

# Composition and physicochemical properties of two protein fractions of bovine blood serum

*Composição e propriedades físico-químicas de duas frações do soro de sangue bovino*

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## Abstract

Animal blood from slaughterhouses represent an important source of food ingredients which have been so far underutilized. Bovine blood production in Brazil was about  $3.6 \times 10^6$  in the year 2005. Besides wasting a good food quality protein, underutilization of this byproduct from the animal industry is an important cause of soil and water resources pollution. Protein concentration of both freeze-dried and spray-dried bovine albumin (BSA) and globulin (BSG) was around 85.0%. Essential Amino Acid Scoring (EAAS) was 72.7% for freeze-dried BSA and 89.3% for BSG. Emulsifying and foaming properties of both protein fractions were good mainly at pH 5.5. Foaming and emulsifying capacity was higher for BSA. Nevertheless, both foam and emulsion stabilities were better for BSG. NaCl tended to decrease both surfactant properties independently of pH. Considering solubility, heat stability, emulsifying and foaming properties, both BSA and BSG should be considered good functional ingredients for the manufacturing of food products. The study not only confirmed data already reported in the literature, but also enabled exploring new features, which amplifies the potential of applications.

**Keywords:** BSA; emulsification; foaming; globulin; functional properties.

## Resumo

O sangue animal de abatedouros representa uma fonte importante de ingrediente alimentício subutilizado. A produção de sangue bovino no Brasil foi aproximadamente  $3,6 \times 10^6$  tons em 2005. Além do desperdício de uma proteína de boa qualidade, a subutilização deste subproduto da indústria animal é uma fonte importante de poluição dos recursos hídricos e do solo. A concentração de proteína albumina (BSA) e globulina (BSG) bovinas secas em spray ou liofilizadas foi, aproximadamente, 85,0%. O score de aminoácidos (EAAS) foi 72,7%, para BSA liofilizada, e 89,3%, para BSG. As propriedades emulsificantes e espumantes de ambas as frações protéicas foram altas, principalmente a pH 5,5. A capacidade emulsificante e espumante foi maior para BSA, no entanto, tanto a estabilidade da emulsão quanto a da espuma formada foram melhores para a BSG. A adição de NaCl apresentou uma tendência de reduzir as duas propriedades surfactantes, independentemente do pH. Considerando a solubilidade, a estabilidade ao calor, propriedades emulsificante e espumante, tanto a BSA quanto a BSG deveriam ser consideradas bons ingredientes funcionais para a manufatura de produtos alimentícios. O estudo não apenas confirmou dados já reportados na literatura, como também explorou novas propriedades, o que amplifica o potencial de aplicações do soro de sangue bovino.

**Palavras-chave:** BSA; emulsificação; espuma; globulina; propriedades funcionais.

## 1 Introduction

Animal blood from slaughterhouses represent a very important source of food ingredients and nutrients which, up to now, have been underutilized (GOMES-JUAREZ et al., 1999). In Brazil, about  $3.6 \times 10^6$  tons of blood protein could be recovered annually, only from bovine slaughterhouses, which corresponds approximately to 28 mi animals sacrificed in 2005 (IBGE, 2006).

Blood can easily be fractionated into blood cells (erythrocytes), blood heme groups, globin protein isolates, plasma protein concentrates, and blood serum protein concentrates. All of these blood components find applications in Food Science and Nutrition. Bovine blood erythrocytes, as hemoglobin and the heme radical have been used as sources of heme iron in food supplements to correct anemia in humans, particularly in

children (WISMER-PEDERSEN, 1979; WALTER et al., 1993). Bovine plasma protein concentrates were used as food ingredients to improve formulated foods nutrition and functional (technological) properties (TYBOR; DILL; LANDMAN, 1975; YU; DAMODARAN 1991; DUARTE; SIMÕES; SGARBIERI, 1999). Blood serum proteins, mainly bovine serum albumin, have been extensively studied from their physicochemical and structural aspects. However, a reduced number of studies were performed aiming at technological applications of these proteins (AL-MALAH; AZZAN; OMARI, 2000).

Many methods available for the fractionation of proteins have been discussed by several authors (KUBOTA; TATSUMOTO; SANO, 1999; RITO-PALOMARES; DALE; LYDDIATT, 2000; MOURE; RENDUELES; DIAZ, 2003). In

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general, the methods involve the interaction of reagents in the protein solubility. The main advantages in the use of ammonium sulphate are high solubility causing precipitation of the majority of the proteins, low heat of dissolution, limiting bacterial growth, and protecting many proteins from denaturation (ENGLARD; SEIFTER, 1990). Comparing to other methods of blood protein recovery, it presents the additional advantage of recovering non-denatured globulins in the process of isolating the albumin fraction (BSA). The disadvantage is the need of dialysis or ultrafiltration for the salt removal.

The aim of the present paper was to fractionate and to characterize some functional properties of the bovine serum globulin fraction (BSG) and Bovine Serum Albumin (BSA) of bovine blood serum.

## 2 Materials and methods

### 2.1 Blood serum

Bovine blood was collected from healthy animals in a slaughterhouse directly from the jugular vein (*Arcus Aortae*) using a vampire knife and it was delivered in a vapor sterilized stainless steel container. Coagulation was performed in a controlled temperature (20 °C) room. The coagulum was fragmented and then retained on a stainless steel sterilized screen. The filtered liquid was centrifuged (Westfalia MTA-9 centrifuge, 9000 g, 15 minutes). The serum was packed in 5 kg polyethylene bags, quickly frozen in a freezing tunnel and stored in a freezer (-25 °C). This procedure was performed in cooperation with a commercial firm (TECSORO).

### 2.2 Fractionation and isolation

Proteins were fractionated by sequentially salting out with ammonium sulphate at 30, 50, 65, 80, and 100% salt saturation. During the salt addition, the solution was maintained under agitation in an ice bath. After each point of saturation, agitation continued for another 30 minutes at 5 °C for 8-12 hours. After each precipitation step, the proteins fractions were isolated by centrifugation (9000 g, 15 minutes, 4 °C) in a Sorvall RC 26 plus centrifuge, USA, resuspended and dialyzed against deionized water. To each supernatant, the necessary quantity of salt to reach the next saturation was added.

Both protein fractions were dehydrated by freeze-drying, at final temperature in the chamber not superior to -10 °C (Atlas Mark, 500 L, Liotécnica, Brazil), or by spray drying (A/S Niro Atomizer, Copenhagen, Denmark). Drying temperatures were 250 °C at the entrance and 80 °C at exit of the chamber.

### 2.3 Centesimal composition of the protein fractions

Crude protein (%N x 6.25), ash, and moisture contents were determined by the AOAC (1990) procedures. Total lipids were determined by the Bligh and Dyer method (1959).

### 2.4 Polyacrylamide gel electrophoresis

Electrophoresis (SDS-PAGE) was performed in both protein concentrates by the method of Laemmli (1970). Protein samples

for SDS-PAGE were prepared by mixing them with sample buffer (10 mM Tris-HCl, 2.5% SDS, 5%  $\beta$ -mercaptoethanol, 0.01% bromophenol blue, pH 8). Proteins were separated in a gradient 8-25% separation gel and stained with Coomassie Brilliant Blue. A Pharmacia Phastsystem was used for the separation, and densitometric readings were performed in a Pharmacia image 1 D densitometer. The following reference standards (Sigma, St Louis, MO, USA) were used: phosphorylase b (94 kD), BSA (65 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20 kD), and  $\alpha$ -lactalbumin (14.4 kD).

### 2.5 Amino acid determination

Amino acids were determined after acid hydrolysis (HCl 6N, 110 °C, 22 hours) of the proteins and complete elimination of HCl, by vacuum evaporation at 50 °C, in a Dionex Dx-300 autoanalyzer. Separation was done in a cation exchange column and quantification was accomplished by post-column ninhydrin reaction with reference to a Pierce standard amino acid mixture. Tryptophan was determined by the colorimetric method of Spies (1967) based on a stand curve of pure tryptophan (Sigma, St Louis, MO, USA) and reading at 590 nm, in a Hitachi U-2000 spectrophotometer.

### 2.6 Functional properties

#### *Protein solubility*

Solubility of the proteins fractions was determined by the method of Morr et al. (1985), under the following conditions: 1% (w/v) sample in water, at pH 3.0; 4.0; 4.5; 5.0; 5.5; 6.0; and 7.0 (25 °C). The amount of soluble protein (%N x 6.25) was determined by the semimicro-Kjeldahl procedure (AOAC, 1990) and expressed as a percentage of the total protein in the sample.

#### *Emulsifying capacity*

Emulsifying capacity (EC) was determined according to De Kanterewicz et al. (1987) at pH 3.0; 5.5 and 7.0 in water or in the presence of 0.15 M NaCl. To approximately 10 mL of 1% (w/v) sample solution, soybean oil was added from a buret. The mixture was agitated continuously in an Ultra-Turrax T-25 homogeneizer (IKA®-Labortechnik, Staufen, Germany) at 9000 rpm while maintained in an ice-bath. Saturation (emulsion breaking) was determined visually by the sudden change in consistency of the emulsion. The EC was calculated by the maximum amount of soybean oil incorporated per gram of protein in solution. The EC of the blood protein fractions was compared with whey protein concentrate (WPC), as a reference.

#### *Emulsion stability*

Emulsion Stability (ES) was tested at pH 5.5. The emulsification system consisted of 25 mL solution (1 mg prot.mL<sup>-1</sup>) plus 25 mL soybean oil homogeneized in an Ultra-Turrax T-25 at 9000 g for 3 minutes. The water content was determined in 5 mL of emulsion withdrawn from the bottom of the emulsion

container immediately after emulsification and after having been allowed to stand for 24 hours. Emulsion stability is expressed by:  $ES(\%) = 100 - U^{24h}/100 - U^{original}$ , where U is the determined humidity (ACTON; SAFFLE, 1970).

#### Emulsifying stability index

The procedure was based on Pearce and Kinsella (1978) with modifications. The emulsion was formed by the homogenization of 30 mL solution (10 mg sample/mL) plus 10 mL soybean oil in a mixer (Ultra-Turrax T-25) for 1 minute at 9000 rpm. Immediately after the emulsion formation, aliquots of 1 mL were diluted (1:250) with 0.1% sodium dodecylsulphate (SDS) solution containing 0.1 M of sodium chloride plus 0.1 M of sodium phosphate at pH 7.0. After vigorous shaking, the absorbancies of the diluted emulsions were read at 500 nm in a Hitachi U-2000 spectrophotometer.

The Emulsion Stability Index (ESI) was calculated by:  $IES = T \cdot \Delta t / \Delta T$  where  $\Delta T$  is the change in turbidity, T, occurring during the time interval  $\Delta t$ . The turbidity was calculated by  $T = 2.303 A DF/l$ , where A is the absorbance, DF, the diluting factor (=250) and l is the optical path (0.01 m).

#### Foaming capacity

Foaming Capacity (FC) (volume expansion) was determined by the method described by Mohanty, Mulvihill and Fox (1988) using 1, 3 and 5% sample dispersion in deionized water, at pH 3.0; 5.5; and 7.0 and compared to commercial egg albumin (SOHOVOS). Final volume (50 mL) were agitated in a homogenizer (Walita mod. RI-3164) at 5000 rpm for 5 minutes. Foam volume (FV) was carefully measured and transferred to a 500 mL graduate cylinder and the foam volume (%) was determined by the expression:  $FV(\%) = (\text{initial foam volume (mL)}/\text{initial volume of solution}) \times 100$ .

#### Foam stability

Foam stability (FS) was determined by the method of Patel, Stripp and Fry (1988) and the kinetics of foam destabilization was followed by a procedure proposed by Elizalde et al. (1991) based on the percentage decrease of foam volume or in the rate of drained liquid as a function of time, respectively. The pH used was 5.5, which presented the highest foam expansion.

## 2.7 Statistical analysis

Statistical analysis was applied in all experiments and the results were submitted to analysis of variance for statistical differences ( $p < 0.05$ ). Differences among means were determined by the Duncan's test. The Statistical Software 5.0 was used.

## 3 Results and discussion

Salting out with ammonium sulphate seemed to be a good alternative method for recovering above 90% of the serum proteins with a yield of 42 and 56%, respectively, as albumins and globulins representing a 77% recovery of the total serum albumin and 89% of the globulins. Main recovery of globulin (BSG) was at 50% saturation and of BSA at 80%. Other methods used for recovering the animal blood proteins resulted in a maximum overall protein recovery of 62% using an aqueous two-phase process (RITO-PALOMARES; DALE; LYDDIATT, 2000) and 63% using alcoholic precipitation from human plasma (COHN et al., 1950). Tanaka (1987) recovered 76.6% of serum albumin by associating the method 6 of Cohn et al. (1950) and SCHNEIDER, WOLTER, and McCARTY (1976).

JIANG, HE and FOUNTOULAKIS (2004) compared some protein precipitation methods for human plasma. They found that precipitation with TCA and acetone and ultrafiltration resulted in an efficient sample concentration and desalting for protein analysis. Ammonium sulfate fractionation can efficiently remove albumin, which represents more than 50% of the plasma proteins.

The partial chemical composition of BSA and BSG, the two major blood serum protein fractions obtained in the present study and dehydrated by spray drying and by freeze-drying, is presented in Table 1.

Looking at the composition of the two protein fractions it becomes apparent that there are no substantial differences in the protein contents of BSA and BSG, regardless of the dehydration method applied. In general, BSA showed higher contents of ash, moisture, and total lipids compared with BSG dehydrated by the two different methods. Total lipids were considerably higher in BSA compared with BSG. The higher participation of undetermined substances in BSG is likely to represent a higher content of carbohydrate associated with this fraction. On the other hand, besides the free fatty acids binding property of BSA, one can not exclude the possibility of a co-precipitation with

**Table 1.** Partial chemical composition of Bovine Serum Albumin (BSA) and the globulin (BSG) fractions dehydrated by spray drying or freeze drying.

Component (g.kg <sup>-1</sup> )	BSA		BSG	
	Spray-dried	Freeze-dried	Spray-dried	Freeze-dried
Protein (N x 6.25)	833.5 ± 0.3 <sup>a</sup>	833.3 ± 0.3 <sup>a</sup>	843.0 ± 0.9 <sup>a</sup>	853.6 ± 0.4 <sup>a</sup>
Ash	6.4 ± 0.3 <sup>a</sup>	4.2 ± 0.1 <sup>b</sup>	3.9 ± 0.1 <sup>a</sup>	2.6 ± 0.5 <sup>b</sup>
Moisture	98.1 ± 1.1 <sup>a</sup>	74.6 ± 1.3 <sup>b</sup>	61.4 ± 0.6 <sup>a</sup>	63.6 ± 2.1 <sup>a</sup>
Total lipids	47.5 ± 4.0 <sup>b</sup>	54.2 ± 0.5 <sup>a</sup>	16.9 ± 0.4 <sup>b</sup>	17.0 ± 0.5 <sup>b</sup>
Undetermined (difference)	14.5	34.0	74.8	63.2

\*Results are means ± sdm of three replications,  $p < 0.05$ . Different small superscript letters (lines) indicate statistical difference between spray-dried and freeze-dried samples. Statistical comparisons are between dehydration processes, not between protein fractions.

BSA of lipids and other hydrophobic compounds interacting with the protein.

The amino acid profiles of freeze-dried and spray-dried BSA, and freeze-dried BSG are shown in Table 2. The amino acid composition of the two protein fractions is quite different. BSA showed higher contents of lysine, leucine, aspartic acid, glutamic acid, aromatic amino acids (phenylalanine plus tyrosine), and sulphur amino acids (methionine plus cysteine) than the BSG fraction. However BSG seems to be nutritionally more equilibrated than BSA, which is suggested by its higher essential amino acid score (EAAS). The most limiting essential amino acid in freeze-dried BSA was tryptophan (72.7%), FAO/WHO/UNU (1990) reference; while in BSG the most limiting amino acid was isoleucine (89.3%) of the FAO reference. Spray drying promoted a decrease in tryptophan concentration resulting in a drop of the BSA (EAAS) from 72.7 to 54.5%.

Some significant differences were observed compared with the essential amino acid pattern reported by Penteadó, Lajolo, and Santos (1979). In the present paper, values for lysine and total sulphur amino acids, for BSA, were higher than the values reported by the above cited authors. On the other hand, they reported for BSG a much higher content (159 g.kg<sup>-1</sup> protein) of total aromatic amino acids (phenylalanine plus tyrosine) compared to 87 g.kg<sup>-1</sup> protein reported in this paper (Table 2). Marquéz et al. (2005) also encountered lower values of lysine and methionine in bovine plasma and blood.

When submitted to polyacrylamide gel electrophoresis (SDS-PAGE), the BSG fraction showed three protein bands with molecular mass 30 kD (14.8%), 57 kD (37.6%), and 67 kD

(47.6%) of the total protein fraction. For BSA two protein bands were revealed with molecular mass (MM) 65 kD (95.3%) and 76 kD (4.6%) of total protein fraction. The number and % of the total mass suggested that the BSG fraction contained an important protein band (47.6% MM 67 kDa) very close in molecular mass to BSA.

The solubility of BSA in water (25 °C) practically did not change with pH as it occurred with the majority of the proteins. Solubility reached near 100% at pH 5.0 and 6.0 and 95% at pH 3.0 and 7.0. Ramos-Clamont et al. (2003) also found that the solubility of serum albumin from swine blood was little affected by pH reaching the maximum solubility of 90%. The difference in the maximum solubility of serum albumin from bovine and swine must be attributed to the different amino acid composition in the two species. The globulin fraction (BSG) solubility was more dependent on pH than BSA. Minimum solubility was about 60% at pH 5.5 coinciding with the mean isoelectric point of globulin fraction but increased to values around 80-85% at pH 3.0 and 7.0.

The emulsifying properties of the bovine serum protein fractions were evaluated in the present investigation by determining the emulsifying capacity and emulsion stability. Table 3 shows the emulsifying capacity (EC) of 1% (w/v) solution of freeze-dried BSA or BSG fraction in water or 0.15 M NaCl at pH 3.0, 5.5, and 7.0. BSA presented better EC than BSG under the conditions tested.

For BSA, EC in water decreased with the increase in pH from 3.0 to 7.0. This suggests that in acidic conditions BSA must exhibit a more adequate hydrophilic-hydrophobic balance,

**Table 2.** Comparative amino acid composition of BSA (freeze-dried or spray-dried) and of BSG (freeze-dried).

Amino acid (g.kg <sup>-1</sup> prot)	BSA		BSG	FAO <sup>1</sup>
	Freeze-dried	Spray-dried	Freeze-dried	
Lys	124	127	72	58
Thr	58	58	67	34
Val	59	58	65	35
Leu	117	120	75	66
Ile	25	26	25*	28
Trp	8*	6*	19	11
His	4.3	39	27	19
Cys	51	48	26	-
Met	10	8	10	-
Met + Cys	61	56	36	25
Tyr	51	50	45	-
Phe	67	69	42	-
Phe + Tyr	118	119	87	63
Asp	116	119	84	-
Ser	50	46	79	-
Glu	177	178	114	-
Pro	47	49	50	-
Gly	24	23	35	-
Ala	65	62	41	-
Arg	59	55	47	-
EAAS <sup>2</sup>	73 (Trp)	55 (Trp)	89 (Ile)	-

<sup>1</sup>FAO/WHO/UNU recommendations (children 2 to 5 years of age); \*most limiting essential amino acids; and <sup>2</sup>EAAS = essential amino acid score.

**Table 3.** Emulsifying capacity (1% w/v protein solution) of freeze-dried BSA and BSG, in water or 0.15 M NaCl solution at different pH (in mL oil.g<sup>-1</sup> sample).

pH	BSA		BSG	
	Water	0.15 M NaCl	Water	0.15 M NaCl
3.0	626.0 ± 24.7 <sup>aA</sup>	486.8 ± 7.3 <sup>aB</sup>	443.5 ± 20.1 <sup>bA</sup>	402.0 ± 1.5 <sup>bA</sup>
5.5	522.6 ± 23.5 <sup>bA</sup>	486.0 ± 12.2 <sup>aA</sup>	490.0 ± 1.7 <sup>bA</sup>	394.6 ± 9.0 <sup>bB</sup>
7.0	451.9 ± 7.3 <sup>cB</sup>	481.9 ± 21.0 <sup>aA</sup>	531.5 ± 30.9 <sup>aA</sup>	441.3 ± 6.8 <sup>aB</sup>

\*Results are means ± sdm of three replications; <sup>a,b</sup>different small superscript letters (columns) indicate statistical difference ( $p < 0.05$ ) for BSA or BSG as influenced by pH; and <sup>A,B</sup>different capital letters (lines) indicate statistical difference ( $p < 0.05$ ) as influenced by NaCl.

which favors the surface film formation and emulsification. Comparing the BSA behavior in water and in 0.15 M NaCl, EC decreased in NaCl at pH 3.0 and 5.5 and presented a tendency to increase at pH 7.0. Regarding BSG, EC was higher at pH 7.0 both in the absence or presence of NaCl. No difference on BSG emulsifying capacity was found at pH 3.0 in water and 0.15 M NaCl solution. However, at pH 5.5 and 7.0, the EC of BSG decreased, in 0.15 M NaCl. Both protein fractions showed good emulsifying property when compared to other protein systems such as milk and soybean proteins (data not shown) largely used for this purpose in food systems (ELIZALDE; BARTHOLOMAI; PILOSO, 1996).

The results of emulsion stability estimated by two different methods are shown in Table 4. The values presented refer to the Emulsion Stability (ES) or Index Of Emulsion Stability (IES) with 1% sample dispersion at pH 5.5 based on the finding that at this pH both protein fractions showed similar EC.

Acton and Saffle (1970) based their procedure on the emulsion creaming process by analyzing the quantity of water separated from the emulsion on standing for an established time period. The Index of Emulsion Stability (IES) measures the destabilization of the emulsion based on the principle of coalescence being calculated by the ratio of the emulsion turbidity difference after a certain period. A lower index value indicates lower emulsion stability. In stable emulsions, the interfacial area does not change with time, consequently their turbidity tend to remain constant. The time used for the calculation of the IES corresponded to the last two turbidimetric measurements because after some time the emulsions should have reached their stationary state and given lower variations between measurements.

Bovine Serum Globulin (BSG) presented the highest emulsion stability using both methods of evaluation when compared with BSA and Whey Protein Concentrate (WPC), which did not differ among themselves. WPC was used as reference since this protein concentrate is commonly used as emulsifier in food products.

Proteins with native compact structures such as BSA tend to produce thick and less stable films. On the other hand, more flexible and partially randomized protein structure tend to form thin and more flexible film with a higher capacity to accommodate at the fat droplets surface conferring higher stability to the emulsion (PHILLIPS; HAQUE; KINSELLA, 1987).

Proteins that form cohesive films covering the oil droplets promote a barrier capable of steric hindrance without significant

**Table 4.** Stability of emulsions prepared with 1% (w/v) aqueous solutions of BSA, BSG, and WPC measured by two different procedures.

Sample	Procedures	
	ES (%) <sup>*</sup>	IES (minutes) <sup>*</sup>
Bovine Serum Albumin (BSA)	60.4 ± 0.7 <sup>b</sup>	222 ± 24 <sup>b</sup>
Bovine Serum Globulin (BSG)	91.0 ± 3.7 <sup>a</sup>	320 ± 30 <sup>a</sup>
Whey Protein Concentrate (WPC)	62.5 ± 2.0 <sup>b</sup>	240 ± 12 <sup>b</sup>

\*Values are the results of three independent determinations ± sdm; different superscript letters (columns) indicate statistical difference ( $p < 0.05$ ); ES(%) = emulsion stability, Acton and Saffle (1970); and IES (minutes) = index of emulsion stability measured after 100 minutes, Pearce and Kinsella (1978).

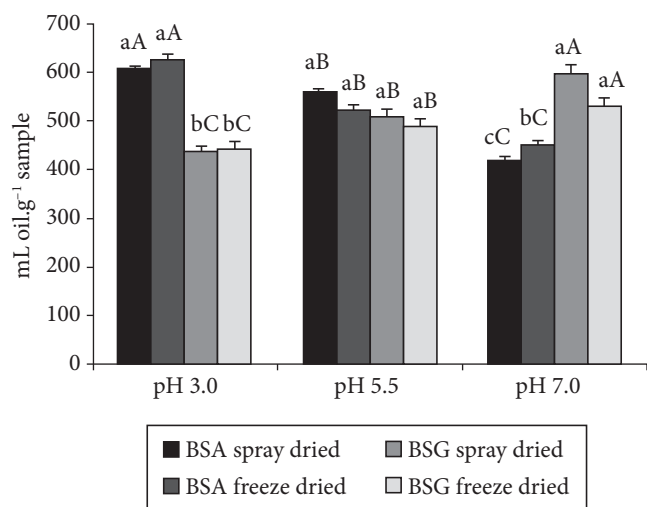
decrease in the surface tension, which seems to work better in terms of ES than certain surfactants that function primarily by reducing surface tension (FRIBERG, 1976).

In the two fractions, BSA and BSG, an increase in ionic strength promoted a decrease in emulsifying capacity. This fact may be attributed to a decrease in the electrostatic repulsion, which would promote aggregation (flocculation) of proteins in solution (TORNERG; EDIRIWEERA, 1988).

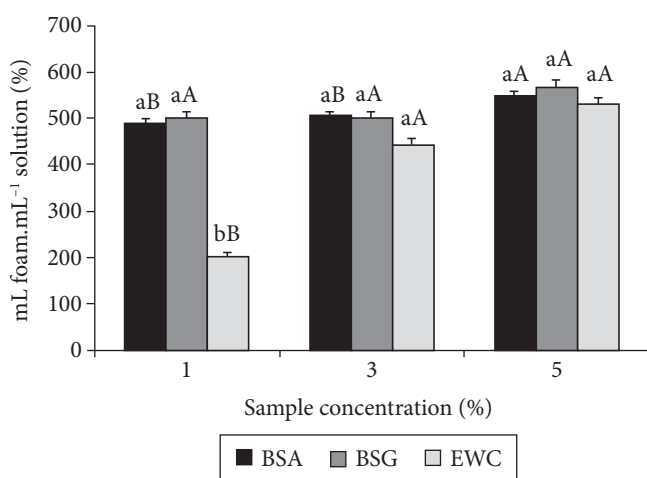
The effect of the drying process on the EC of BSA and BSG at the pH 3.0, 5.5, and 7.0 is shown in Figure 1. At pH 3.0, the EC was not affected by spray drying compared to freeze-drying. Nevertheless, independently of the drying method BSA showed a significantly higher EC than BSG ( $p < 0.05$ ) at pH 3.0. On the other hand, at pH 7.0 the emulsifying capacity was higher for BSG than for BSA, and in both samples EC was not affected by spray drying. At pH 5.5, both protein fractions behaved similarly independently of the drying process. Comparing pH 3.0 and 7.0, a decrease of EC of BSA was observed independently of the drying method. On the other hand, BSG showed an increase in the EC as the pH increased from 3.0 to 5.0, and to 7.0, independently of the drying method.

Comparing the effect of freeze-drying and spray drying on bovine blood plasma, Raeker and Johnson (1995) reported that the process did not alter the denaturation temperature of the samples by using Differential Scanning Calorimetry (DSC); however the spray dried sample showed evidence of some denaturation based on the enthalpy reduction. This may have occurred because plasma is composed of thermo labile proteins such as fibrinogen and more thermo stable proteins for example, BSA and alpha-globulin.

Figure 2 compares the effect of protein (sample) concentration on Foaming Capacity (FC) of BSA, BSG, and Egg White Concentrate (EWC) used as a reference sample. At 1%, sample concentration BSA and BSG showed higher foaming capacity



**Figure 1.** Effect of pH and drying method on the emulsifying capacity of BSA and BSG (1% sample). Different small superscript letters indicate statistical difference between samples at the same pH.; and the capital letters for the same sample at different pH ( $p < 0.05$ ).

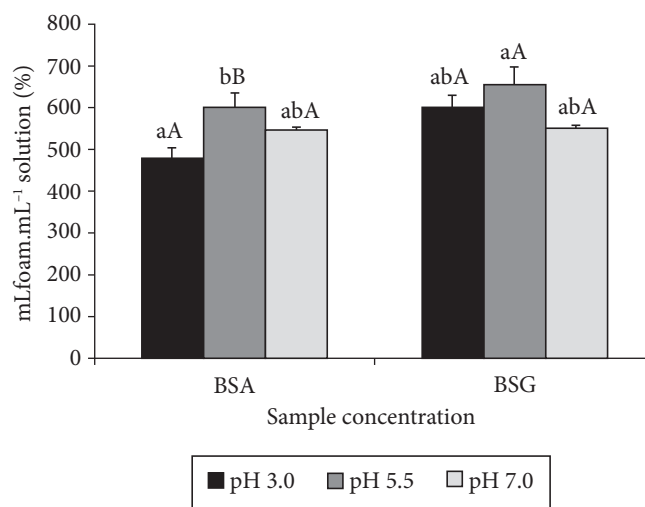


**Figure 2.** Foaming capacity of BSA, BSG fractions, and Egg White Concentrate (EWC) dehydrated by freeze drying. Different small superscript letters indicate statistical difference between different samples in the same concentration; and different capital letters, indicate statistical difference for the same sample at different concentrations ( $p < 0.05$ ).

than the EWC. At 3 and 5% sample concentration, this difference disappeared. The influence of protein concentration on FC was much stronger for EWC than for BSA and BSG.

Figure 3 shows the FC of freeze-dried BSA and BSG at various values of pH. BSA showed lower FC at pH 3.0, which was improved at the pH 5.5 and 7.0. Although no statistical difference could be demonstrated between FC at the last two values of pH, the highest absolute value of FC was found at pH 5.5 for BSA. Howell and Taylor (1995) observed an improvement of BSA foam expansion with low pH in the presence of ascorbic acid.

BSG showed no statistical difference in FC at the pH 3.0, 5.5, and 7.0. Again, the highest absolute value for BSG FC was



**Figure 3.** Foaming capacity of BSA and BSG freeze dried fractions at different values of pH. Different small superscript letters indicate statistical difference for the same sample at different pH; and capital letters, for different samples at the same pH ( $p < 0.05$ ).

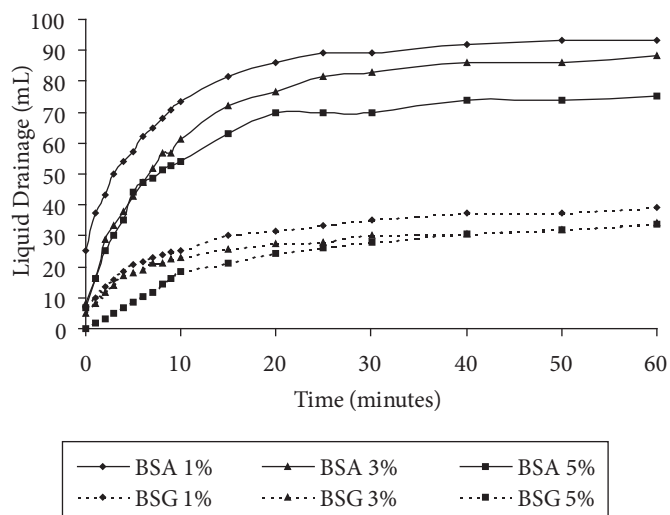
found at pH 5.5. The pH 5.5 is closer to the isoelectric point of these proteins, condition that tends to increase the foaming capacity for most proteins.

The kinetic for destabilization of freeze-dried BSA and BSG stabilized foams, based on liquid drainage as a function of time and protein concentration, is illustrated in Figure 4. Foam stability increased with sample concentration in both BSA and BSG. Nevertheless, regardless of the sample concentration BSG showed much higher foam stability (slower destabilization) than BSA. The drainage of liquid was much faster and in larger volume for the stabilized BSA foam than for the BSG foam. The quickest phase of destabilization lasted for 10 minutes in both protein fractions. BSA continued to release liquid faster than BSG, up to 30 minutes, when the rate of water drainage seemed to stabilize in both samples.

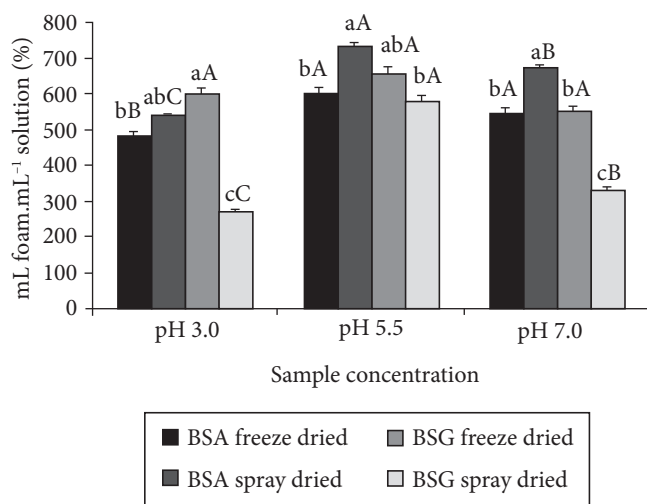
According to Raeker and Johnson (1995), the alpha globulin fraction may contain up to 40% carbohydrate which increases the interstitial lamella viscosity decreasing foam drainage.

The influence of pH and dehydration method on the FC of BSA, and BSG are shown in Figure 5. At pH 3.0, spray drying affected BSG more drastically than BSA. Similar effect was observed for BSG at pH 7.0. At pH 5.5, spray drying increased BSA FC ( $p < 0.05$ ) and did not alter FC of BSG ( $p > 0.05$ ). The data of Figure 5 seem to indicate that pH 5.5 favors FC for both BSA and BSG with some detrimental effects of the drying process on BSG. Spray drying affected negatively the FC of BSG at pH 3.0 and 7.0. It seems probable that under acidic (pH 3.0) and neutral (pH 7.0) conditions, BSG undergoes denaturation on spray drying beyond the ideal degree of denaturation for foam formation.

According to Graham and Phillips (1979), the most limiting factor in protein surfactant properties (foaming and emulsification) is not the continuous dispersant phase but rather the structural nature of the protein. In the studies cited above, the



**Figure 4.** Kinetic of liquid drainage from Bovine Serum Albumin (BSA) and Bovine Serum Globulin (BSG) stabilized foams produced at three different sample concentration, pH 5 at 25 °C.



**Figure 5.** Effect of the drying method and pH on the foaming capacity of BSA and BSG (1% sample) at different values of pH. Different small superscript letters indicate statistical difference between samples (same pH); and capital letters, for different pH (same sample) ( $p < 0.05$ ).

authors compared the interfacial behavior of structural different proteins such as  $\beta$ -casein, BSA, lysozyme, and fosvitin. The following order of adsorption and film formation at air-water interface was established at pH 7.0:  $\beta$ -casein > BSA > lysozyme > fosvitin. The most flexible and partially randomized structure of  $\beta$ -casein rapidly reached the air-water interface and acquired a new conformation resulting in rapid formation of an interfacial film (good EC). On the other hand, BSA and lysozyme, due to their globular and more organized structures, showed a lag phase before reaching the interface resulting in lower adsorption compared to  $\beta$ -casein. Lastly, fosvitin (a highly phosphorylated egg yolk protein) did not form an interfacial film at pH 7.0 due to electrostatic repulsion of the large number of negative charges

in the phosphate residues. Nevertheless, BSA and lysozyme formed a thicker and hydrodynamically more stable film than did  $\beta$ -casein. Therefore, the protein properties that facilitate film formation at interfaces are not necessarily the same properties that stabilize the film. Molecular flexibility helps film formation but does not guarantee film stability.

Phillips, Haque and Kinsella (1987) demonstrated that proteins with native highly ordered structure, as the case of BSA and other globular proteins are not capable of forming interfacial films highly cohesive, responsible for high emulsion stabilization.

The adsorption process of proteins at interfaces involves various stages. First, the free movement of the protein in the solution to reach the interface, the accommodation (relaxation) of the protein molecules on the interface, and the interactions between protein molecules to form a viscoelastic protein film with good hydrodynamic property. Depending on the interacting capacity of the protein molecules during film formation and stabilization, protein molecules can be released from the interface contributing to destabilization of the film (NORDE; GIACOMELLI, 2000).

Higher difficulties of structural adjustments and stabilization of the protein film at the air-water or oil-water interfaces may explain the lower stability of BSA emulsion and foam when compared with BSG, for the same properties.

According to Damodaran (1994), the basic requirement for a protein to be a good foaming agent are the ability to be rapidly adsorbed at the air-water interface during whipping or bubbling, undergoing a rapid conformational change and rearrangement at the interface, and forming a cohesive viscoelastic film via intermolecular interactions. These properties are related to high molecular flexibility, charge density, and hydrophobicity. It should be noticed that foam stability is highest at pH 4.5 to 5.5. This is to be expected because most protein-stabilized foam show maximum stability at or near the isoelectric pH (KIM; KINSELLA, 1985).

Partial heat denaturation of proteins often results in improvement of foaming properties (DE WIT; KLARENBECK; ADAMSE, 1986). This is attributed primarily to an increase in surface hydrophobicity and flexibility of denatured proteins.

#### 4 Conclusions

Based on Essential Amino Acids Composition (EAAS), the BSG fraction seems to be more nutritive than the bovine serum albumin fraction. BSA is limited in tryptophan and the globulins in isoleucine. The Emulsifying Capacity (EC), both in water and 0.15 M NaCl, was higher for BSA than for BSG, but the emulsion stability was higher for BSG. Acidic pH favored serum albumin while neutral pH favored globulin EC. The pH 5.5 favored foaming capacity both in freeze-dried and spray-dried samples. Spray drying at pH 3.0 and 7.0 affected foaming capacity of the BSG fraction. BSG stabilized foam retained significantly more liquid than BSA foam, and higher protein concentration increased stabilization in both samples.

Considering the properties such as solubility, relatively high heat stability, good emulsifying, and foaming properties, both BSA and BSG proved interesting ingredients to be used in emulsified and foamed food products.

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## References

- ACTON, J. C.; SAFLE, R. L. Stability of oil-water emulsion 1. Effects of surface tension, level of oil, viscosity and type of meat protein. **Journal of Food Science**, v. 35, n. 9, p. 852-855, 1970.
- AL-MALAH, K. I.; AZZAM, M. O. J.; OMARI, R. M. Emulsifying properties of BSA in different vegetable oil emulsions using conductivity technique. **Food Hydrocolloids**, v. 14, n. 5, p. 485-490, 2000.
- AOAC – ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis** 15 ed. Washington DC, 1990. v. 1-2.
- BLIGHT, E. C.; DYER, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, v. 37, n. 8, p. 911-917, 1959.
- COHN, E. J. et al. System for the separation of the components of human blood: quantitative procedures for the separation of the protein components of human plasma. **Journal of the American Chemical Society**, v. 72, n. 9, p. 465-474, 1950.
- DAMODARAN, S. Structure-function relationship of food proteins. In: HETTIARACHCHY, N. S.; ZIEGLER, G. R. (Eds.) **Protein Functionality in Food Systems**. New York: Marcell Dekker Inc., 1994. p. 1-37.
- DE KANTEREWICZ, R. J. et al. Water-oil absorption index (WOAI): a simple method for predicting the emulsifying capacity of food proteins. **Journal of Food Science**, v. 52, n. 5, p. 1381-1383, 1987.
- DE WIT, J. N.; KLARENBECK, J.; ADAMSE, E. M. Evaluation of functional properties of whey protein concentrates and whey protein isolates. 2. Effects of processing history and composition. **Netherlands Milk and Dairy Journal**, v. 40, p. 41-48, 1986.
- DUARTE, R. T.; SIMÕES, M. C. C.; SGARBIERI, V. C. Bovine blood components: fractionation, composition and nutritive value. **Journal of Agricultural and Food Chemistry**, v. 47, n. 1, p. 231-236, 1999.
- ELIZALDE, B. E.; BARTHOLOMAI, G. B.; PILOSO, A. M. R. The effect of pH on the relationship between hydrophilic/lipophilic characteristics and emulsification properties of soy proteins. **Food Science and Technology-Lebensmittel-Wissenschaft & Technologie**, v. 29, n. 4, p. 334-339, 1996.
- ELIZALDE, B. E. et al. Kinetics of liquid drainage from protein-stabilized foams. **Journal of Food Science**, v. 56, n. 1, p. 24-26, 1991.
- ENGLAND, S.; SEIFTER, S. Precipitation techniques. **Methods in Enzymology**, v. 182, 1990.
- FAO/WHO/UNU. FOOD AND AGRICULTURE ORGANIZATION/ WORLD HEALTH ORGANIZATION. **Report of a Joint FAO/WHO Expert Consultation on Protein Quality Evaluation**. Bethesda, Maryland, 1990.
- FRIBERG, S. E. Emulsion Stability. In **Food Emulsions**. Friberg, S. E.; Larsson, K. (Eds.). 3 ed. Nova York: [s.n.], 1976. 582 p.
- GÓMEZ-JUÁREZ, C. et al. Protein recovery from slaughterhouse wastes. **Bioresource Technology**, v. 70, n. 2, p. 129-133, 1999.
- GRAHAM, D. E.; PHILLIPS, M. C. Proteins at liquid interfaces. I. Kinetics of adsorption and surface denaturation. **Journal of Colloid Interface Science**, v. 70, n. 2, p. 403-414, 1979.
- HOWELL, N. K.; TAYLOR, C. Effect of ascorbic acid on the foaming and gelling of globular proteins. **International Journal of Food Science and Technology**, v. 30, n. 3, p. 321-334, 1995.
- IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Produção Animal**, n. 4, 4º Trimestre, 2006. Disponível em: <[http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/producaoagropecuaria/abate\\_leite\\_couro\\_ovos\\_200604.pdf](http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/producaoagropecuaria/abate_leite_couro_ovos_200604.pdf)>. Acesso em: 19 junho 2007.
- JIANG, L.; HE, L.; FOUNTOULAKIS, M. Comparison of protein precipitation methods for sample preparation prior to proteomic analysis. **Journal of Chromatography A**, v. 1023, n. 2, p. 317-320, 2004.
- KIM, S. H.; KINSELLA, J. E. Surface activity of food proteins: relationship between surface pressure development, viscoelasticity of interfacial films and foam stability of bovine serum albumin. **Journal of Food Science**, v. 50, n. 6, p. 1526-1530, 1985.
- KUBOTA, N.; TATSUMOTO, N.; SANO, T. Recovery of serum proteins using cellulosic affinity membranes modified with tannic acid. **Carbohydrate Polymers**, v. 40, n. 2, p. 107-113, 1999.
- LAEMMLI, N. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T<sub>4</sub>. **Nature**, v. 227, p. 680-685, 1970.
- MÁRQUEZ, E. et al. Proteins, isoleucine, lysine and methionine content of bovine, porcine and poultry blood and their fractions. **Food Chemistry**, v. 93, n. 3, p. 503-505, 2005.
- MOHANTY, B.; MULVIHILL, D. M.; FOX, P. F. Emulsifying properties of acidic caseins and sodium caseinate. **Food Chemistry**, v. 28, n. 1, p. 17-30, 1988.
- MORR, C. V. et al. A collaborative study to develop a standardized food protein solubility procedure. **Journal of Food Science**, v. 50, n. 6, p. 1715-1718, 1985.
- MOURE, F.; RENDUELES, M.; DIAZ, M. Coupling process for plasma protein fractionation using ethanol precipitation and ion exchange chromatography. **Meat Science**, v. 64, n. 4, p. 391-398, 2003.
- NORDE, W.; GIACOMELLI, C. E. BSA structural changes during homomolecular exchange between the adsorbed and the dissolved states. **Journal of Biotechnology**, n. 79, n. 1, p. 259-268, 2000.
- PATEL, P. D.; STRIPP, A. M.; FRY, J. C. Whipping test for the determination of foaming capacity of protein: a collaborative study. **International Journal of Food Science and Technology**, v. 23, n. 1, p. 57-63, 1988.
- PEARCE, K. N.; KINSELLA, J. E. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. **Journal of the Agricultural and Food Chemistry**, v. 26, n. 3, p. 716-723, 1978.
- PENTEADO, M. V. C.; LAJOLO, M. F.; SANTOS, N. P. Functional and Nutritional Properties of Isolated Bovine Blood Proteins. **Journal of the Science of Food and Agriculture**, v. 30, n. 8, p. 809-815, 1979.
- PHILLIPS, L. G.; HAQUE, Z.; KINSELLA, J. E. A method for the measurement of foam formation and stability. **Journal of Food Science**, v. 52, n. 4, p. 1077, 1987.



- RAEKER, M. O.; JOHNSON, L. A. Thermal and functional properties of bovine blood plasma and egg white proteins. **Journal of Food Science**, v. 60, n. 4, p. 685-690, 1995.
- RAMOS-CLAMONT, G. et al. Functional properties of protein fractions isolated from porcine blood. **Journal of Food Science**, v. 68, n. 4, p. 1196-1200, 2003.
- RITO-PALOMARES, M.; DALE, C.; LYDDIATT, A. Generic application of an aqueous two-phase process for protein recovery from animal blood. **Process Biochemistry**, v. 35, n. 7, p. 665-673, 2000.
- SCHNEIDER, W.; WOLTER, D.; McCARTY, L. J. Alternatives for plasma fractionation. **Vox sanguinus**, v. 31, n. 2, p. 141-151, 1976.
- SPIES, J. R. Determination of tryptophan in proteins. **Analytical Chemistry**, v. 39, p. 1412-1415, 1967.
- TANAKA, K. **Fracionamento do plasma bovino para isolamento da albumina e sua liofilização**. São Paulo, 1987. Dissertação - (Mestrado em Tecnologia Bioquímica Farmacêutica), Faculdade de Ciências Farmacêuticas, USP.
- TORNBERG, E.; EDIRIWEERA, N. Factors that influence the coalescence stability of protein-stabilised emulsions, estimated from the proportion of oil extracted by hexane. **Journal of the Science Food and Agriculture**, v. 46, n. 1, p. 93-113, 1988.
- TYBOR, P. T.; DILL, C. W.; LANDMAN, W. A. Functional properties of protein isolated from bovine blood by a continuous pilot process. **Journal of Food Science**, v. 40, n. 1, p. 155-159, 1975.
- WALTER, T. et al. Effect of bovine-hemoglobin-fortified cookies on iron status of schoolchildren: a nationwide program in Chile. **American Journal Clinical Nutrition**, v. 57, p. 190-194, 1993.
- WISMER-PEDERSEN, J. Utilization of animal blood meat products. **Food Technology**, v. 33, n. 8, p. 76-80, 1979.
- YU, M.; DAMODARAN, S. Kinetics of protein foam destabilization: evaluation of a method using BSA. **Journal of Agricultural and Food Chemistry**, v. 39, p. 1555-1562, 1991.