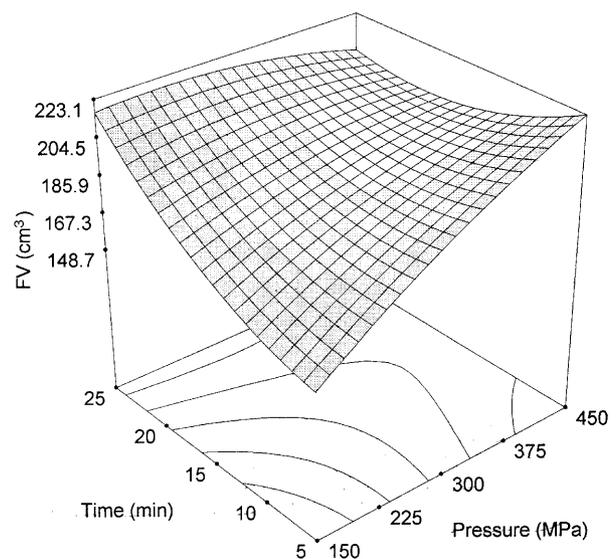


Fig. 1. Effect of high pressure and treatment time on FV of WPI solution (0.005% w/v) at pH 7.0.

pressure and treatment-time were observed to improve the FV at pH 7.0. 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 disrupts hydrophobic interactions and ionic bonds resulting in protein molecules being more flexible to adsorb at a faster rate (Dickinson, 1989). The effect of treatment time seemed to be more pronounced in the pressure range (150–300 MPa) compared with pressure-intensity (Fig. 1). However, pressurisation time effect appeared to be avoided as the pressure is increased.

High-pressure treatment at pH 6.0 and 5.0 reduced the foaming ability of WPI (Figs. 2 and 3). A slight improvement in foamability was observed at pH 6.0 at 150 MPa as the treatment time increased up to 25 min (Fig. 2). The progressive increase in the FV may indicate a gradual alteration in the protein structure as the treatment time is extended. However, the foaming ability was lost as the treatment time was increased at pressures higher than 150 MPa. No marked effect of high pressure in the range of 150–450 MPa on foamability was observed (Fig. 2) when the protein solution was treated for 5 min at pH 6.0. This suggests that both the intensity of high pressure and the duration of application is needed to be optimized to obtain the structural alterations in the protein structure that coincide with the functional requirements. Reduction in the FV at <pH 7.0 can be explained with the loss of solubility of WPI. A significant reduction in the solubility of whey proteins after pressurisation has been revealed by several authors (Funtenberger et al., 1995; Pittia et al., 1996). The decrease is most pronounced when pressurisation is performed near the isoelectric point of the whey proteins. Therefore, subsequent decrease in foaming ability upon pressure-treatment at pH 5.0 (Fig. 3), which is very

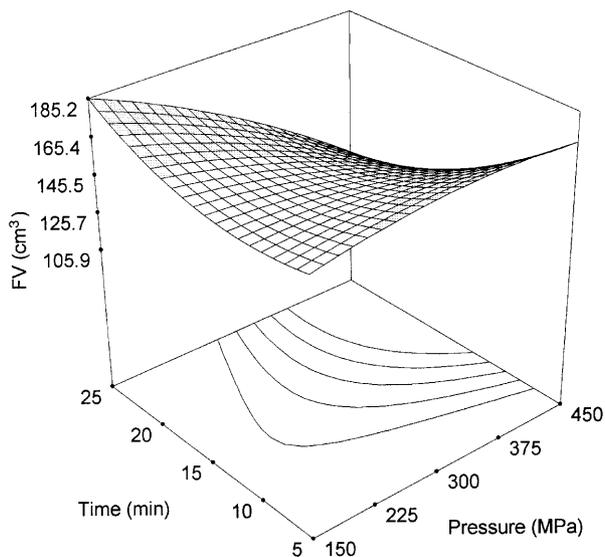


Fig. 2. Effect of high pressure and treatment time on FV of WPI solution (0.005% w/v) at pH 6.0.

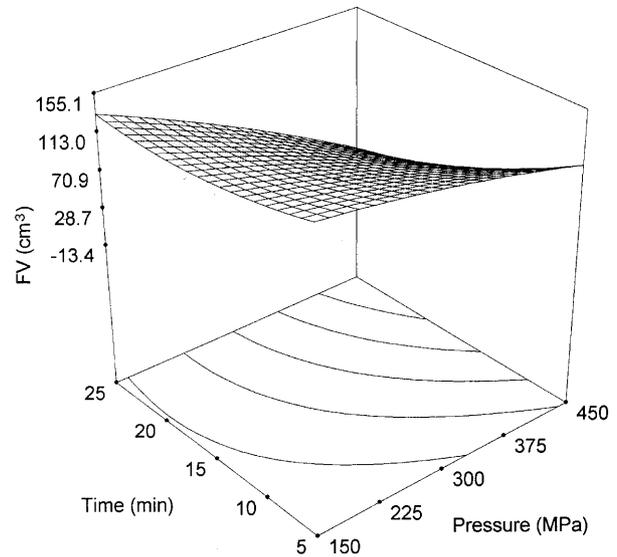


Fig. 3. Effect of high pressure and treatment time on FV of WPI solution (0.005% w/v) at pH 5.0.

near to pIs of whey proteins, can be explained by this fact. Adverse effects on emulsifying properties and solubility were seen by pressurisation around pI (Denda & Hayashi, 1992). The loss in overall solubility is explained by aggregation, which is caused by the association of unfolded proteins through hydrophobic interactions and facilitated due to the absence of repulsive forces near pI of proteins. Above pI, the repulsion between negatively charged carboxyl groups reduces the protein aggregation. It was revealed (Cheftel, 1992) that the major protein component can primarily determine the functional behaviour of WPI under high pressure. Van Camp et al. (1997) demonstrated that it is  $\beta$ -lactoglobulin which is significantly losing its solubility, while no significant changes are noticed in  $\alpha$ -lactalbumin in WPC. The reduced overall functionality of WPI in the foaming experiments may result from the large content of  $\beta$ -lactoglobulin in WPI.  $\beta$ -lactoglobulin was observed to be losing its foaming ability near pI upon pressurisation at 300 MPa for 15 min (unpublished data).

Fig. 4 shows the change in FS with pressure (150–450 MPa) and treatment time (5–25 min) at pH 7.0. It was observed that FS increases up to 300 MPa and then decreases as the pressure-intensity is increased. Although foamability is enhanced at pH 7.0 (Fig. 1) due to the increase in the hydrophobicity of protein upon pressure-treatment (Pittia et al., 1996), the reduction in stability above 300 MPa may be explained as the detrimental effect of unfolding to stability by lessened viscoelasticity of the film with reduced number of interactions between molecules. Influence of treatment time on FS appears to be more extensive (Fig. 4). An increase in the duration of treatment time results in the improvement in FS between 150 and 450 MPa. This may be due to protein aggregation upon pressure-release.

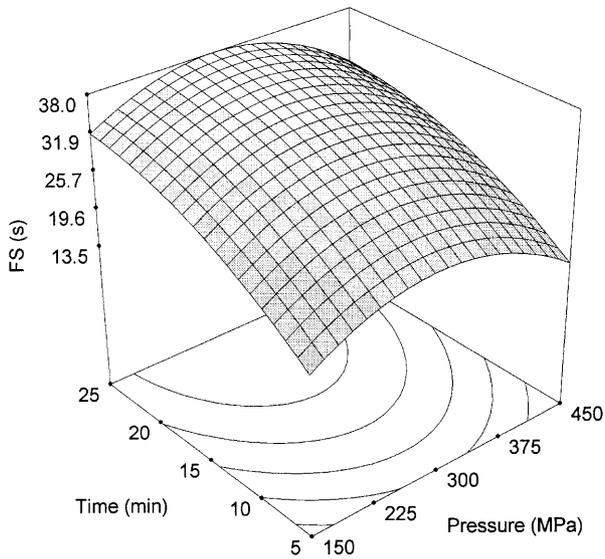


Fig. 4. Effect of high pressure and treatment time on FS of WPI solution (0.005% w/v) at pH 7.0.

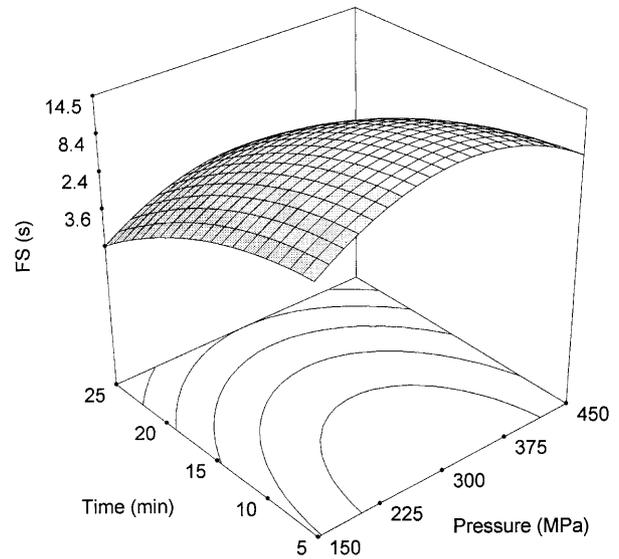


Fig. 6. Effect of high pressure and treatment time on FS of WPI solution (0.005% w/v) at pH 5.0.

Protein aggregation imparts thickness to the film and retards the drainage of lamella liquid (Zhu & Damodaran, 1994). At pH 6.0 FS gradually increases and then decreases with high pressure and duration of pressurisation (Fig. 5). The optimum treatment is at 300 MPa for 15 min to have maximum FS at this pH. An extended treatment time (15–25 min) or high-pressure exposure (300–450 MPa) reduces the stability. At pH 5.0 FS is improved with the pressurisation in the range of 150–450 MPa for a treatment time of 5 min (Fig. 6). Increase in treatment time reduces the FS at any pressure. Protein aggregation takes place more readily at acidic pHs due to reduced electrostatic repulsion. Therefore high-pressure treatment diminishes the amount of protein available for adsorption. This is

consistent with the reduced foaming ability at pHs near pI (Figs. 2 and 3). Thus, depletion of the concentration of protein or formation of aggregates with reduced diffusion properties could result in an adsorbed layer, which is less resistant to liquid drainage and external disturbances.

When 300 MPa pressure was applied for 15 min to 1% and 2% WPI solutions at pH 7.0 to see the effect of concentration of pressurised sample (Fig. 7), a reduction in FV and FS of the sample of high concentration was

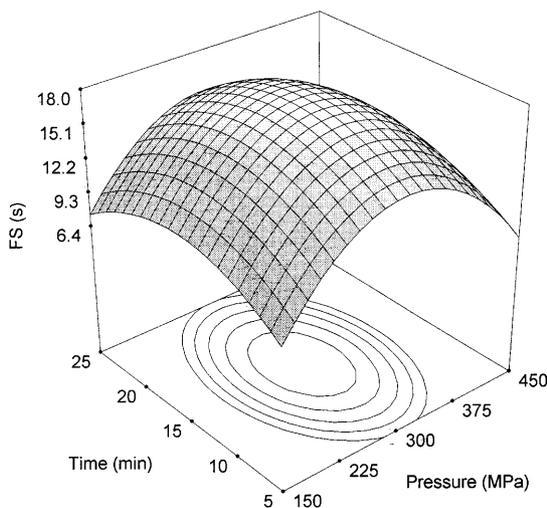


Fig. 5. Effect of high pressure and treatment time on FS of WPI solution (0.005% w/v) at pH 6.0.

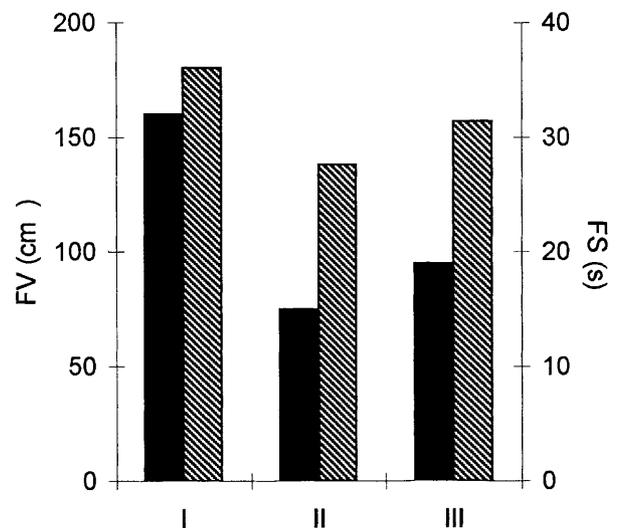


Fig. 7. FV and FS of WPI solution (0.005% w/v): (a) WPI solution (1% w/v) pressurised at 300 MPa for 15 min in 50 mM phosphate buffer at pH 7.0; (b) WPI solution (2% w/v) pressurised at 300 MPa for 15 min in 50 mM phosphate buffer at pH 7.0; (c) WPI solution (1% w/v) pressurised at 300 MPa for 15 min in 100 mM phosphate buffer at pH 7.0. Solid bar shows the foam stability and shaded bar represents the foam volume.

observed. This may indicate a severe aggregation taking place at high concentration. The formation of hydrophobic interactions upon pressure-release may be extensive in the solutions in which the molecules are in close proximity. Also foaming properties appeared to be reduced when buffer molarity was doubled to 100 mM (Fig. 7). An extensive aggregation of the protein with high-buffer molarity may occur compared to the sample with low buffer molarity. Previous experiments revealed a decrease in protein solubility proportional to the increase in buffer molarity (Funtenberger et al., 1995) and the formation of high molecular weight aggregates for  $\beta$ -lactoglobulin isolate solutions when pressurised at a higher concentration (Dumay, Kalichevsky, & Cheftel, 1994).

In order to verify the validity of the models obtained for FV and FS, independent experiments whose conditions were chosen arbitrarily within the range of variables were conducted. After pressure-treatment at 350 MPa for 12 min, WPI solution at pH 6 gave FV of 160.0 cm<sup>3</sup> and FS of 18.0 s. The calculated values from the regression equations (Table 2) for the same conditions were 156.9 cm<sup>3</sup> and 19.5 s for FV and FS, respectively.

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