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Functional and Physical Properties of Bovine Plasma Proteins as a Function of Processing and pH, Application in a Food Formulation

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Abstract: The combined effects of ultrafiltration and freeze drying stages, and the incorporation of a stabilizing agent on selected functional and physical properties of bovine plasma proteins, were evaluated in the pH range of 3.0-9.0. The raw material was also characterized and was compared with the processed one. The results show that the process had a positive effect on solubility, emulsion capacity and emulsion stability in all the pH range, while the foaming capacity was similar to the raw material, having a better foam stability. The content of salts and denatured proteins was reduced in the processed plasma. This product was used in the formulation of a minced meat, which had a high stability and adequate moisture, improving the consistence according to the sensorial analysis. Therefore, the use of processed proteins in formulation of food products may be enhanced, and a higher added-value protein can be obtained.

Key words: Freeze-drying, minced meat, protective agent, protein characterization, ultrafiltration

INTRODUCTION

Many natural and processed foods consist of dispersions or at least, they have been in a dispersed state at some point during their formation. Most of these food dispersions are emulsions and foams. These dispersions include traditional food formulations (such as bakery, confectionery or meat products, fat-based products, ice cream, dressings, toppings, mousses, beer and sparkling wine) or new formulations (including low-fat and instant foods, high- or low-alcohol food formulations, functional foods, etc.,) (Borcherding *et al*., 2008; Gatade *et al*., 2009; Kamath *et al*., 2008; Rodríguez Patino *et al*., 2008). Food dispersions do not form spontaneously. Indeed, in order to produce dispersion, a large amount of mechanical energy must be supplied. However, from a practical point of view, it is possible to form dispersions that are kinetically stable (or metastable) by adding substances known as emulsifiers.

Two types of emulsifiers are used in foods: low-molecular weight emulsifiers and macromolecules (proteins and some polysaccharides). Thermodynamically, these emulsifiers decrease the surface tension and thus, the free energy of the system, imparting the desired kinetic stability to dispersions (emulsion or foam), (Rodríguez Patino *et al*., 2008). The decrease in surface tension by proteins and amphiphilic macromolecules is usually caused by the following processes: (i) the surface active entity moves by diffusion from the bulk phase to the subsurface layer, immediately adjacent to the fluid interface, (ii) the adsorption and unfolding of the macromolecule take place at the interface, and (iii) the adsorbed segments rearrange at the fluid interface. In addition, amphiphilic macromolecules form continuous viscoelastic films at the interface via non-covalent intermolecular interactions (Benichou *et al*., 2007; Glaser *et al*., 2007). The stability and formation of foams and emulsions depend on the following variables: the type of protein used, protein solubility and concentration, pre-treatment, manufacturing process and environmental factors including mineral composition, ionic strength, degree of denaturation, pH value, presence of other components in a mixture, etc., (Borcherding *et al*., 2008; Glaser *et al*., 2007; El-Salam *et al*., 2009; Nikovska, 2010).

Animal blood is a highly contaminating by-product of slaughterhouses; however, it contains a high quality protein content which has, among others, emulsifying properties. In a previous work (Rodriguez Furlán *et al*., 2009) it was found that a product with less denatured protein can be obtained, processing plasma proteins in a saccharose solution, with membrane technology and freeze dried. Besides, protein-polysaccharide interactions play a significant role in the structure, stability and improvement of functional properties of many processed foods.

The objective of this study is to assay functional and rheological properties, as a function of pH, of plasma bovine processed by membrane technology and a freeze drying, using sucrose as protective agent of proteins, and

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to compare these results with plasma proteins as raw material. The information is a key factor in getting a product with the desired textural characteristic and functional properties in the formulation of food products. An application of the processes protein in a minced meat formulation had been evaluated.

MATERIALS AND METHODS

This study was conducted in the period from February 2009 to July 2009 at the Membrane Laboratory of INTEQUI, National San Luis University, San Luis, Argentine.

Raw materials: Spray dried bovine plasma (Yerubá S.A. Argentina) has been used. The molecular weights of proteins are in the range of 15.000 to 80.000 Da. The composition is 76±5% proteins, <0.1% fat, 10% ash, 4% water, 1% low molecular weight compounds. Commercial sucrose (Ledesma S.A. Argentina) with a purity of 99.99% has been employed as protein stability agent.

Ultrafiltration and freeze drying of bovine plasma proteins: The feed solution consisted of bovine plasma and sucrose dissolved in de-ionized water at a concentration of 3 % w/v and 10 % w/v, respectively. The process of concentration of bovine plasma proteins through ultrafiltration (UF) and freeze drying was assayed, as described by Rodriguez Furlán *et al*. (2009, 2010). A porous support (Viledon F 02431 D, Germany) and a membrane of microfiltration (pore size of 60 mm, Gora, Argentina) were used to remove macroscopic aggregates (mainly, denatured proteins) and reduce the amount of bacteria and spores, protecting the UF membrane from fouling. The UF was performed using Pellicon cassette module (Millipore, Bedford, MA, USA), containing modified polyethersulfone membranes with a molecular weight cut-off of 10-kDa. The concentration of proteins by UF was 4% (w/v) and sucrose 12% (w/v), determined with a portable refractometer (Arcano). The operating conditions were the following: transmembrane pressure of 1.5 bar, flow rate of 2.9±0.05 L/min and a temperature of 10 ºC. A discontinuous diafiltration (DD) process was applied to removal salts and other contaminant of low molecular weight. The cleaning of the fouled membrane was performed by the procedure described by Rodriguez Furlán *et al*. (2010). The bovine plasma protein concentrate, obtained by UF was placed on stainless steel trays and frozen in a freezer at -40ºC and freeze-dried using a lyophilizer (Rificor, Argentina) at 1 bar for 48 h. Three replicates of the procedure have been performed, for statistical analysis.

Determination of protein content (total and denatured): The total protein content of the bovine plasma used as raw material (RM) and the processed bovine plasma (PBP) was determined by the Kjeldhal method using a Digestion Systems and semi-automatic distillatory (Selecta S.A, Spain), (AOAC 15017, Association of Official Analytical Chemists, 1995). The conversion factor used to express the results was 6.24. All tests were carried out in triplicate.

The denatured protein content was determined in three replicates after isoelectric precipitation of denatured/aggregated protein using a refrigerated ultracentrifuge (Beckman, USA) and then, the absorption of protein concentration at 280 nm in the supernatants was measured. Measurements were performed at room temperature and were described in detail elsewhere (Rodriguez Furlán *et al*., 2010). The results were expressed as percentage of denatured protein content.

Ash content: Ash content measurement of RM, PBP and minced meat samples, was carried out in three replicates in a muffle furnace (Indef, Model 132) using a controlled temperature program to reach gradually 520ºC. Salt reduction percentage was determined by the difference between the initial and final weight of the samples, (AOAC, 1995).

Determination of functional properties: All test were carried out by triplicate by gram of product, in spite of the different protein content between raw material and processed plasma, with the aim of compare the quality as product, to be used as additive or ingredient in a food formulation.

For protein solubility measurements at room temperature, dispersions of protein concentrate were centrifuged and then analyzed by absorption spectroscopy (Double beam Shimadzu Spectrophotometer, USA) at 280 nm as described elsewhere (Rodriguez Furlán *et al*., 2010). The results were expressed as percentage of protein in supernatant, divided by the percentage of total protein in the original solution.

The emulsifying capacity (EC) expressed as volume (mL) of oil emulsified per gram of protein concentrate was determined as described previously by Rodriguez Furlán *et al*. (2010). The pH was varied between 3 and 9, using 0.1 N of NaOH and HCl. The equipment for emulsion characterization assays was hermetically closed as to maintain a constant air volume in each run. The agitation time and volume used were standardized.

Foaming capacity (FC) and foaming stability (FS) of the protein concentrate were investigated using the technique described by Rodriguez Furlán *et al*. (2010). The pH was varied between 3 and 9 with 1.0 N NaOH and HCl. The equipment employed for foam is the same as for emulsion characterization.

Samples of RM and PBP used in foam and emulsion capacity determinations were analyzed with an optical

microscopy (Arcano XSZ1008N) with x40 magnification. The emulsions were photographed each 24 h for eight days, and foams, each 10 for 60 min. The images were analyzed by Image-Pro Plus 6.0 (Media Cybernetics Inc, Bethesda, USA) software. Foam and emulsion stability was assayed measuring the diameters of bubbles and drops, respectively. As some bubbles and drops were not completely spherical, length was also considered. When there was superposition between bubbles and drops (at least upon 250 units), they were measured manually, and analyzed with the software. The particle size distribution was determined by the statistic software GraphPad InStat. The data were divided into classes rounding the ±0.01 mm.

Water holding capacity was measured weighing one gram of each protein concentrate into pre-weighed 15 mL centrifuge tubes. For each sample, 10 mL of de-ionized water was added and mixed using a mixer at high speed for 5 min. After the mixture was thoroughly wetted, samples were allowed to stand at room temperature for 30 min, and then centrifuged at 20000 rpm at 5ºC for 30 min. The supernatant was decanted and the centrifuge tube containing the sediment was weighted and dried in a stove for 30 min or until it reached constant weight. Water holding capacity (grams of water per gram of protein) was calculated as follows: WHC = $(w_2 - w_1) / (w_0)$, where w_0 is the weight of the dry sample (g), w_1 is the weight of the tub plus the dry sample (g), and w_2 is the weight of the tub plus the sediment (g) (Yu *et al*., 2007).

Heat- induced gels preparation and rheological measurement: Gel strength was determined in three replicates according to the procedure described by Chakraborty (1986) and Yu *et al*., (2007). Protein suspensions containing 7.5 % (w/v), 10 % (w/v) and 12.5% (w/v) of protein concentrates were prepared, and the pH was adjusted to 7.4 with 1.0 N NaOH or HCl. Viscosities of these protein suspensions were measured by a programmable Brookfield DV-III + Viscometer (Brookfield, USA) at room temperature (22±1)ºC. Samples were heated at 90ºC in a shaking water bath and kept for 30 min, then cooled to room temperature without stirring. Gel viscosity was determined using the viscometer at different shear rates (10 to 70 s^{-1}) at room temperature. The rheological behavior of the fluid was characterized by means of Power-Law:

$$\eta = K\gamma^{n-1}$$

where η : apparent viscosity of the fluid (Pa s); γ : shear rate (s^{-1}); K : flow consistency index (Pa s^n); n : flow behavior index (<1 for pseudoplastics or shear-thinning fluids).

Scanning electron microscopy: The microstructure of RM and PBP was analyzed by Scanning Electron Microscopy (SEM) using an LEO1450VP equipment (Zeiss, Germany). Fragments of the freeze-dried samples were mounted on metal stubs and gold-coated to obtain surface micrographs.

Preparation of minced meats: The meat mixtures (500 g) were prepared in function of the effective legal regulations (moisture <68 %, meat content >30 % in the final product), using RM and PBP in the formulation. The ingredients for kg of bovine meat were: bovine fat 150 mL, broth 650 mL, protein (RM or PBP) 22 g, cornstarch 4 g, wheat flour 1 g, sodium diphosphate 3.75 g, sodium triphosphate 3.75 g, salt 22.5 g, sucrose 1.75 g, nitrite 17.5 mg, ascorbic acid 0.5 g, citric acid 1.5 g, sodium citrate 1.5 g, onion 3 g, garlic 1 g, pepper 2 g, paprika 2 g, parsley 1.5 g, oregano 1 g, beet (betaine coloring) 1 ml.

The proceeding used for the elaboration was: the frozen meat was cut in small pieces and cooked in a pot of boiling water (1L per kg of meat), during 20 min. The broth was reserved for later use. The meat was ground in a machine using a 1 mm disk. Bovine fat was melted and mixed with the broth and the protein (RM or PBP) for 5 min at 60ºC for better training of the emulsion. Afterwards minced meat was added and the preparation was mixed for 10-15 min. Then, the remaining ingredients were added and continued stirring continuously at 80± 5ºC for 10-15 min. The mixture was processed into a paste. After that, 90 g of pulp was put in sealed glass containers and were sterilized at 115ºC for 60 min. Finally, the samples were submerged in a bath with cold water to reach room temperature. The content of moisture was determined according to AOAC 950.46 B.

Minced meat stability test: The method of Mun *et al*. (2009) with some modifications was used to determine the stability of samples. 5 g (F_0) of each sample were transferred to test tubes that were stored at 50ºC for 48 h. After storage, the emulsions were placed in centrifuge tubes and processed for 15 min at 5,000 rpm to remove the top oil layer. The weight of the precipitated fraction (F_1) was measured, and the emulsion stability was characterized as (%) = $F_1/F_0 \times 100$.

Minced meat sensorial analysis: The design described by Babiker *et al*. (1996) and Viana *et al*. (2005), with some modifications, was used for evaluating the color, the aroma, the taste and the consistency (degree of firmness) of the samples. 20 g of each sample was served in random order to the panelists. The samples were tested at 25ºC, in a uniformly illuminated room, by a 25-member panel selected from a pool of students and staff members of our department. The attributes were estimated on a five-point scale (from 1 = I disliked very much to 5 = I liked very much). Water was provided for rinsing between samples.

Table 1: Chemical determinations of raw material (bovine plasma) and processed bovine plasma

	RM	PBP
Total protein content, (g/100g of product)	73.7±0.60	27.80±0.40
Denatured protein, (w/v)	34.0±0.60	26.00±0.51
Ash content, (g/100g of product)	9.34±0.08	2.80±0.13
Carbohidrate content, (°Brix)	--	64.00±1.00

Statistical analysis: The obtained data were statistically evaluated by the Tukey-Kramer multiple comparison test in the cases where 2 or more comparisons were being considered, assuming that a $p<0.05$ (SAS, 1989) was statistically significant.

RESULTS AND DISCUSSION

Chemical determinations of protein concentrates: The chemical determinations of RM and PBP are given in Table 1. By UF-DD step, proteins were concentrated eliminating insoluble macroscopic components (mainly, denatured proteins), reducing the saline content (Belhocine *et al.*, 1998). The results showed that using sucrose as protective agent, the proteins were better preserved from denaturation. Indeed the determination of denatured protein content on concentrated bovine plasma processed without incorporation of sugars was 39.25±2.83% (w/v) ($p< 0.05$). With respect to mineral content, it was reduced a 70.5±0.5% in the PBP.

Solubility determination: From a practical point of view, data about solubility characteristics are quite helpful to determine the optimal conditions of protein extraction and purification, as well as the separation of protein fractions. Solubility, under different conditions, is also a good indicator of potential applications of proteins and it influences other functional properties (Cheftel *et al.*, 1989; Yu *et al.*, 2007).

Freeze-drying, is a dehydration process that has desirable effects on bovine plasma protein since causes less damage than other dehydration methods using higher temperatures. However, freeze-drying may affect the functionality of protein because of a partial protein denaturation. The presence of protectants (lyoprotectants) prevents denaturation of proteins during freeze-drying and subsequent storage. Ultrafiltration and freeze-dried of bovine plasma proteins, significantly increased protein solubility in the pH range 3.0-9.0 compared to RM as shown in Fig. 1. This is in agreement with the findings of Del Hoyo *et al.* (2008), who reported that plasma protein deionized by ultrafiltration increased its solubility. The solubility differences between these products reaches 13 and 10% at pH 7 and 3, respectively; with a difference around 4% at the other pH's tested. Besides, the variations with pH were not significant with a maximum variation

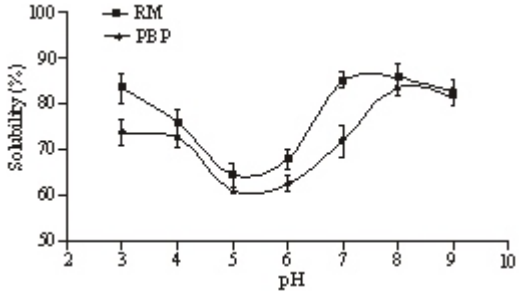

Fig. 1: Protein solubility of RM and PBP as a function of pH

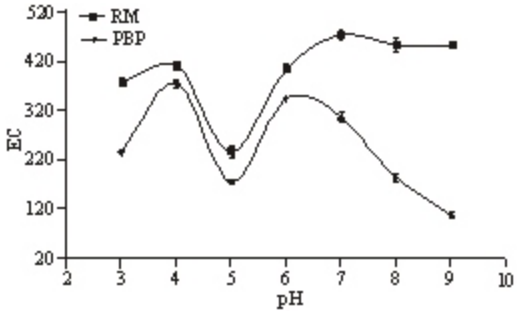

Fig. 2: Emulsion capacity (EC) of RM and PBP

around 22% in the pH range tested (3-9) for the standard and processed plasma. This increase shows the effective protection of sugar, which preserves the native structure of proteins, presumably due to a rapid embedding in a glassy matrix (Buera *et al.*, 2005; Wolkers *et al.*, 1998). Also, the saccharides in solution operate as charges that adsorb on the protein surface diminishing protein-protein interaction, and thus, the protein tends to remain in solution (Cheftel *et al.*, 1989; Toldrà *et al.*, 2008; Yusuf *et al.*, 2008).

RM and PBP presented a minimum of solubility at the isoelectric point of the plasma (pH 4-6), and maximum solubility at pH 8 for both samples. The occurrence of minimum solubility close to the isoelectric pH is due primarily, to the lack of electrostatic repulsion, which promotes aggregation and precipitation via hydrophobic interactions (Cheftel *et al.*, 1989; Fennema, 1996).

The results of solubility showed by PBP are important in the elaboration of dairy products, drinks, meat products and bread.

Emulsion capacity and stability: Emulsifying properties of food proteins are usually described as: (i) emulsion capacity or emulsion activity, reflecting the ability of the proteins to aid formation of the newly created emulsion, and (ii) emulsion stability, which reflects the ability of the proteins to impart strength to emulsion for resistance to stress (Liu *et al.*, 2008).

The results for RM and PBP emulsion capacity are shown in Fig. 2. It can be observed that PBP emulsifying

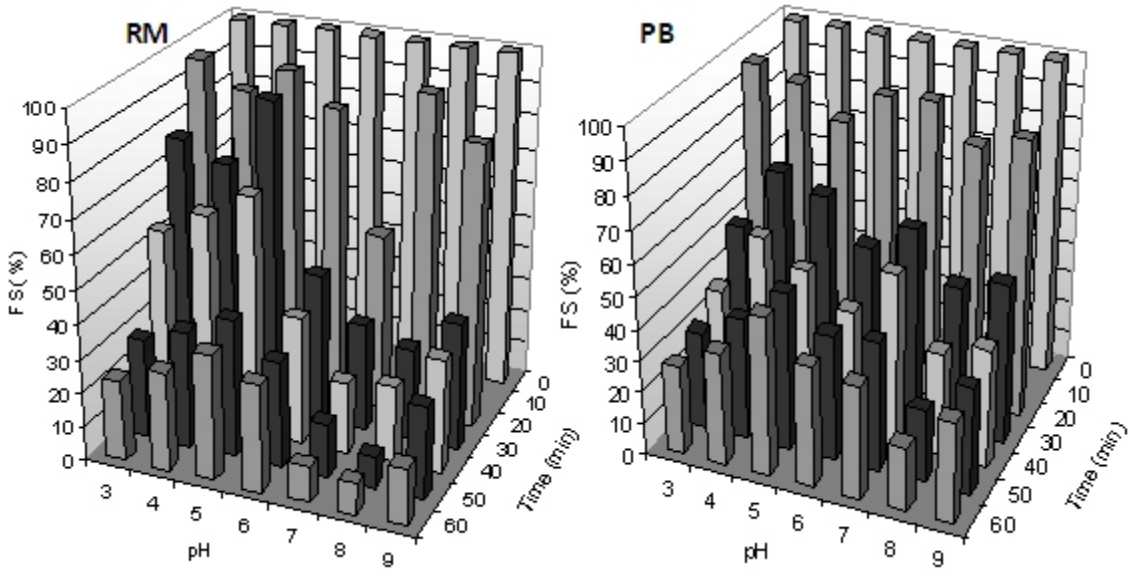

Fig. 3: Foam stability (FS) of RM and PBP as a function of pH, during 60 min (Temperature: 28±2ºC)

capacity is much higher than RM capacity in the studied pH range, with maximums of 476 ml/g for PBP at pH 7 and 378 ml/g for RM at pH 3. Besides, present minimums at the isoelectric point, according to solubility results (Rodiles-López *et al*., 2008). As known, proteins have low solubility at the isoelectric pH becoming poor emulsifiers. For RM, an important decrease of emulsion capacity took place when close to extreme pH, due to higher interactions with the environmental ions. This affects protein diffusion and unfolding, reducing the absorption velocity at the interphase and therefore, the protein emulsion capacity (Zayas, 1997). In the case of PBP, solubility was practically constant in all the pH range, attributed to the neutralization of charges by sugars (Fennema, 1996).

The improved emulsion stability and capacity are important in the elaboration of whipped products and emulsified sauces such as mayonnaise, frozen desserts and sausages, cakes and meat products.

Foamability and foam stability: The properties of protein-based foams were measured by two key parameters: foamability and foam stability. Foamability relates to the capacity of the continuous phase to include air or another gas, and foam stability relates to the stability of the lamellae and the ability to retain the gas for a given time (Glaser *et al*., 2007). For effective foam formation, proteins must be capable of rapid migration and orientation to form encapsulating films around the gas bubbles, in order to prevent destabilization. As the bubbles emerge from the liquid phase during foam formation, they move toward each other until they almost touch, but remain separated by a thin aqueous film stabilized by disjoining forces (Glaser *et al*., 2007; Kinsella, 1981).

The results showed a similar foaming capacity (FC) between RM and PBP in the pH range, with the maximum of 1.9±0.5 at pH 3 and a minimum of 1.3±0.1 at pH 9. Analogous results were found by Huh Yang *et al*. (2002) for porcine blood globin. A higher denatured protein content of the RM produces an improvement in the interface orientation, increasing the foaming capacity.

Figure 3 exhibits foaming stability for RM and PBP for 60 min as a function of pH, at room temperature. The PBP proteins showed higher foam stability in all the pH range. This behavior can be attributed to saccharide-protein mixtures which form a complex with superficial activity that increases system viscosity and diminishes foam drainage, becoming an essential role in the control of foam stability (Benichou *et al*., 2007; Cheftel *et al*., 1989; Fennema, 1996; Fidantsi and Doxastakis, 2001; Leser and Michel, 1999; Rodríguez Patino *et al*., 2008; Rouzes *et al*., 2002). The results show that for both samples, the most unstable pH was 8 and the most stable was 5. These values are consistent with the fact that foam stability is expected to occur most readily near protein pI, where the net surface charge on a protein is closer to zero. This facilitates the ability of protein molecules to interact with one another and form an interfacial film that encircles air cells (Agyare *et al*., 2009; Glaser *et al*., 2007).

PBP exhibited desirable foam capacity and stability making it a good candidate for many food formulations such as ice-creams, souffle´s and bread.

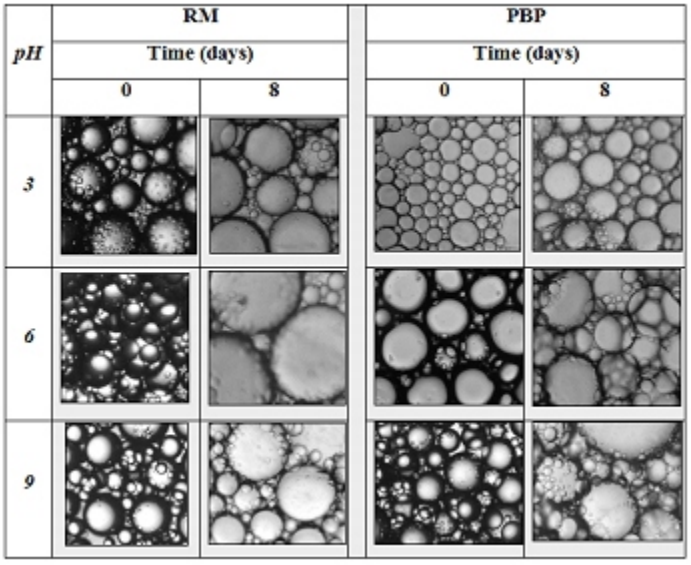

Fig. 4: Images of drops emulsion of RM and PBP as a function of pH (x 40)

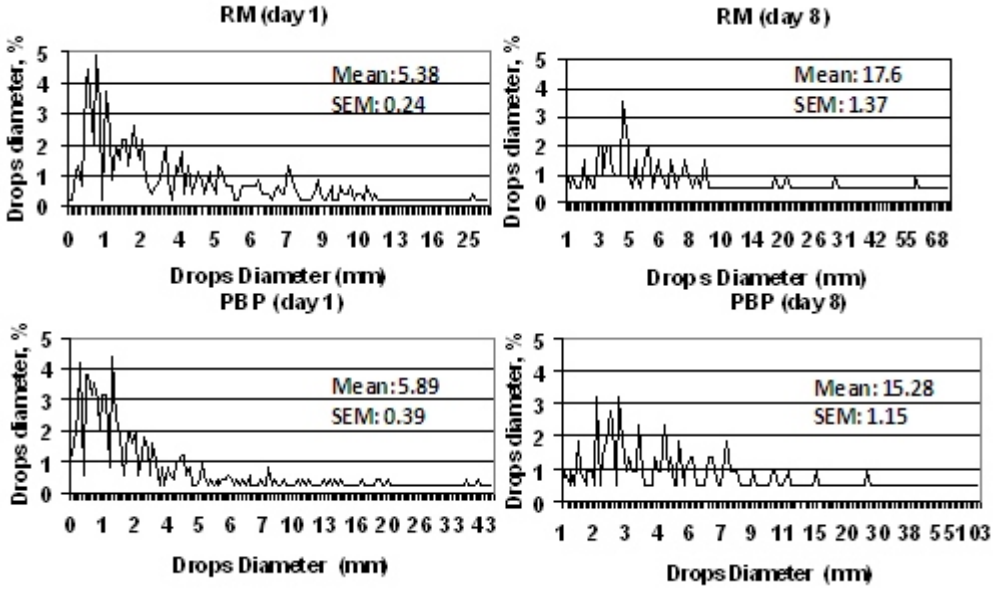

Fig. 5: Variation of the average diameter distribution of emulsion drops for RM and PBP during the experimentation

Effect of pH upon bubble and drop size distribution. Microscopy study: Foams and emulsions of RM and PBP were analyzed by means of an optic microscopy to quantify bubble and drop size as a function of pH. Usually, foam and drained liquid volumes are the parameters used to describe foams. However, they do not show foam internal conditions, as size particle distribution (Glaser *et al*., 2007).

Figure 4 shows the images of RM and PBP initial and final drops (8 days), as a function of pH. Emulsion stability for both samples was similar in all cases, with close average drop sizes. As expected, it can be seen that drop size increases with time, being the emulsion more unstable.

Figure 5 presents the average diameter distribution of emulsion drops for RM and PBP. It can be seen a re-distribution to higher drop sizes and a decrease of the emulsion stability with time. A similar distribution was observed by the average bubble diameter of RM and PBP foams.

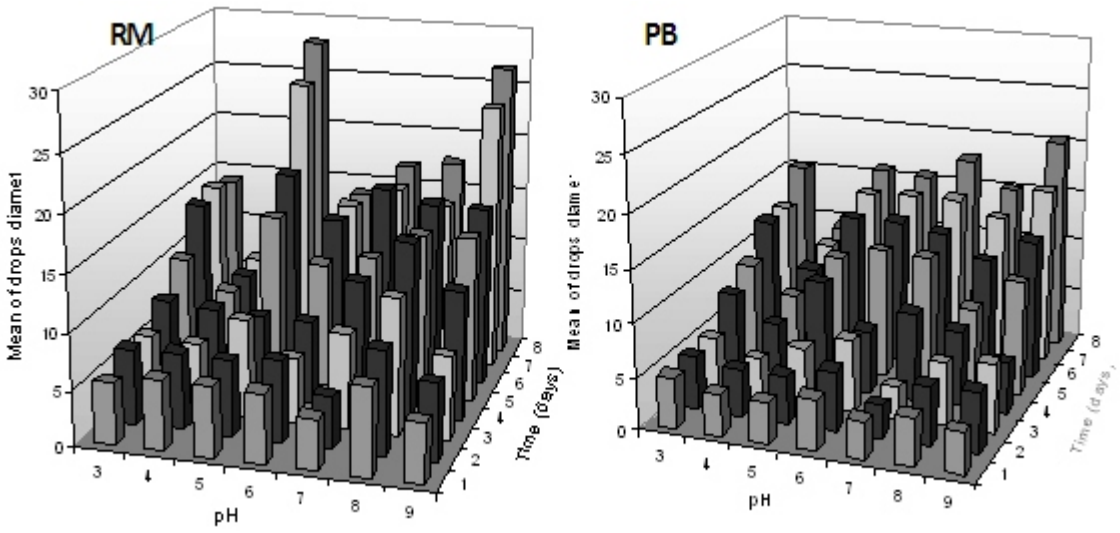

Fig. 6: Means diameter of emulsion drops of RM and PBP at different pH values, during 8 days

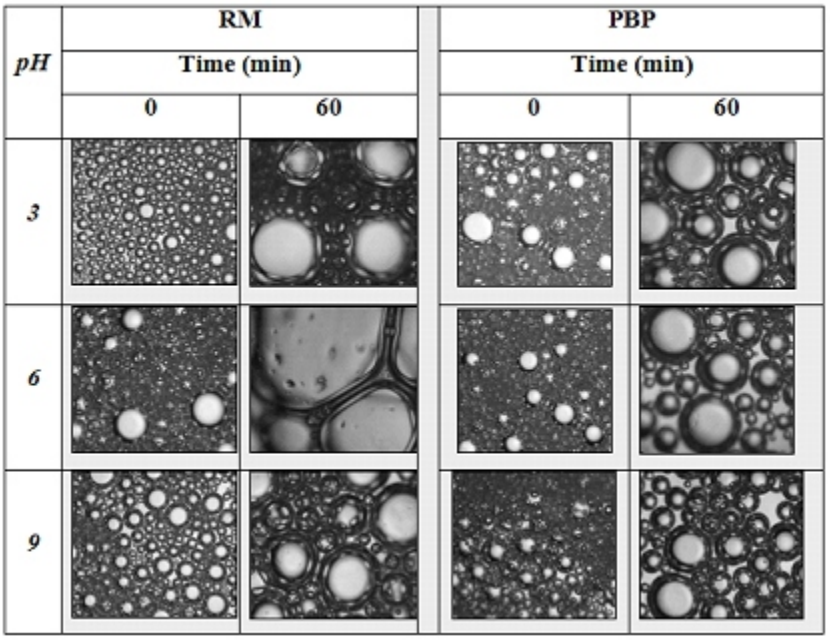

Fig. 7: Images of bubbles foams of RM and PBP for different pH values (x 40)

In Fig. 6, the average emulsion drop diameters of RM and PBP after 8 days of storing at room temperature as function of pH, are compared. The PBP presented a higher stability in practically all the range of pH, particularly at pH 5 and 9. At any pH, the rate protein-saccharide is an important parameter that determines the drop stability (Leal-Calderon *et al*., 2007). In fact, emulsion stability depends on protein-protein interactions and the presence of saccharides can reinforce the interactions (Ghoush *et al*., 2008; Glaser *et al*., 2007). Emulsion stability is increased because the saccharide adsorbs quickly and saturates the whole surface of the drops (Leal-Calderon *et al*., 2007; Mun *et al*., 2008). The results show that for RM the more unstable pH was 5 (higher average diameter, near the isoelectric point); the lack of electrostatic repulsive interactions among

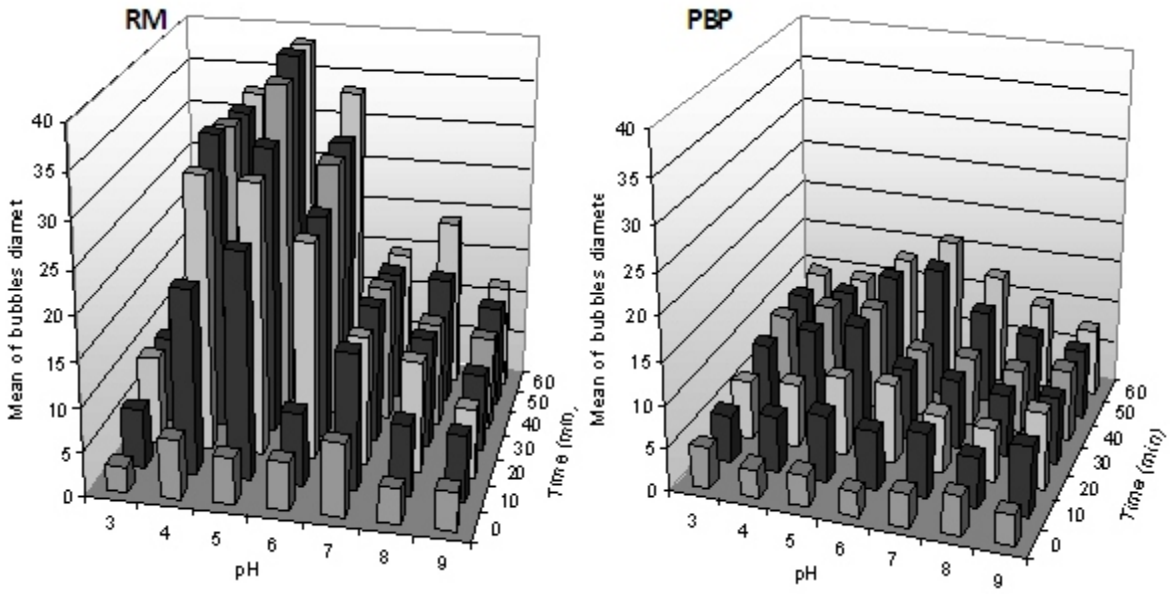

Fig. 8: Means diameter of foam bubbles of RM and PBP to different pH values, during a period of 60 sec

emulsion particles may promote flocculation and coalescence, and thus a decrease in the emulsion stability (Cheftel *et al*., 1989; Fennema, 1996).

Figure 7 shows foam photographs of RM and PBP for initial and final stage (60 min) as function of pH, where an increment in bubbles size with time is observed.

In Fig. 8, RM and PBP average foam bubble diameters for each pH are compared. PBP foams have lower diameters and so, higher stability in all the pH range as explained above. This may be attributed to the use of sugar as protective agent, which also stabilizes the foam (Cheftel *et al*., 1989). Destabilization or stabilization of foams may be produced by a number of mechanisms. The drainage or loss of liquid leading to the destabilization of foams is reduced to a great extent by the presence of interfacial low molecular tensions and a higher superficial viscosity of the liquid phase close to pI (Cheftel *et al*., 1989). Therefore, the presence of large size bubbles in RM and PBP at pH 4, 5 and 6 was the result of a high stability of foams at pH near pI, corroborating the information obtained in previous studies mentioned above.

Rheological properties of PBP: Viscosity is an important property of foods that affects taste, texture and mechanical handling of fluid materials. Gel formation occurs in two cases: (i) when the protein is at high concentrations, and (ii) as a consequence of a sol-gel transformation, due to protein denaturation usually caused by a thermal treatment. Therefore, gel formation takes place because of a controlled aggregation of the protein molecules after cooling, forming a tridimensional matrix that confines the liquid (de Man, 1999; Yu *et al*., 2007).

In Figure 9, the rheological study carried out for PBP as a function of pH is presented. The samples of RM, with a protein concentration of 12.5% (w/v), have similar pseudoplastic behavior than PBP. Due to the formation of pasty gels, they could not be adequately determined and therefore, the data are not shown.

Suspension of 7.5% (w/v) became solution after heating, 10% (w/v) suspension produced soft gel while 12.5% (w/v) suspension turned into a firm gel. These results are in agreement with Huh Yang *et al*. (2002) for porcine blood globin.

The viscosity of PBP solutions was sensitive to pH, presenting the highest values at pI. Similar results were found by Jindal and Grandison (2007), for chhana whey powders. This behavior may be due to the rate of structure of the three-dimensional network of the gel, which occurs more easily when the electrostatic repulsion diminishes (Cheftel *et al*., 1989; Hall, 1995). At pH 7, 8 and 9, gelification does not take place, or only a weak one occurs. This behavior may be due to the little or no aggregation of protein molecules in the state of pre-gel (Hall, 1995).

Consistency and flow indexes are of practical value for identifying flow, and for engineering design. Both indices can be used in determining power requirements (Wei *et al*., 2001). The Power Law model was applied to describe flow behavior of PBP, determining the flow behavior index, n, and the consistency index, K. These rheological parameters are presented in Table 2. The n

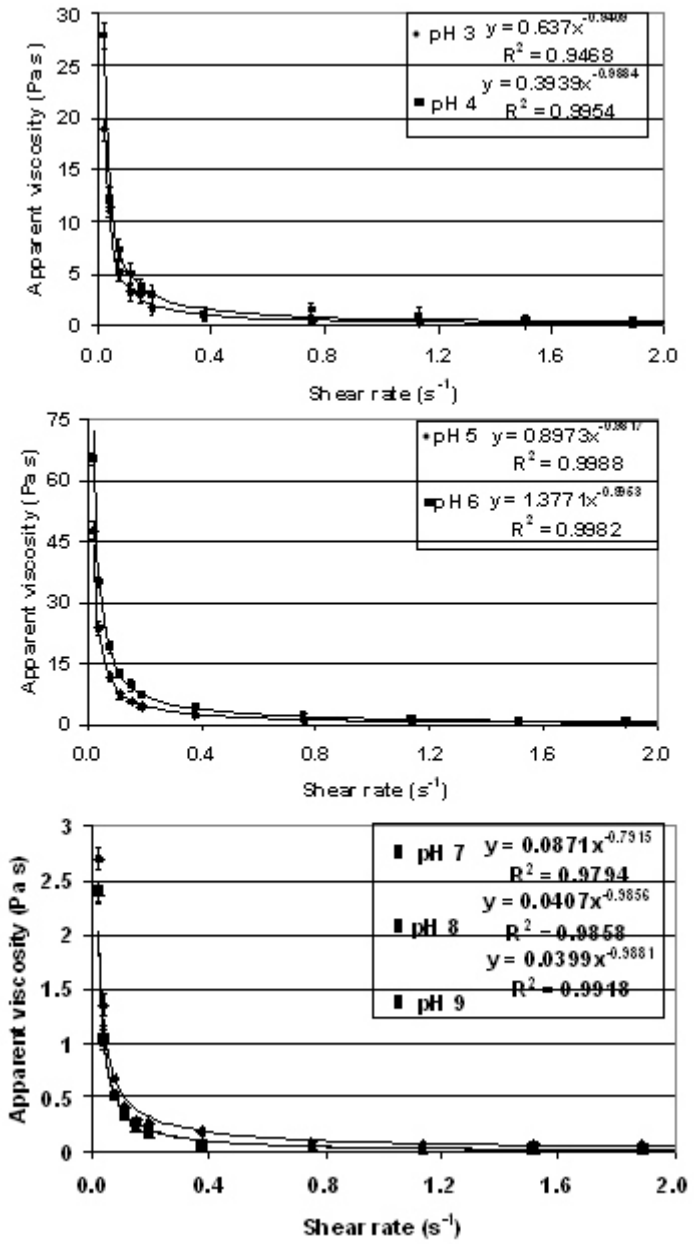

Fig. 9: Rheological behavior of PBP to 12.5% (w/v) to different pH values after heating 90 ºC during 30 min, under different shear rates (Spin 31)

values (<1) correspond to a pseudoplastic behavior. At pH <7, an increase in the consistency index and in the apparent viscosity of the solutions was obtained. This can be explained considering that the bonds among the protein molecules increase the viscosity or the consistency index of the solutions as a function of the pH (Gauchea *et al*., 2008).

Microstructure analysis: The objective of the microstructure analysis was to elucidate the relationships between powder functional properties and powder structure as suggested by Autio and Laurikainen (1997) and Marco and Rosell (2008). Powder samples of freeze-dried plasma without sucrose observed by Scanning Electron Microscopy (SEM) showed a disrupted-like structure where the proteins present a significantly affected microstructure (Fig. 10a). When sucrose (10%, w/v) was added, an uniform distribution of a globular structure of proteins hold together by the saccharide was observed, suggesting the protein structure conservation,

Table 2: Rheological properties of PBP at different pH

pH	Apparent viscosity, η (Pa s)	Flow behavior index, *n*	Flow consistency index, *K* (Pa s^n)
3	0.545±0.029	0.0591	0.6370
4	0.266±0.028	0.0116	0.3939
5	0.600±0.032	0.0183	0.8973
6	0.960±0.021	0.0047	1.3771
7	0.071±0.003	0.2085	0.0871
8	0.030±0.002	0.0144	0.0407
9	0.030±0.002	0.0119	0.0399

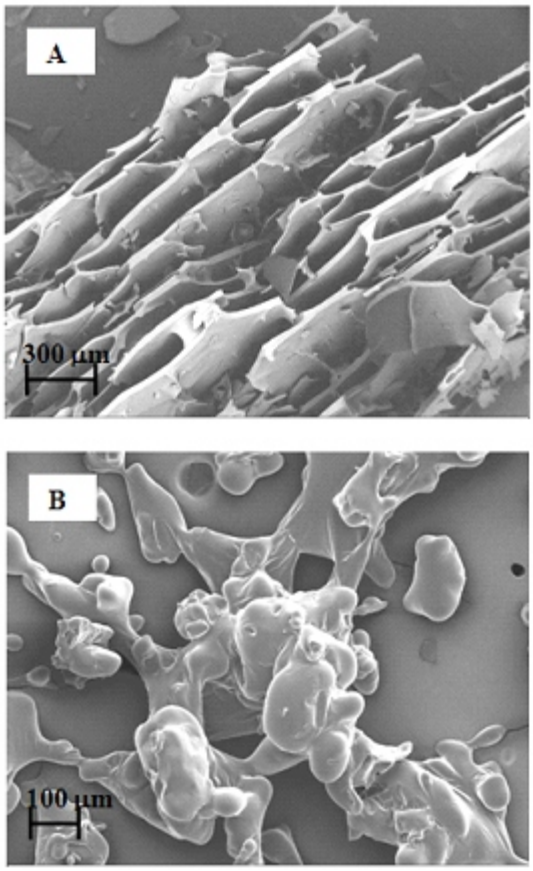

Fig. 10: Scanning electron micrographs of the freeze-dried products: (A) without sucrose (magnification, 50X), (B) with sucrose, 10% w/v (magnification, 200X

which is integrated in the carbohydrate matrix, improving the functionally of the system (Fig. 10b). Besides, plasma proteins displayed a gel-like structure (Fig. 10b). This structure was also found by Marco and Rosell (2008) for hydrated soybean protein isolate.

Analysis of minced meat samples: The results show that the minced meat with PBP presented a significant increase in the moisture content (59.4±0.29%), compared with minced meat with RM (55.6±0.32%). These results may be explained considering the higher WHC of the PBP (7.31±0.03%) front RM (6.83±0.09%), thus the processed proteins have higher ability to trap moisture and reduce moisture loss (Mun *et al*., 2009). The ash content was 4.08±0.09% and 3.9±0.07%, for RM and PBP respectively, the reduction is due to UF- DD step for processed proteins. The pH of the samples was 5.97±0.02.

The minced meat with PBP presented a stability of 95.20±0.57%, higher than the formulation with RM, with a value of 91.02±0.68%. The creaming is the main problem in minced meat, which is usually prevented by adding a thickening agent such as a gum, starch, flour or proteins to the aqueous phase to slow down the droplet movement (Mun *et al*., 2009). Thus, our minced meat showed a good stability because of the increased viscosity of the aqueous phase by the addition of protein concentrate, wheat flour and cornstarch. Besides, the higher stability of the formulation with PBP is due to the improved functional properties of the protein concentrate.

The results of sensorial analysis showed that no significant difference was observed between the sensorial quality of the samples with BPB and RM for the aroma, color and taste. However, the consistency of the minced meat with PBP has higher acceptance by the panelists. This fact can be due to the better functional and rheological properties of the processed proteins.

CONCLUSION

In this work, properties of bovine plasma processed with UF-DD, and freeze drying using sugar as protective agent were characterized in a broad pH range, for food formulations. Protein behavior was compared with plasma proteins as raw material. The denatured protein content was reduced in the PBP, improving many functional properties such as solubility, emulsion capacity and foam stability. Additionally, the salt natural content was reduced as well.

Functional property profiles for RM and PBP were pH-dependent. The solubility of both products had similar profiles with the lowest solubility at pH 4-6, other than protein solubility of PBP was improved ($p<0.05$) respect to raw material. The functional properties of both products were similar in terms of foam capacity. However, PBP showed an improvement in emulsion capacity, emulsion and foam stability ($p<0.05$). Also, scanning electron micrographs of the freeze-dried samples confirmed that the usage of sucrose can be promising to produce protein with improved functionality, by better preservation of protein native configuration. PBP presented desired rheological properties to become a suitable candidate for dietary formulations that require heating-induced gelation.

The proteins of bovine plasma obtained by the processes described can become an alternative to other proteins as soybean and milk proteins in the production of emulsions and foams. An application has been assayed for minced meat elaboration, employing RM and PBP, having

this last higher moisture content, improved stability, and better consistency, while no change in the aroma, taste and color was observed. Thus, these results indicate that PBP was beneficial to the quality of minced meat suggesting that might be used as stabilizing and emulsifying.

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