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Assessment of Functional Properties of Bovine Plasma Proteins Compared with Other Protein Concentrates, Application in a Hamburger Formulation

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ABSTRACT

World population growth has brought quantitative and qualitative issues related to food supply, particularly protein. This study investigated the rheological, functional and physicochemical properties of plasma bovine proteins comparing them with milk and soy-milk proteins. A membrane process including microfiltration and tangential ultrafiltration was used to purify and concentrate the protein solutions and then concentrates were freeze-dried at -40°C. During all the steps, bovine plasma proteins were processed alone (as control) and protected using a stabilizer agent (sucrose) in order to reduce the factors responsible for protein denaturation. The results showed that plasma protein with sugar, improved all the functional properties studied with respect to plasma without sugar, furthermore the solubility, water holding capacity, foam capacity, foam stability and gelling properties were better than the other protein sources evaluated. The different protein concentrates, were used in the formulation of a beef hamburger. Cooking characteristics and sensory properties of the product were assayed. Samples with bovine plasma and sucrose presented lower weight and moisture losses ($p < 0.05$), than hamburgers without protein addition or with milk and soy-milk proteins. Furthermore, the addition of 1% (w/w) of protein had no effect on sensory properties ($p > 0.05$). The procedure applied improves performance of plasma proteins for use in food formulations.

Key words: Protein concentrates, ultrafiltration, freeze-drying, functional properties, protective agent

INTRODUCTION

Proteins are complex macromolecules that constitute 50% or more of the dried weight of cells and play a fundamental role in cell structure and function. Among them, food proteins are tasty, digestible, non-toxic and economically viable for humans. Unfortunately, there is a deficit in the availability of proteins at worldwide scale due to an increasing world population. In order to satisfy the steady growth of protein demand, several investigations are currently headed to find new protein sources and technologies that yield a greater availability, quality and efficiency of the productivity (Benichou *et al.*, 2007; Kaur and Singh, 2007; Yu *et al.*, 2007; Selmane *et al.*, 2008; Toldra *et al.*, 2008; Kamara *et al.*, 2009; Kanu *et al.*, 2009; Kain *et al.*, 2009). Functional and

physical-chemical properties are affected by intrinsic factors of proteins such as molecular structure and size and many environmental factors including the method of production pH, ionic strength and the presence of other components in the food system (Yu *et al.*, 2007).

Although animal blood is a highly contaminating byproduct of slaughterhouses, it contains proteins of a high biological value, making it valuable for recovery and processing (Belhocine *et al.*, 1998; Torres *et al.*, 2002). However, the methods used in its processing diminish protein functional properties and so; it cannot be used in high concentrations in food formulations (Toldra *et al.*, 2008; Del Hoyo *et al.*, 2007). With a usual method of food preservation as freeze-drying, the functional properties of proteins are reduced by loss of protein structures. In this sense, the use of saccharides as stabilizing agent of protein denaturation has been previously described (Hinrichs *et al.*, 2001; Furlan *et al.*, 2009). During freeze-drying process, sugars exert two protecting effects: (i) they replace the water that hydrates proteins and form with the protein hydrogen-bridge bonds and (ii) the protein is encapsulated within a vitreous structure avoiding its unfolding and thus preserving its conformation (Carpenter *et al.*, 1993; Buera *et al.*, 2005).

Functional and physicochemical characteristics of food proteins are important in food processing and food formulation, since they contribute to obtain consumers desired characteristics and quality. Membrane technology allows the purification and concentration of proteins, reducing salt content with efficiency and in a less expensive way than other treatments such as vacuum evaporation used for concentration of animal blood (Fernando, 1981; Belhocine *et al.*, 1998; Torres *et al.*, 2002; Xu *et al.*, 2008). The main properties studied here are solubility, water holding capacity, oil binding capacity, foaming capacity and stability, emulsifying capacity and stability, viscosity and gelation. Furthermore, the behavior of the different proteins concentrates in a beef hamburger formulation was assayed; the food products were congealed and then cooked to evaluate the functional and sensory parameters. Hence, the objective of the present study was to study the effect of saccharose as protective agent during plasma protein concentration by MF-UF and freeze-drying and to compare the results with the raw material without protection and with two other classic sources of proteins as bovine milk and soy milk.

MATERIALS AND METHODS

Raw materials: The raw materials were the following: (i) Bovine Milk (BM) was a partially skim-milk supplied by Milkaut Argentina Industry (3.9% proteins, 1.5% fat, 0.73 ash) (ii) Soy Milk (SM) was a commercial soy food (3.2% proteins; 1% fat and 0.6 % ash and (iii) Bovine Plasma (BP) was a spray dried bovine plasma (Yerubá S.A., Argentina) (76±5% proteins; <0.1% fat; 10% ash; 4% humidity, 1% low molecular weight compounds). All compositions were provided by providers as % w/w.

Saccharose (Analar) was used as protective agent of bovine plasma proteins (BP+S).

Protein purification and concentration: The soy and bovine milks were used directly for membrane processes. The plasma powder was dissolved to a final concentration of 30% (w/v) to be in a similar protein concentration than the two others. The plasma solutions was prepared as follows: i) a fraction in de-ionized water and ii) a fraction in a solution containing saccharose at 10% w/w in de-ionized water. A mixer was used at low revolutions, avoiding the formation of a vortex and minimizing the appearance of foam. The solution was passed through a porous support (Viledon F 02431 D, Germany) to remove macroscopic aggregates (Furlan *et al.*, 2009).

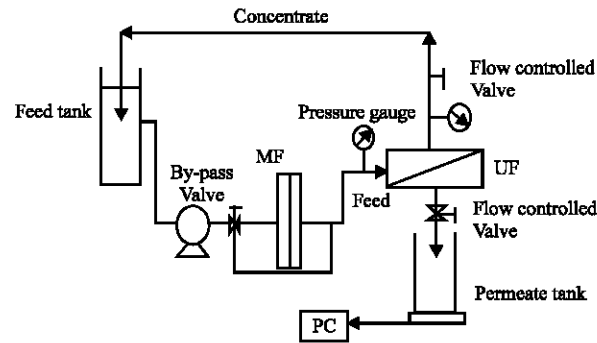


Fig. 1: Schematic experimental unit for ultrafiltration

The feed solutions were purified and concentrated using membrane technology including successive Microfiltration (MF) and Ultrafiltration (UF). Figure 1 shows a schematic diagram of the equipments set up used in this study. MF was used to filter colloidal and suspended particles, as well as bacteria and was performed by a frontal flow polyethylene microfiltration filter with a pore size of 5-10 μm (Pall Corporation, USA) for SM and BM and for BP and BP+S, a frontal flow stainless steel filter, with a pore size of 60 μm (Gora, Argentine). The filters were cleaned and sanitized after each experiment and exchanged periodically. UF was used to concentrate the solutes by continuously removing the permeate stream. The filtration unit was a tangential flow Pellicon cassette (Millipore, Bedford, MA and USA) with asymmetric membranes of polyethersulfone (molecular weight cut off: 10 kDa) and a membrane area of 0.5 m^2 . The runs were conducted at constant transmembrane pressure (2 bar) and constant feed flow rate (2.9 ± 0.05) L min^{-1} . The experimental temperature was $22 \pm 1^\circ\text{C}$ for SM and BM and 10°C , for BP and BP+S. There was no microorganism proliferation because of the short processing times. An analytic balance connected to an electronic interface and to a PC with acquisition data (ADQ12-B Converter A/D 12 bits Micro Axial) were used as auxiliary elements. The cleaning of the fouled membrane was performed by applying a Cleaning in Place (CIP) procedure according to the manufacturers instructions. Measurements of normalized water permeability were determined in order to verify recovery of flow through the membrane.

Freeze-drying stage: Protein concentrates obtained by UF of BM, SM and BP (with and without saccharose, 4% (w/v) were placed on stainless steel trays and frozen in a freezer at -40°C and freeze-dried with a lyophilizer (Rifcor S.A., Argentina) at 1 bar for 48 h. The samples temperature was controlled by a temperature sensor.

Characterization of protein concentrates: The characterization of freeze-dried concentrates was conducted by various techniques. All assays were carried out in triplicate:

- The total protein content of each concentrate was determined by the Kjeldhal method (AOAC, 1995). The conversion factors used to express the results were: 6.38 for BM, 6.25 for BP and 5.8 for SM
- Protein solubility was determined as described earlier (Furlan *et al.*, 2010a)
- Water holding capacity (WHC) was investigated using the technique described by Furlan *et al.* (2010b) and was expressed as grams of water per gram of product

- Lipid Binding Capacity (LBC) was determined using the method of Chakraborty (1986)
- The Emulsifying Capacity (EC) expressed as volume (mL) of emulsified oil per gram of product was measured as described previously (Furlan *et al.*, 2010a)
- All the formed emulsions were stored in a refrigerator (4°C) and were monitored daily for 16 days. Each preparation had also 0.05% (w/v) sodium azide added to prevent microbial contamination. The emulsions were visually examined every day for signs of creaming, oiling off or other physical separation attributes (Ee *et al.*, 2009). The height of the Separation Layer (SL) was measured in relation to the total emulsion height (E) as an indicator of the emulsion stability (ES): $ES = (E/SL)$
- Foaming Capacity (FC) and Foaming Stability (FS) of protein concentrates were investigated using the technique described before by Butt and Batool (2010)
- Gel strength was determined in triplicate according to the procedure described by Furlan *et al.* (2010b), the pH of suspensions was adjusted to 7.4 with 1.0 N NaOH and HCl
- Ash content measurement was carried out in a muffle furnace (Indef 132, Argentina) using a controlled temperature program to reach gradually 520°C. Salt reduction percentage was determined by the difference between the initial and final weight of the samples

Hamburger preparation: Meat was mixed in a mixer for 1 min. The ingredients (NaCl 0.5%, protein 0.75%, pepper 0.1%) were incorporated and the sample mixed again for 1 min. Beef hamburgers (55 g) were prepared using a manual burger former (9 cm diameter) and placed in airtight bags with toothed zip fastener (Ziploc, S.C. Johnson and Son), protecting the product from contact with oxygen and water vapor. Hamburgers were frozen at -25°C and storage for 5 days, after that, they were cooked for 12 min in a forced air oven at 170°C to bring the adequate temperature at the centre of the product (Lopez-Lopez *et al.*, 2010). Each sample was prepared in duplicate.

Chemical analysis of the hamburgers: In order to determine the weight loss, the hamburgers were weighed with an electronic balance prior to cooking and after removal from the oven. The total weight loss (L_T) was determined to be the difference in weight before and after cooking and is expressed as a percentage of the original weight (Braeckman *et al.*, 2009). The contents of water were measured in both, the raw and cooked meat to enable the calculation of water losses. Moisture content of the samples was determined using the AOAC Official Method 950.46 (AOAC, 1995). Water losses were calculated as a percentage of the original weight of the meat:

$$L_M = \frac{m_i - m_f}{m_i} \times 100 \quad (1)$$

where, L_M is the moisture loss, m_i is the moisture content before (g) and m_f the moisture content after thermal processing (g). Fat losses were calculated as the difference between the weight and water losses.

The pH was monitored with a digital pH-meter.

Sensory evaluation: A quarter portion of each beef hamburger was given to the panelist with a code to identify each product. The samples were tested at 25°C, in a uniformly illuminated room,

by a 20-member panel selected from a pool of students and staff members of our Department. The challenge for each panelist was to determine whether there are differences between them classifying if their attributes using a ten points descriptive scale were 10 = excellent and 1 = extremely poor. The control sample was identified with the letter (C) and it was analyzed the grade of approach to the reference; different characteristics were analyzed taste, aroma, color and consistency (degree of firmness). Water was provided for inning between samples.

Statistical analysis: Statistical analysis was performed using the software GraphPad InStat for Windows (State of California, U.S.A.). Data were analyzed using a one-way analysis of variance (ANOVA). The normal distribution and the homogeneity of variances were checked. The test of Tukey-Kramer of multiple comparisons was used in the cases where two or more comparisons were being considered. Otherwise, the T-test was used. The level of significance for all tests was set at $p < 0.05$ (SAS, 1989).

RESULTS AND DISCUSSION

Protein solubility: From a practical point of view, data about solubility characteristics are quite helpful in determining the optimal conditions of protein extraction and purification, as well as the separation of protein fractions. Under different conditions, solubility is also a good indicator of the potential applications of proteins and influences other functional properties (Fernando, 1981).

Figure 2 shows the solubility of protein concentrates across the pH range tested (pH 3.0-9.0). It is observed that the solubility of BP and BP+S as BM and SM is practically pH-dependent, having their minimum solubility at precipitation pH (isoelectric point, pI) and maximum solubility at alkaline pH. The occurrence of minimum solubility near the isoelectric pH is due primarily, to the lack of electrostatic repulsion which promotes aggregation and precipitation via hydrophobic interactions (Fennema, 1996). Results showed that BP proteins have a maximum of solubility at pH 7.5 and a decrease between pH 4 and 6. The results obtained are comparable to those of Del Hoyo *et al.* (2008) referred to plasma protein but they concluded that the standard plasma (raw material) has better attributes than the plasma processed by ultrafiltration, since standard plasma protein should be less denatured than the proteins of the processed plasma. In present study the processed plasma with sugar (BP+S), has improved its solubility with respect to BP, being its behavior similar to SM proteins. This result for SM is in accord to that obtained by Chove *et al.*

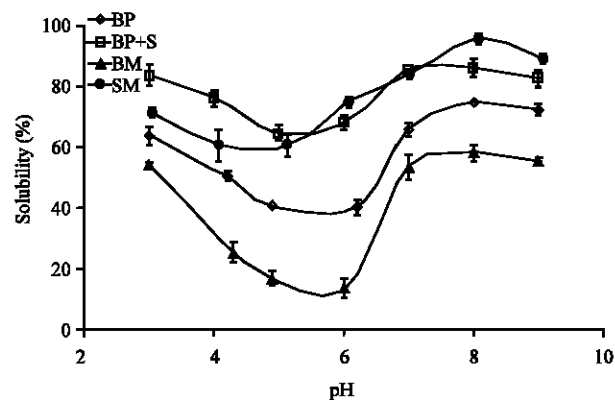


Fig. 2: Solubility of protein concentrates at different pH values ($22 \pm 1^\circ\text{C}$)

Table 1: Characteristics of the lyophilized protein concentrates

Properties	BP	BP+S	BM	SM
Proteins total contain (% w/w) WHC	73.70±0.6	27.80±0.4	34.80±0.3	28.80±0.2
(mL of water g ⁻¹ of product)	5.21±0.08	7.31±0.03	1.00±0.05	2.05±0.06
LBC (mL of oil g ⁻¹ of product)	2.20±0.2	2.40±0.1	3.90±0.11	2.30±0.08
EC (mL of oil g ⁻¹ of product)	289.00±7.8	445.00±5	790.00±9	260.00±7
FC (mL mL ⁻¹)	1.83±0.08	1.22±0.02	0.00	0.00
FS (%)	21.70±1.2	50.60±0.4	-	-
Ash (%) (w/w)	5.20±0.1	2.50	6.70	7.50

(2007) who reported that the soy protein fractions of higher molecular weight retained by a membrane were the most soluble ones, particularly at pH>6. BM proteins exhibited a lower solubility than plasma protein (p<0.05). This may be because the proteins concentrated by the membrane procedure, mainly caseins, are rather hydrophobic and so they are slightly soluble in water. This could be related to the associated mineral concentration by UF (Table 1) which enhances the interactions protein-protein and diminishes the interactions protein-water (Zhang *et al.*, 2004).

Water holding capacity: The water holding capacity of the protein ingredients plays a major role on the texture quality of a number of foods, particularly in ground meats and pastries. Moreover, this property is fundamental to viscous foods such as soups, sauces and baked products. The water adsorption without protein dissolution leads swelling and provides properties such as consistence, thickening, viscosity and adherence (Yu *et al.*, 2007).

The results obtained from the characterization of the protein concentrates of BP (with and without protective agent), SM and BM are shown in Table 1. It can be seen that BP+S concentrate exhibits the highest WHC. The intrinsic factors affecting the WHC of food include amino acid composition, protein conformation and surface polarity/hydrophobicity (De Man, 1999). Basically, WHC depends on two fundamental factors: (i) the size of the free space where the water is retained in the protein structure and (ii) the existence of molecules that contribute with charges and allow dipole-dipole interactions as was explained by Wolkers *et al.* (1998) and Ee *et al.* (2009). For these reasons, it is expected that BP+S has a higher WHC. The value of PB+S is also higher than the results found for other protein sources as peanut flour or protein isolates from different chickpeas (Kaur and Singh, 2007; Yu *et al.*, 2007). Furthermore, the low values found for BM may be related to protein hydrophobicity and minor availability of polar groups to fix water (Zhang *et al.*, 2004).

Lipid binding capacity: The lipid binding capacity of proteins is quite important in the formulation of fried products and for taste retention. Furthermore, it decreases the development of oxidative staling and consequently, increases the stability throughout storage. Data obtained in this study show that BP without a protective agent has the lowest value (Table 1). This fact could be due to protein denaturation during processing. This effect has been reported by Yu *et al.* (2007) when roasting peanut flour. It can be seen how the use of saccharose improves this property, being better than soy and very close to milk proteins which present the highest value due to its folding structure and global hydrophobicity (Yusuf *et al.*, 2008).

Emulsifying capacity and stability: Food emulsions are thermodynamically unstable mixtures of immiscible liquids (water and oil). The formation and stability of emulsion is very important in

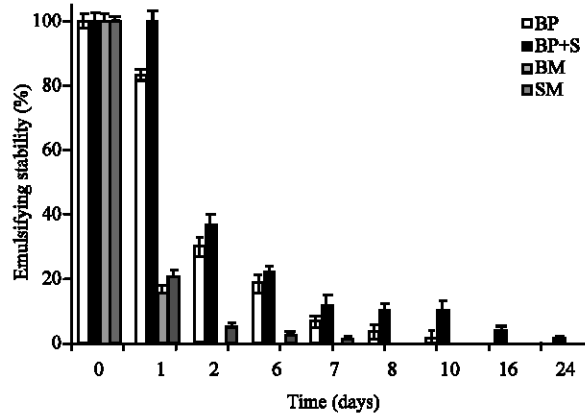


Fig. 3: Emulsion stability of the BP, BM and SM proteins ($22\pm 1^\circ\text{C}$)

food systems such as salad dressings, ice cream, confectionary or meat products (Yu *et al.*, 2007; Patino *et al.*, 2008). Table 1 shows that BP and BP+S concentrates present good emulsifying characteristics comparing with the other samples. This result may be because plasma proteins present good overall hydrophobicity capacity as a result of the distribution of the hydrophobic charged group along the polypeptide chains and the manner in which the chains are folded. Besides, these proteins have flexible polypeptide chains, so that they are able to orient and unfold at the interface, making them effective emulsifiers (De Man, 1999). In effect, the emulsifying capacity of these concentrates is higher than the values reported for other protein sources as benniseed and bambara groundnut or peanut protein concentrate (Yu *et al.*, 2007; Yusuf *et al.*, 2008).

Table 1 shows that the presence of the disaccharide increases the emulsifying capacity of plasma proteins since they decrease the surface tension and thus, the free energy of the system and impart the desired kinetic stability to dispersions (emulsions or foam) (Patino *et al.*, 2008). Moreover, Fig. 3 shows that PB+S emulsions have higher stability than other concentrated proteins. This may be because the rate protein-saccharide is an important parameter that determines drop stability or coalescence. In fact, emulsion stability depends on protein-protein interactions and the presence of saccharides may reinforce or weaken the interactions. To an optimal concentration of sugar, the stability of emulsion is enhanced because the saccharide adsorbs quickly and saturates the whole surface of the drops as was described by Glaser *et al.* (2007), Leal-Calderon *et al.* (2007) and Mun *et al.* (2008). The value obtained for SM is consistent with the result reported by Chove *et al.* (2007) for conventional soy protein isolates and concentrates with microfiltration membranes. The emulsion stability of BM is poor considering that at a higher protein concentration, as by ultrafiltration process a protein layer saturates the interface. Thus, emulsion instability is due to flocculation and/or creaming as in the results of Sanchez and Patino (2005) and Patino *et al.* (2008).

Foaming capacity and stability: The formation of foam is analogous to the formation of emulsion. In the case of foam water molecules surround air droplets and air is the non-polar phase. Theoretically, the amphipathic character of proteins makes them good foaming agents that study at air-water interface to prevent bubble coalescence (Yu *et al.*, 2007).

Table 1 show that plasma protein with the addition of saccharose impairs formability but improves foam stability. This fact was reported by Sikorski (2007) who explained that the positive effect of sugars on foam stability is due to increased bulk-phase viscosity which reduces the rate of drainage of lamella fluid. The depression in foam overrun is mainly due to enhanced stability of protein structure in sugar solutions. Because of this, the protein molecule is less able to unfold upon adsorption at the interface, decreasing the ability of the protein to produce large interfacial areas and large foam volumes during whipping. This effect has been reported by Fidantsi and Doxastakis (2001) for amaranth seed protein isolates, Patino *et al.* (2008) in foam formulations and Ee *et al.* (2009) in wattle seed. Comparing with blood proteins processed by high pressure (Toldra *et al.*, 2008), BP+S proteins have better foaming capacity and better foam stability.

On the other hand, BM and SM showed negligible foam capacity, although their high interfacial properties. This may be due to the fact that UF concentrates lipids, besides proteins (Brans *et al.*, 2004). It is well-known that fat is a foam depressor because it competes with proteins at the air-water interface and may displace the absorbed protein layer (Dickinson and Stainsby, 1982). This behavior was found by Selmane *et al.* (2008) studying the functional properties of proteins from slaughterhouse. Furthermore, Kamath *et al.* (2008) studied on foaming properties of milk and they reported that the adsorption of free-fat acids leads to disruption of protein-protein interactions and as a result, milk proteins are unable to form a stable viscoelastic film around the air bubbles, causing a decrease in foam stability and an increase in the free-fat acid content of milk.

Rheological properties of protein concentrate: Viscosity is an important property of foods that affects taste, texture and mechanical handling of fluid materials. Gel formation arises when the protein is at high concentrations and 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 the controlled aggregation of protein molecules after cooling, forming a tridimensional matrix that confines the liquid (Yu *et al.*, 2007).

The results shown in Table 2 indicate that BP and BP+S protein concentrates present a higher viscosity and capacity to form gels than SM and BM. For the solutions of BP+S heated with concentrations higher than 10%, a marked increase in viscosity is observed, from a value of 1.98±0.23 to 3.322±0.150 cP after gel formation (p<0.001 considered extremely significant). BP had a similar behavior, somewhat more doughy than BP+S (data not shown). In this sense, Selmane *et al.* (2008) explained that most meat proteins extracted from by-products may be denatured easily by heating due to their highly-ordered secondary and tertiary structures. Heat treatment as the spray dried of our raw material, should therefore, lead to protein aggregation and favor water-holding, contributing to irreversible protein gels by heating. Furthermore, the presence

Table 2: Viscosity of the protein solutions before and after heating to 90°C for 30 min

Protein concentrate	Concentrations process	Viscosity (mPa.s)		
		75 g L ⁻¹	100 g L ⁻¹	125 g L ⁻¹
BP+S	Not heated	1.75±0.07	1.91±0.07	1.98±0.23
	Heated	2.76±0.08	4.14±0.07	0.30±0.15
SM	Not heated	5.05±0.02	5.03±0.03	10.00±0.01
	Heated	7.26±0.02	13.05±0.03	15.97±0.02
BM	Not heated	1.85±0.03	2.07±0.02	2.58±0.03
	Heated	2.62±0.01	2.38±0.02	2.80±0.03

Table 3: Moisture and fat losses and pH control, during hamburger cooking. (C) Control; 1-4: 1% (w/w) of each protein concentrate

Sample	Moisture losses (%)	Fat losses (%)	pH
C	13.38±0.49 ^a	22.14±0.18 ^a	6.04±0.06 ^a
1: BP	10.48±1.32 ^a	18.37±1.94 ^b	6.06±0.01 ^a
2: BP+S	8.92 ±1.02 ^b	18.08±1.02 ^b	6.02±0.05 ^a
3: BM	12.63±0.52 ^a	18.37±0.52 ^b	6.05±0.05 ^a
4: SM	12.08±1.22 ^a	17.92±1.86 ^b	6.06±0.01 ^a

Means within columns followed by the same letter are not significantly different (p<0.05)

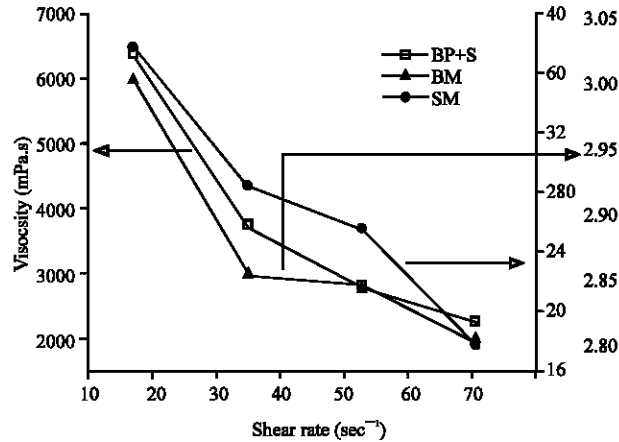


Fig. 4: Rheological behaviour of protein concentrates, at 12.5% (w/v) after heating at 90°C for 30 min, under different shear rates

of sodium chloride may decrease the gel strength. For BP+S, salt concentration is negligible (Table 1), contributing to have a gel of outstanding stiffness (Chove *et al.*, 2007). In addition, its high water holding capacity improves the gel properties.

The rheological behavior of protein concentrates at 12.5% (w/v) after heating at 90°C is shown in Fig. 4. BP+S showed a pseudoplastic behavior as SM and BM.

Influence of protein content on total weight, moisture and fat loss: The incorporation of BP+S to the hamburger formulation reduce the total losses of water and fat as it is shown in Table 3 (p<0.01). BP+S presented a lower moisture loss than BM and SM (p<0.05). However, BP did not show a difference statistically significant (p>0.05) compared with BM and SM. The reduced water loss in hamburger with bovine plasma proteins with sugar added may be attributed to the better water holding capacity of the PBP+S. While the fat loss, is lower with protein added to the hamburger formulation but did not show difference statistically significant (p>0.05) between all the samples with the protein concentrates. Similar results were observed by Serdaroglu (2006) with 0-4% w/w of whey powder in meatball, additionally Hale *et al.* (2002) found that patties with added textured whey protein had lower cooking losses than all beef patties. No statistically significant difference was found between the different values of pH.

Sensory evaluation: Table 4 shows sensory attributes evaluation. Color, aroma and consistence, of hamburger after cooking were no affected (p>0.05) by the addition of the different protein concentrates. The only sensory parameter affected by the formulation was taste. In the

Table 4: Effect of incorporation of protein concentrates on sensory attributes of cooked hamburgers

Treatments/parameters	Taste	Aroma	Color	Consistency
C	7.2±0.4 ^a	8.2±0.3 ^a	8.1±0.5 ^a	8.8±0.4 ^a
BP	8.6±0.5 ^a	8.5±0.4 ^a	7.9±0.3 ^a	8.7±0.4 ^a
BP+S	6.9±0.6 ^a	8.4±0.5 ^a	7.8±0.4 ^a	8.5±0.7 ^a
BM	8.8±0.5 ^b	8.1±0.3 ^a	7.7±0.6 ^a	8.4±0.5 ^a
SM	5.2±0.7 ^b	8.0±0.6 ^a	8.2±0.5 ^a	8.6±0.8 ^a

Means within columns followed by the same letter are not significantly different ($p < 0.05$)

formulations that contained BM and BP some panelists detected a different taste from the one commonly encountered in hamburger but they found it pleasant and more palatable than the control. The hamburgers containing SM were detected as less agreeable compared with the control ($p < 0.05$) while the samples with BP+S were found pleasant and palatable as the control ($p > 0.05$).

CONCLUSION

Functional properties of BP protein concentrates with and without the addition of saccharose as protective agent were compared to those from SM and BM, obtained through ultrafiltration and drying by freeze-drying. One of the most important characteristics is protein solubility, since it influences the other functional properties. It was demonstrated that through the addition of sugar during the treatment, solubility was improved being similar to soy proteins which had a high solubility in the range considered. Indeed all the functional properties of the plasma proteins investigated were enhanced by the use of a protective agent to prevent protein denaturation and loss of functionality, except for foam capacity. It is important to highlight that the BP+S has less protein content than BP per gram of product. The functional properties of BP+S were comparable to and in some cases, better than soy and milk proteins. The protein concentrate of BP+S exhibits the desired rheological properties, provided that during suspension pumping, it presents a low viscosity for an easier manipulation but after heating, gel formation with higher viscosities is appropriate for the manufacture of soups, sausages and meat analogues. Therefore, plasma protein processed with sugar is a suitable candidate for dietary formulations that require heating-induced gelation. The incorporation of the protein concentrates to a formulation of a beef hamburger was beneficial in reducing the water and fat losses during cooking. In accordance of the improved functional properties of the BP+S, the sample containing this concentrate had the better moisture retention ($p < 0.05$). The addition of protein concentrates 1% (w/w) did not significantly alter sensory properties of hamburgers ($p > 0.05$). BM and SM are considered proteins having outstanding functional properties in comparison with bovine plasma proteins. The procedure improves the performance of the product, as per gram of raw material (bovine plasma) was obtained 0.99 g for BP and 4.16 g for BP+S by the addition of sugar while the attributes and functional properties of bovine proteins are improved using a protective agent of low cost as saccharose during the processing and therefore, plasma proteins processed with saccharose may be considered as a feasible option to be used as dietary supplement and/or in the development of human food formulations. In this way, proteins obtained from blood may have a higher added value than the traditional ones.

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REFERENCES

- AOAC, 1995. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
- Belhocine, D., H. Grib, D. Abdessmed, Y. Comeau and N. Mameri, 1998. Optimization of plasma proteins concentration by ultrafiltration. *J. Membr. Sci.*, 142: 159-171.
- Benichou, A., A. Aserin, R. Lutz and N. Garti, 2007. Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. *Food Hydrocolloids*, 21: 379-391.
- Braeckman, L., F. Ronsse, P.C. Hidalgo and J. Pieters, 2009. Influence of combined IR-grilling and hot air cooking conditions on moisture and fat content, texture and colour attributes of meat patties. *J. Food Eng.*, 93: 437-443.
- Brans, G., C.G.P.H. Schroen, R.G.M. van der Sman and R.M. Boom, 2004. Membrane fractionation of milk: State of the art and challenges. *J. Membr. Sci.*, 243: 263-272.
- Buera, P., C. Schebor and B. Elizalde, 2005. Effects of carbohydrate crystallization on stability of dehydrated foods and ingredient formulations. *J. Food Eng.*, 67: 157-165.
- Butt, M.S. and R. Batool, 2010. Nutritional and functional properties of some promising legumes protein isolates. *Pak. J. Nutr.*, 9: 373-379.
- Carpenter, J.F., S.J. Prestrelski and T. Arakawa, 1993. Separation of freezing- and drying-induced denaturation of lyophilized proteins using stress-specific stabilization: I. Enzyme activity and calorimetric studies. *Arch. Biochem. Biophys.*, 303: 456-464.
- Chakraborty, P., 1986. Coconut Protein Isolate by Ultrafiltration. In: *Food Engineering and Process Applications*, 2, LeMeguer, M. and P. Jelen (Eds.). Elsevier Applied Science Publishers, New York, pp: 308-315.
- Chove, B.E., A.S. Grandison and M.J. Lewis, 2007. Some functional properties of fractionated soy protein isolates obtained by microfiltration. *Food Hydrocolloids*, 21: 1379-1388.
- De Man, J.M., 1999. Principles of Food Chemistry. 3rd E dn., Springer-Verlag, USA., ISBN-13: 978-0834212343, pp: 520.
- Del Hoyo, P., F. Moure, M. Rendueles and M. Diaz, 2007. Demineralization of animal blood plasma by ion exchange and ultrafiltration. *Meat Sci.*, 76: 402-410.
- Del Hoyo, P., M. Rendueles and M. Diaz, 2008. Effect of processing on functional properties of animal blood plasma. *Meat Sci.*, 78: 522-528.
- Dickinson, E. and G. Stainsby, 1982. *Colloids in Foods*. Applied Science Publishers, London, UK.
- Ee, K.Y., A. Rehman, S. Agboola and J. Zhao, 2009. Influence of heat processing on functional properties of Australian wattle seed (*Acacia victoriae* Bentham) extracts. *Food Hydrocolloids*, 23: 116-124.
- Fennema, O.R., 1996. *Food Chemistry*. 3rd Edn., Marcel Dekker, New York, USA., ISBN-13: 9780824796914, pp: 1069.
- Fernando, T., 1981. Concentration of animal blood by ultrafiltration. *Biotechnol. Bioeng.*, 23: 19-27.
- Fidantsi, A. and G. Doxastakis, 2001. Emulsifying and foaming properties of amaranth seed protein isolates. *Colloids Surf. B. Biointerfaces*, 211: 119-124.
- Furlan, L.T.R., A.P. Padilla and M.E. Campderros, 2009. Procedure for obtaining a concentrated animal protein, protein concentrate obtained and uses. Argentine Patent AR067798 A1.
- Furlan, L.T.R., A.P. Padilla and M. Campderros, 2010a. Functional and physical properties of bovine plasma proteins as a function of processing and pH, application in a food formulation. *Adv. J. Food Sci. Technol.*, 2: 256-267.

- Furlan, L.T.R., A.P. Padilla and M.E. Campderros, 2010b. Inulin like lyoprotectant of bovine plasma proteins concentrated by ultrafiltration. *Food Res. Int.*, 43: 788-796.
- Glaser, L.A., A.T. Paulson, R.A. Speers, R.Y. Yada and D. Rousseau, 2007. Foaming behavior of mixed bovine serum albumin-protamine systems. *Food Hydrocolloids*, 21: 495-506.
- Hale, A.B., C.E. Carpenter and M.K. Walsh, 2002. Instrumental and consumer evaluation of beef patties extended with extrusion-textured whey proteins. *J. Food Sci.*, 67: 1267-1270.
- Hinrichs, W.L.J., M.G. Prinsen and H.W. Frijlink, 2001. Inulin glasses for the stabilization of therapeutic proteins. *Int. J. Pharm.*, 215: 163-174.
- Kain, R.J., Z. Chenc, T.S. Sonda and J.C. Abu-Kpawoh, 2009. Study on the effects of enzymatic hydrolysis on the physical, functional and chemical properties of peanut protein isolates extracted from defatted heat pressed peanut meal flour (*Arachis hypogaea* L.). *Pak. J. Nutr.*, 8: 818-825.
- Kamara, M.T., Z. Huiming, Z. Kexue, I. Amadou and F. Tarawalie, 2009. Comparative study of chemical composition and physicochemical properties of two varieties of defatted foxtail millet flour grown in China. *Am. J. Food Technol.*, 4: 255-267.
- Kamath, S., T. Huppertz, A.V. Houlihan and H.C. Deeth, 2008. The influence of temperature on the foaming of milk. *Int. Dairy J.*, 18: 994-1002.
- Kanu, P.J., J.B. Kanu, E.H. Sandy, J.B.A. Kandeh, P.M.P. Mornya and Z. Huiming, 2009. Optimization of enzymatic hydrolysis of defatted sesame flour by different proteases and their effect on the functional properties of the resulting protein hydrolysate. *Am. J. Food Technol.*, 4: 226-240.
- Kaur, M. and N. Singh, 2007. Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. *Food Chem.*, 102: 366-374.
- Leal-Calderon, F., F. Thivilliers and V. Schmitt, 2007. Structured emulsions. *Curr. Opin. Colloid Interfac. Sci.*, 12: 206-212.
- Lopez-Lopez, I., S. Cofrades, A. Yakan, M.T. Solas and F. Jimenez-Colmenero, 2010. Frozen storage characteristics of low-salt and low-fat beef patties as affected by Wakame addition and replacing pork backfat with olive oil-in-water emulsion. *Food Res. Int.*, 43: 1244-1254.
- Mun, S., Y. Cho, E.A. Decker and D.J. McClements, 2008. Utilization of polysaccharide coatings to improve freeze-thaw and freeze-dry stability of protein-coated lipid droplets. *J. Food Eng.*, 86: 508-518.
- Patino, J.M.R., C.C. Sanchez and M.R.R. Nino, 2008. Implications of interfacial characteristics of food foaming agents in foam formulations. *Adv. Colloid Interfac. Sci.*, 140: 95-113.
- SAS, 1989. User guide: Statistic. SAS Inst. Inc., Cary, NC, USA..
- Sanchez, C.C. and J.M.R. Patino, 2005. Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration in solution. *Food Hydrocolloids*, 19: 407-416.
- Selmane, D., V. Christophe and D. Gholamreza, 2008. Extraction of proteins from slaughterhouse by-products: Influence of operating conditions on functional properties. *Meat Sci.*, 79: 640-647.
- Serdaroglu, M., 2006. Improving low fat meatball characteristics by adding whey powder. *Meat Sci.*, 72: 155-163.
- Sikorski, Z.E., 2007. Chemical and Functional Properties of Food Components. 3rd Edn., CRC Press, Boca Raton, USA., ISBN-13: 978-0849396755, pp: 544.

- Toldra, M., E. Davila, E. Saguero, N. Fort, P. Salvador, D. Pares and C. Carretero, 2008. Functional and quality characteristics of the red blood cell fraction from biopreserved porcine blood as influenced by high pressure processing. *Meat Sci.*, 80: 380-388.
- Torres, M.R., F.R. Marin, A.J. Ramos and E. Soriano, 2002. Study of operating conditions in concentration of chicken blood plasma proteins by ultrafiltration. *J. Food Eng.*, 54: 215-219.
- Wolkers, W.F., M.G. Van Kilsdonk and F.A. Hoekstra, 1998. Dehydration-induced conformational changes of poly-L-lysine as influenced by drying rate and carbohydrates. *Biochim. Biophys. Acta.*, 1425: 127-136.
- Xu, J., A.A. Mohamed, P.M. Hojilla-Evangelista and J.D. Sessa, 2008. Rheology of defatted ultrafiltration-diafiltration soy proteins. *Am. J. Food Technol.*, 3: 294-302.
- Yu, J., M. Ahmedna and I. Goktepe, 2007. Peanut protein concentrate: Production and functional properties as affected by processing. *Food Chem.*, 103: 121-129.
- Yusuf, A.A., H. Ayedun and L.O. Sanni, 2008. Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indicum*) and bambara groundnut (*Vigna subterranean*). *Food Chem.*, 111: 277-282.
- Zhang, Z., D.G. Dalgleish and H.D. Goff, 2004. Effect of pH and ionic strength on competitive protein adsorption to air/water interfaces in aqueous foams made with mixed milk proteins. *Colloid Surf. B: Biointerfac.*, 34: 113-121.