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Emulsifying and gelling properties of porcine blood plasma as influenced by high-pressure processing

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Abstract

The emulsifying properties and the characteristics of heat-induced gels (texture and water-holding capacity) prepared from pressurised blood plasma solutions of different pH (5.5–7.5) were investigated. Changes in the plasma, that affected its behaviour as an emulsifier, occurred after pressurisation. The highest emulsifying activity was found for samples treated at 400 MPa. At pressures above 400 MPa, the emulsifying activity and stability, at all pHs, decreased with increasing pressure. The hardness of heat-induced gels decreased significantly as the pressure increased above 400 MPa, this effect being more noticeable with decreasing pH. However, although a 600 MPa pressurisation induced a further decrease in the firmness of gels from solutions at pH 6.5 and 7.5, it increased the hardness of gels at pH 5.5. For treatments up to 500 MPa, the highest elasticity was found in gels from solutions at pH 7.5. Pressure treatments above 400 MPa improve the water-holding capacity of heat-induced gels prepared from plasma solutions at pH $\geqslant$6.5. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Porcine blood plasma; High pressure processing; Emulsifying properties; Heat-induced gels; Water holding capacity; Texture; pH dependence

1. Introduction

There is increasing interest by the food industry in high-pressure processing as a technology to obtain high quality traditional and novel products. The pressures involved in the treatment of food (100–1000 MPa) cause a variety of changes, including destruction of microorganisms, alteration of enzyme-activity, control of phase changes and altered conformation of biopolymers, leading to changes in functional properties, which may be beneficial to product quality (Galazka & Ledward, 1998).

Many authors have reviewed the effects of high pressure on proteins (Balny & Masson, 1993; Balny, Mason, & Travers, 1989; Gross & Jaenicke, 1994; Heremans, Van Camp, & Huyghebaert, 1997; Mozahev, Heremans, & Frank, 1994; Silva & Weber, 1993). Pressure-induced denaturation of proteins is a complex phenomenon that depends on the protein structure, pressure, temperature, pH, ionic strength, and solvent composition (Masson, 1992). Studies, carried out on volume changes in proteins, have shown that the interactions, responsible for the stabilisation of the tertiary and quaternary structures, such as hydrophobic and electrostatic interactions, are the most pressure-sensitive (Balny & Masson, 1993; Galazka & Ledward, 1998; Heremans, 1982, 1992). Hydrogen-bonding, which stabilises the α helical and β pleated sheet forms of proteins, and covalent bonds, are little-affected by pressure. Covalent bonds are stable, since their formation gives a negative volume change (-10 ml.mol^{-1}), and almost zero values for exchanges between covalent bonds (Mozahev, Heremans, & Frank, 1994). However, at pressures about 300 MPa, sulfhydryl groups may oxidise to form -S-S- bridges (Galazka & Ledward, 1998).

By-products, such as blood from slaughtered animals, are known to be potential sources of nutritional and functional protein. Many authors have suggested the utilisation of blood proteins as functional ingredients in foods (Raeker & Johnson, 1995; Tybor, Dill, & Landmann, 1975; Wismer-Pedersen, 1979, 1988). Among the functional properties of proteins, the capacity to form a gel on heating and emulsifying properties are important in food processing. Gels may give texture, consistency and a medium that enables retention of water, nutrients

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and flavour and plasma proteins have been shown to have good gelling properties (Hermansson, 1982; Howell & Lawrie, 1984) and to be good emulsifying agents (Hermansson & Tornberg, 1976; Saito, Ichikawa, & Taira, 1988; Tornberg & Jönsson, 1981).

Native bovine serum albumin undergoes significant unfolding and aggregation following pressure treatment (Galazka, Summer, & Ledward, 1996), and a loss of secondary structure, depending on the magnitude of applied pressure (Hayakawa, Kajihara, Morikawa, Oda, & Fujio, 1992; Hayakawa, Kanno, Tomita, & Fujio, 1994). Since serum albumin is the main component of plasma proteins (55–64%), a change in the functional properties of pressurised plasma may be expected.

The aim of the present study was to determine the influence of high-pressure treatment on the functionality of plasma proteins. Several experiments were conducted to assess the efficiency of pressure-treated plasma to aid in the dispersion of the oil phase when used as emulsifier, as well as the stability of the emulsions obtained. The textures and water-holding capacities of heat-induced gels, from pressurised plasma, were also determined.

2. Materials and methods

2.1. Preparation of protein solutions

Porcine blood plasma powder (Batch 614-5103), purchased from Harimex (Loenen, Netherlands), was used in this study. A 10% (w/v) solution of plasma powder in distilled water was prepared to adjust the concentration to that of the original liquid plasma. The pH was adjusted to 7.5, 6.5, or 5.5 with 1 M HCl. The pH was adjusted to 7.5 because this is the natural pH of liquid plasma, and to pH 6.5 and 5.5 because plasma is regarded as a potential functional ingredient in food products with a neutral or slightly acidic pH. Our previous studies, on the effect of pH on blood plasma heat-induced gels, indicated that the plasma would not be a good gelling agent, nor good for water-holding, in products with a pH of $\leqslant 5.5$ (Parés, Saguer, Saurina, Suñol, & Carretero, 1998). Five aliquots (30 ml) of each sample were packed into plastic bags and sealed for the emulsifying activity studies. To determine the gelling properties, five aliquots (50 ml) of each sample were packed into flexible cylindrical plastic tubes (23 mm diameter). In both cases, one aliquot was kept as a non-treated control while others were pressurised for 15 min at 300, 400, 500, or 600 MPa, respectively.

2.2. High-pressure treatment

The treatments were carried out using a prototype Stansted "Food lab" high-pressure apparatus (Stansted Fluid Power, Essex, UK). Samples were introduced into the working chamber (300 ml capacity), containing the low compressibility fluid (2:8 of a mixture of caster oil: ethanol). The equipment was installed in an air-conditioned laboratory at 20°C. The temperature in the pressurisation chamber increased during compression reaching a maximum after 2 min (the maximum temperature achieved in all treatments was 30°C, corresponding to the treatment at 600 MPa), then decreased to 20°C in 0.5 min, and remained stable at 20°C until the end of the cycle. The compression and de-compression times were about 1–2 min and 0.5 min, respectively. After treatment, the control and the treated samples were kept at 5°C for 16 h.

2.3. Preparation of emulsions

The required dilution in distilled water was prepared from each sample just before production of the emulsion. 50 ml of commercial corn oil and 150 ml of the aqueous protein solution were shaken together and homogenised using a hand-operated laboratory piston-type homogeniser (MFC Microfluidizer™ Series 5000, Microfluidics Corporation, Newton, MA, USA). The emulsion was collected after five passages through the homogeniser at a homogenisation pressure of 6 MPa. Temperature was maintained at 20°C. Duplicate preparations were carried out for each sample.

2.4. Measurements of turbidity

Preliminary experiments were carried out to standardise the experimental conditions. Some of the variables affecting turbidimetric measurements of protein-stabilised emulsions, such as the concentration of plasma powder solution and the dilution of the emulsions, were studied. Immediately and 10 min after homogenisation, aliquots (20 μl) of the emulsion were diluted with 0.1% sodium dodecyl sulphate (SDS) to give a final dilution of 1/2500. The absorbance of the diluted emulsions on duplicate aliquots was then determined in a 1-cm pathlength cuvette at a wavelength of 500 nm in a Philips PU 8620 UV/VIS/NIR spectrophotometer (Pye Unicam Ltd, Cambridge, UK).

Results are reported as Emulsifying Activity Index (EAI) for each solution (Pearce & Kinsella, 1978): $\mathrm{EAI} = 2T/\phi C$, where T is turbidity, ϕ is the volume fraction of dispersed phase, and C is the weight of protein per unit volume of aqueous phase before the emulsion is formed. EAI has units of area of interface stabilised per unit weight of protein (m^2/g protein). An Emulsion Stability Index (ESI) was also calculated: $\mathrm{ESI} = T \times \Delta t/\Delta \mathrm{T}$, where ΔT is the change in turbidity, T, occurring during the interval Δt.

2.5. Preparation of heat-induced gels

Samples were heated at 80°C for 45 min, in a water bath, cooled to room temperature (25°C) and kept at 5°C for 16 h.

2.6. Texture analysis

Gel cylinders were cut into portions (23 mm dia×14 mm height) and Texture Profile Analysis (TPA) was performed by means of a TA.XT2 texture analyser (Stable Micro Systems, Surrey, UK). A flat cylindrical probe (35 mm dia) was used to compress samples twice until a 30% strain was attained. The rate of uniaxial compression was 1 mm s^{-1}. The mean of four TPA measurements for each combined treatment (pressure and pH) was used to calculate the hardness and elasticity values of every single gel. Hardness, defined as the maximum force attained during the first compression cycle, is expressed in Newtons. Springiness, defined as the extent to which a compressed sample returns to its original size when the load is removed, was calculated as the ratio of the height of the sample at the beginning of the second compression cycle to its original height.

2.7. Water holding capacity (WHC)

A technique combining filtration and centrifugation, based on the method proposed by Kocher and Foegeding (1993) with slight modifications (Parés, Saguer, Saurina, Suñol, & Carretero, 1998), was used to determine WHC. Cylinders (10 mm length and 8 mm dia) of gels were placed into bags (1×5 cm) made from a GORE-TEX® filter membrane (pore dia 0.45 μm) (W.L. Gore y Asociados S.L., Barcelona, Spain). The bags containing the samples were suspended at 2 cm from the bottom of centrifuge tubes and centrifuged at 4000×$\boldsymbol{g}$ for 10 min at 15°C (Sorvall RC 5C Plus, DuPont Co., Newtown, Co) and the weight of released water measured. WHC was calculated as the percentage (w/w) of water released to the weight of the sample prior to centrifugation. Four measurements were performed for each gel.

2.8. Statistical analysis

Three samples of plasma solution for each pH value were treated on different days. The data were analysed by the Statistical Analysis System (SAS Institute Inc., 1990). The Barlett test was used to assess the homogeneity of variances. The data corresponding to the emulsifying properties (EAI and ESI) were transformed into logarithms. Analyses of variance, using the general linear model procedure (GLM), were applied to the different response variables. The significance level for all tests was $P=0.05$. The treatment-by-pH interaction was included in the model. When the effect of the interaction was significant, differences among least square means (LSM) were determined at $P\leqslant 0.05$. The Tukey–Kramer test with least significant differences at $P\leqslant 0.05$ was used to compare sample means if the interaction was not significant.

3. Results and discussion

3.1. Emulsifying activity and emulsion stability

The dependence of the absorbance values on plasma concentration increased almost linearly up to 0.75% plasma, and then remained constant at higher concentrations (Fig. 1). Tornberg, Olsson, and Perrson (1997) found a concentration dependence for the interfacial tension for blood plasma up to 1%. However, in the present study, the EAI, calculated for the 0.5 and 0.75% solutions, were similar (363.8 and 362.0, respectively), indicating that the maximum interfacial area that can be stabilised per unit weight of plasma proteins was achieved at a concentration of 0.5%. The study of the effect of high-pressure was conducted on 0.5% v/v plasma solutions, since the relative emulsifying efficiency of a protein can be undervalued if the concentration used is higher than that necessary to obtain the maximum absorbance (Santiago, Gonzalez, Remondetto, & Bonaldo, 1998). The minimum and the maximum absorbances obtained with a 1/2500 dilution of the emulsions were 0.133 and 0.640, respectively. At concentrations giving absorbances higher than 0.4, the relationship between turbidity and interfacial area becomes non-linear, but errors are less than 7% for absorbances up to 0.8 (Pearce & Kinsella, 1978). The pressure-treated plasma solutions at the three pH values varied in both emulsifying activity (Fig. 2) and emulsion stability (Fig. 3) with treatment and pH.

Studies by Gekko and Hasegawa (1986, 1989) have suggested, from compressibility changes of globular

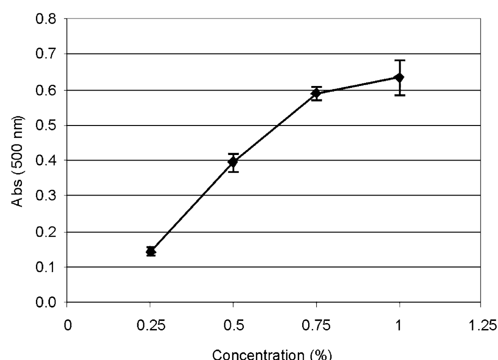

Fig. 1. Absorbance at 500 nm of emulsions stabilised by porcine blood plasma powder as a function of concentration (% w/v) (pH 7.5, oil volumetric fraction = 0.25, 1/2500 dilution in 0.1% sodium dodecyl sulpahte). All values are the means of three determinations ± S.D.

proteins on denaturation, that incomplete unfolding of pressure-denatured proteins may occur. This partial unfolding of protein is a complex phenomenon which is mainly due to the breaking of intramolecular salt bridges or weakening of hydrophobic interactions, both of which tend to increase the area accessible to the solvent (Tornberg, Olsson, & Perrson, 1997). Our previous studies, by differential scanning calorimetry on plasma proteins, showed that 450 MPa leads to a 20–25% loss of structure and up to 55% loss for treatments at 500 MPa (Parés, Saguer, Saurina, Suñol, Toldrà, & Carretero, 1998; Parés, Saguer, Toldrà, & Carretero, 2000).

Important differences in the EAI between samples are shown in Fig. 2. The interfacial activity of a blood plasma preparation was previously studied by Tornberg and Jönsson (1981), who suggested the serum albumin (55 to 64% of the blood plasma preparation) was a major contributor to the surface activity of the plasma. BSA has been reported to be very stable under pressure (Denda & Hayashi,1992; Hayakawa, Kajihara, Morikawa, Oda, & Fujio, 1992). Denda and Hayashi, working on 1% BSA solutions, reported that pressurisation had little effect up to 500 MPa, apart from leading to a slight improvement in the emulsifying properties. An explanation, for the marked effects of pressurisation in the present study, might be the high protein concentration used (10%). The effects of pressure on protein unfolding appear to be enhanced at higher protein concentrations, possibly because of decreased reversibility due to aggregation (Dumay, Kalichevsky, & Cheftel, 1994; Galazka, Ledward, Summer, & Dickinson, 1997).

Our results showed that the highest emulsifying activity was obtained with samples of pH 6.5 and 7.5, treated at 400 MPa ($P<0.05$). No significant differences between non-treated samples and samples treated at 300 MPa were seen using the LSM test. However, at pressures above 400 MPa, the emulsifying activity at all pHs decreased with increasing pressure, ultimately reaching lower values than the non-treated solution. Kato, Osako, Matsudomi, and Kobayashi (1983) found that the emulsifying activity of BSA decreased with increasing degrees of heat denaturation, as evidenced by decreasing surface hydrophobicity. As surface hydrophobicity is a major factor governing the emulsifying properties of proteins (Galazka, Dickinson, & Ledward, 1996; Kato, Osako, Matsudomi, & Kobayashi, 1983; Tornberg, Olsson, & Perrson, 1997), our results suggest that high pressure treatments between 300 and 400 MPa induce a partial protein unfolding, leading to increased surface hydrophobicity. These results do not agree with Galazka, Dickinson and Ledward (1996) and Galazka, Ledward, Summer and Dickinson (1997) who found that, in BSA, pressure-treatment led to some loss of surface hydrophobicity at all pressures from 300 to 800 MPa. The losses in emulsifying activity at pressures above 400 MPa do, however, agree with the results of the authors mentioned above. Perhaps the discrepancies found at the lower pressures relate to the fact that, in the present study, measurements were made on whole plasma, and the results, therefore, correspond to the effects of pressure treatment on the many proteins present. It has previously been reported that plasma preparations contain a component with good emulsifying activity in itself and interactions may enhance emulsifying activity compared to that expected from the individual proteins (Saito, Ichikawa, & Taira, 1988).

The decreased emulsifying activity of solutions treated at 500 and 600 MPa can be explained by the lower number of hydrophobic groups exposed because of conformational changes (Galazka, Dickinson, & Ledward, 1996) or, more likely, disulphide bond intermolecular interactions (Hayakawa, Kajihara, Morikawa, Oda, & Fujio, 1992). It is known that BSA undergoes extensive aggregation during high-pressure treatment, the extent increasing with the magnitude of applied pressure (Galazka, Summer & Ledward, 1996).

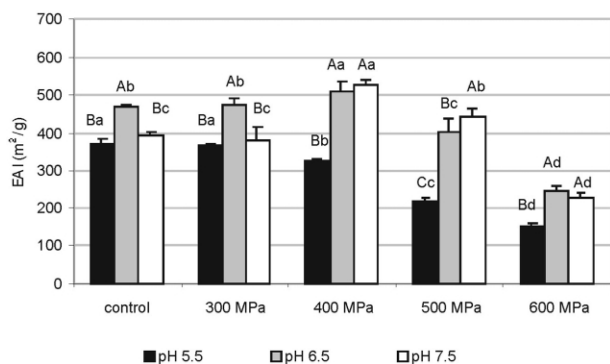

Fig. 2. Emulsifying Activity Index of high-pressure-treated blood plasma solutions at different pH (0.5% w/v, oil volumetric fraction = 0.25, 1/2500 dilution in 0.1% sodium dodecyl sulphate). All values are the means of three determinations ± S.D. Different letters indicate significant differences between pH (capital letters) and treatments (small letters).

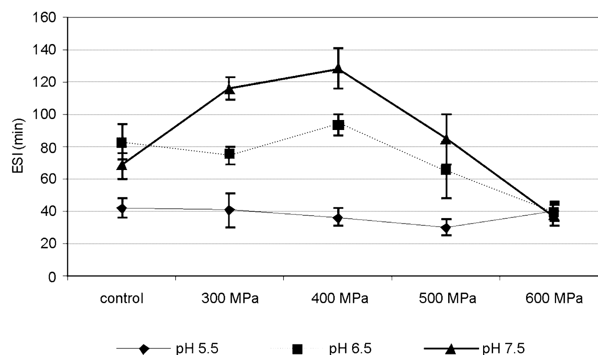

Fig. 3. Emulsion Stability Index of high-pressure-treated blood plasma solutions at different pH (0.5% w/v, oil volumetric fraction = 0.25, 1/2500 dilution in 0.1% sodium dodecyl sulphate). All values are the means of three determinations ± S.D.

Differences in the EAI between samples of different pH were shown to be significant ($P < 0.05$). Of the three groups, those at pH 6.5 had the higher emulsifying activities and those at pH 5.5 the lowest. Hermansson and Tornberg (1976), working with 1% plasma proteins dispersed in distilled water, showed that they had lower solubility (70%) in the isoelectric region, around pH 5. Since it is mainly the soluble part of a protein which acts as an emulsifier, loss of solubility is the probable reason for the reduced emulsifying activity of the plasma at pH 5.5.

It can be seen (Fig. 2) that the treatment effects on EAI were pH-dependent. For the samples treated at pH 5.5, the emulsifying activity decreased with increasing pressure above 300 MPa. Possibly, coagulation (aggregation) between proteins in solution at pH 5.5 counteract the positive effect seen at 400 MPa at the higher pH values. At both pH 7.5 and 6.5, maximum emulsifying activity was found at 400 MPa.

Analysis of the ESI data (Fig. 3), showed that more stable emulsions were formed with plasma treated at 300 and 400 MPa and pH 7.5 compared to the untreated controls ($P < 0.05$). At the other pHs, no such increase in stability was seen. Pressurisation at 500 and 600 MPa led to solutions, at all pHs, that formed emulsions of reduced stability. The effect of pH on the ESI were different from the effects on the EAI, significantly more stable emulsions being obtained with the solutions of pH 7.5, followed by 6.5 and 5.5. Haque and Kinsella (1988) found the highest emulsion stability for BSA to be at pH 4, but these systems required a higher energy input for emulsification than solutions of pH 5 to 7.

As in the case of EAI, the effect of pressure on stability of the emulsions was pH-dependent. No significant effect of pressure was found for solutions of pH 5.5. At pH 6.5 and 7.5, a higher stability was found for the emulsions stabilised with plasma treated at 400 MPa. Pressure treatments at 300 MPa improved the emulsion stability only for solutions of pH 7.5.

3.2. Heat-induced gels

Rheological properties of heat-induced gels prepared from the pressure-treated plasma solutions showed differences in hardness (Fig. 4) and elasticity (Fig. 5), depending on pressure treatment and pH ($P < 0.05$). Therefore, the changes induced by pressurisation affect the gelling behaviour of the plasma on heating.

The effect of pressure on hardness (Fig. 4) followed a similar pattern, whatever the pH, for treatments up to 500 MPa. The highest values were obtained for gels from samples pressurised at 300 MPa, but no significant differences between untreated samples and those treated at 300 and 400 MPa were found ($P > 0.05$). For pressures above 400 MPa, the hardness decreased significantly as pressure increased, this effect being more noticeable with decreasing pH. Although treatment at 600 MPa induced further decreases in the firmness of gels prepared from solutions of pH 6.5 and 7.5, it led to the hardest gels at pH 5.5 ($P < 0.05$). High concentrations and pHs near the isoelectric point favour precipitation of pressure-denatured proteins and it is likely that the increased tendency towards protein-protein interactions induced by treatment at 600 MPa under these conditions, led to aggregation, which altered the characteristics of the protein network formed on heating.

The rheological analysis (Fig. 5) also showed that, for treatments up to 500 MPa, the higher elasticities were always found in gels made from solutions of pH 7.5, followed by 6.5 and 5.5 ($P < 0.05$). These results are in agreement with those reported by Hermansson (1982) who found that the recoverable height of 5% plasma gels increased with pH.

The most elastic gels corresponded to untreated, 300 and 400 MPa-pressurised plasma, with no significant differences between them ($P > 0.05$). Pressurisation at 500 and 600 MPa, at pH 6.5 and 7.5, led to solutions that formed gels with reduced elasticity compared to those treated at lower pressures, the effect being greater at pH 6.5. The decrease in elasticity may have been caused by the increased irregularity and coarseness of the gel structure formed by the highly denatured proteins. However, for gels at pH 5.5, a 600 MPa treatment resulted in a significant recovery of elasticity, although the elasticity was always lower than the control and samples treated up to 400 MPa. It is somewhat surprising that the elasticity of the pH 5.5 gel is significantly higher at 600 compared to 500 MPa, as also that it correlates with increased hardness (Fig. 4). It may be that, at this pH, which is close to the isoelectric point of the plasma proteins, protein–protein interactions are more favoured after treatment at 600 MPa, compared to 500 MPa, leading to both increased hardness and elasticity.

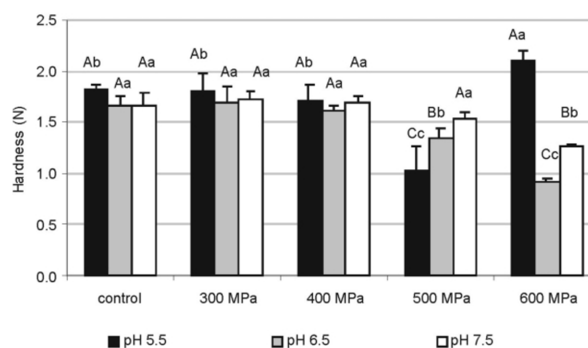

Fig. 4. Hardness of heat-induced gels (45 min at 80°C) from high-pressure-treated blood plasma solutions (10% w/v) at different pH. All values are the means of three determinations ± S.D. Different letters indicate significant differences between pH (capital letters) and treatments (small letters).

The WHC of these gels at the three pH values is shown in Fig. 6. Gels at pH 7.5 had a significantly higher WHC than gels at pH 6.5 and the maximum loss of water was always from gels at pH 5.5, whatever the pressure treatment ($P < 0.05$). It has been previously reported that minimum WHC of the heat-induced gels, from liquid and spray-dried plasma, was at pH 5.5 (Parés, Saguer, Saurina, Suñol, & Carretero, 1998).

Within each pH group, the gels with the highest WHC corresponded to the untreated, and those treated at 300 and 400 MPa, with no significant differences between them ($P > 0.05$). Gels at pH 7.5 gradually increased their WHC as the pressure increased but only the differences between non-treated samples and those pressurised at 600 MPa were significant ($P < 0.05$). The WHC of gels at pH 6.5 was significantly enhanced with increasing pressure at treatments above 400 MPa ($P < 0.05$). However, the opposite effect was found for gels at pH 5.5 ($P < 0.05$). The increased tendency for protein–protein interactions of pressure-denatured proteins, at pHs near the isoelectric point, probably led to stronger gels but with reduced WHC because of a partial disruption of the protein network due to local aggregation phenomena. Further studies on gel microstructures are necessary to confirm this hypothesis.

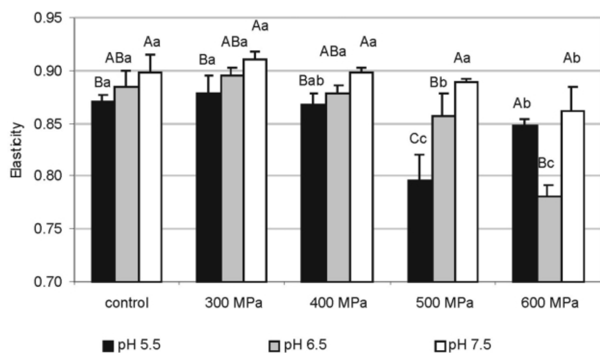

Fig. 5. Elasticity of heat-induced gels (45 min at 80°C) from high-pressure-treated blood plasma solutions (10% w/v) at different pH. All values are the means of three determinations ± S.D. Different letters indicate significant differences between pH (capital letters) and treatments (small letters).

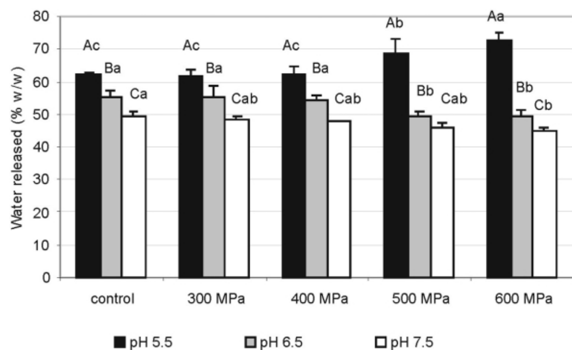

Fig. 6. Percent of water released from heat-induced gels (45 min at 80°C) of high-pressure treated blood plasma solutions (10% w/v) at different pH, after centrifugation at 4000×***g*** for 10 min. All values are the means of three determinations ± S.D. Different letters indicate significant differences between pH (capital letters) and treatments (small letters).

4. Conclusions

While high-pressure treatments up to 300 MPa did not affect the functionality of blood plasma proteins, whatever the pH, the effects of treatments at pressures above 400 MPa caused pH-dependent changes. At acidic pH (5.5), increasing pressures led to a decrease in the emulsifying properties of plasma solutions and provoked changes in the textures of heat-induced gels, which negatively affected their water-holding capacity. Both properties were seriously affected by the changes in structure that the proteins undergo following pressurisation at this pH. At pH $\geqslant$6.5, high-pressure treatments at 400 MPa improved the emulsifying properties of plasma solutions without negatively affecting the characteristics of heat-induced gels. Treatments above 400 MPa led to solutions with reduced emulsifying activity and stability. At pH $\geqslant$6.5, heat-induced gels were always weaker and less elastic but, in some cases (pH 6.5), they showed improved water-holding capacity.

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