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Food Chemistry 88 (2004) 461–468
Food Chemistry
www.elsevier.com/locate/foodchem

Functional properties of a spray-dried porcine red blood cell fraction treated by high hydrostatic pressure

M. Toldrà *, A. Elias, D. Parés, E. Saguer, C. Carretero

Institut de Tecnologia Agroalimentària (INTEA), Escola Politècnica Superior, Universitat de Girona, Avda. Lluís Santaló s/n, 17071 Girona, Spain

Received 16 October 2003; received in revised form 12 January 2004; accepted 12 January 2004

Abstract

The aims of this study were to evaluate the functional properties of a spray-dried porcine red blood cell fraction (RBC), and the effects of the prior application of high hydrostatic pressure (HHP) on RBC protein functionality (solubility, foaming capacity, foam stability, water-holding capacity and texture properties of heat-induced gels) as well as on its microbiological quality and colour. The application of HHP (400 MPa, 15 min, 20 °C) and later dehydration allowed a dried product to be obtained with a reduction in the mesophilic bacterial counts of 3.2 logarithmic units. The colours of the pressurized and non-pressurized spray-dried RBC were the same, which indicated that both samples presented the same susceptibility to the hem group oxidation provoked by dehydration. The application of HHP increased the denaturant effects of spray-drying on hemoglobin (Hb), especially at pH 7 (isoelectric point; p*I*) since, after both processes, a decrease in protein solubility at neutral pH was observed. Spray-dried RBC had a maximum foaming capacity at the p*I* of Hb. The application of HHP decreased the foaming capacity but did not negatively affect the foam stability. Thermal treatment of RBC solutions led to hard and consistent gels at pH 7 whereas, at acid pH, less consistent, more adhesive and more elastic pastes were formed. The latter had higher water-holding capacities than gels at pH 7, in which, the water was retained by capillarity. Neither springiness and adhesiveness nor water-holding capacities of heat-induced gels or pastes were affected by the application of the HHP treatment to the fresh RBC.
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Keywords: Red blood cell fraction; Hemoglobin; High hydrostatic pressure; Spray-drying; Functional properties; Colour; Microbial counts

1. Introduction

The red blood cell fraction (RBC) constitutes 40% of porcine blood and hemoglobin (Hb) represents 90% of the protein content of this fraction (approximately 35%). The high iron and protein content, as well as its good functional properties, make RBC interesting as a food ingredient, provided that the problems of the dark colour and blood flavour, which appear when it is added to food products, are solved. Hb has good solubility, foaming, emulsifying and swelling properties (Nakamura, Hayakawa, Yasuda, & Sato, 1984; Ranken, 1980; Tybor & Dill, 1975; Wismer-Pedersen, 1988). This fraction is also an important source of iron and it is better absorbed than ferrous salts commonly used in food supplies. Moreover, RBC could also be used as a natural colorant in several food products by taking advantage of the colorant properties of the Hb or hem group.

Although closed collection systems have been developed in order to obtain hygienic blood, porcine blood is still collected by means of open systems in many industrial abattoirs. Blood is frequently contaminated by contact with microorganisms present on the skin or in the stomach and the intestinal contents of the slaughtered animal, and by air-borne bacteria. Hygienic precautions, such as disinfecting the animal skin and the knife used for slaughter, mixing the blood flow with an anticoagulant solution, the immediate cooling of the collected blood and storing at 5–7 °C, must be taken during collection to prevent spoilage organisms or even pathogens from growing. Despite this, previous studies undertaken in our laboratory (Toldrà, 2002) showed that raw RBC from porcine blood had quite a high level of microbiological contamination. After hemolysis treatment, including ultrasonication to disrupt erythrocytes

* Corresponding author. Tel.: +34-72-418941; fax: +34-72-418399.
E-mail address: mtoldra@intea.udg.es (M. Toldrà).

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doi:10.1016/j.foodchem.2004.01.060

and subsequent centrifugation at 20,900*g* for 30 min to obtain the Hb solution, the product obtained in that study still showed microbiological counts of around 10^6 cfu ml^{-1}. Spray-drying decreased the total counts of hemolyzed RBC by one logarithmic unit, but the dried product still reflected the high contamination of the raw material. Consequently, the microbial quality of this by-product needs to be improved before it is used as a food ingredient for human consumption.

Due to the fact that RBC and its protein are highly modified or damaged by heat treatments, the use of a non-thermal preservation method is required in order to reduce microbiological counts and to maintain the nutritional quality and the organoleptic and functional properties of these products. High hydrostatic pressure (HHP) treatment gives microbiological stability whilst producing fewer negative effects than classical thermal treatments used in the food industry. Preservation by HHP is based on several effects on biological systems, such as cell membrane permeability and functionality, cellular morphology, biochemical reactions and the genetic mechanisms of microorganisms (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989). Since covalent bonds are not affected by pressure, the nutritive and organoleptic qualities of products can be maintained intact. However, HHP acts on non-covalent interactions (hydrogen bonds and hydrophobic and electrostatic interactions) that stabilize the structure of biomolecules and may induce changes in proteins, such as denaturation, aggregation and gel formation (Van Camp, 1996). Protein denaturation, produced by HHP, may be different from the denaturation caused by high temperatures due to the fact that the interactions which stabilize the protein structure behave in a different way when they are submitted to pressure than when submitted to thermal treatments (Okamoto, Kawamura, & Hayashi, 1990; Morild, 1981; in: Defaye, Ledward, MacDougall, & Tester, 1995).

Our group has reported several experiences of the effects of HHP processing on the microbial contamination and protein functionality of porcine blood plasma (Parés, Saguer, Toldrà, & Carretero, 2000, 2001). HHP treatments at 450 MPa, for 15 min and operating temperatures above 25 °C were effective for improving the microbiological quality of porcine blood plasma. Pressurization at 40 °C was highly effective in reducing the microbial counts of plasma. Although HHP treatments at 40 °C resulted in greater protein denaturation than at 5 °C, the functional properties of heat-induced gels from plasma proteins were not negatively affected by HHP treatment.

The best HHP processing conditions of the RBC had already been determined in a previous study by observing the effects of different pressure, temperature and time combinations, on the microbiological quality as well as some functional characteristics, such as protein solubility, viscosity and colour. We concluded that the most adequate pressurization conditions were 400 MPa at 20 °C for 15 min. This treatment produced a significant improvement in the microbiological quality, did not adversely affect the colour or the protein solubility, and the RBC remained fluid after the treatment (Toldrà, Busquets, Saguer, Parés, & Carretero, 2002).

Since spray-drying is the most common technology employed to preserve blood and its fractions, this study focussed on the determination of functional properties, including solubility, foaming capacity and stability, water-holding capacity (WHC) and texture properties of heat-induced gels, of the pressurized and spray-dried RBC at two different pH values (7 and 4.5). The effects of HHP treatment on the microbiological quality and the colour of RBC powder were also evaluated.

2. Materials & methods

2.1. RBC preparation

Hygienically collected and refrigerated porcine blood from an industrial abattoir was used. Blood contained sodium citrate solution (0.4% w/v) as a anticoagulant. This study was carried out on samples of blood collected on different days but under the same conditions.

RBC was separated by centrifuging the blood for 15 min at 2530*g* at 5–10 °C (Sorvall RC-5C Plus, Dupont Co., Newton, Connecticut, USA) and discarding the supernatant fraction (plasma) by decanting. RBC was hemolyzed by using an ultrasonic probe LabSonic U (B. Braun-Biotech, S.A., Melsungen, Germany) to obtain Hb isolate. Aliquots (300 ml) of erythrocyte fraction were treated three times at 75 W for periods of 2 min, with 1 min intervals between treatments. Samples were kept in an ice bath to prevent the temperature being raised. Hemolyzed RBC was centrifuged for 30 min at 20,900*g* at 10–15 °C in order to remove erythrocytic stroma.

An aliquot of about 500 ml was kept refrigerated to be used as a non-pressurized control, and 500 ml were pressurized in order to reduce microbial contamination. Samples were pressurized and spray-dried within 24 h of the blood collection.

2.2. HHP treatment

High hydrostatic pressure experiments were performed on a batch isostatic press (Alstom, Nantes, France) with a 2-l capacity pressure chamber. Previously sterilized flexible low density PE bottles (Bibby Sterilin Ltd., UK) were filled with 500 ml of hemolyzed RBC without head space and vacuum-sealed in flexible PA/PE bags (Cryovac Europe, Cryovac Packaging Spain S.L., Barcelona, Spain). HHP treatments were carried out at 400 MPa at 20 °C for 15 min, following the method described in Toldrà et al. (2002). Pressure was reached in

1–2 min and decompression at the end of the cycle was achieved in 30–40 s. The maximum temperature reached, during treatments, was 2 °C above the programmed processing temperature. An oil/water emulsion was used as a fluid medium to apply pressure around the bottle inside the pressure vessel. After pressurization, samples were cooled and kept at 5 °C before spray-drying.

2.3. Spray-drying treatment

Pressurized and control RBC samples were spray-dried using a Büchi Mini Spray-Dryer B-191 (Büchi Labortechnik AG, Flawil, Switzerland). The process conditions used were as follows: 140 °C air inlet temperature, 77 °C air outlet temperature, 670 $ml\,h^{-1}$ feed flow of the product, 60 $l\,h^{-1}$ aspirator flow rate, and 5 bar spray air flow pressure. The proximate composition (% w/w) of spray-dried RBC was $5.3 \pm 0.8\%$ water, $94.6 \pm 0.9\%$ protein, $3.0 \pm 0.3\%$ mineral salts and $0.7 \pm 0.2\%$ fat.

2.4. Microbiological analysis

The sanitizing efficiency of HHP treatment on RBC and the effects of spray-drying were determined as the percentage-reduction observed in the total colony counts on Agar plates before and after processing.

Pour plate methods, with Blood Agar Base Oxoid CM 87 (Oxoid Ltd., Basingstoke, England) as a culture medium, were used for the total aerobic mesophile colony counts in non-treated and pressurized RBC before and after spray-drying. Plates were incubated at 37 °C for 24 h. Plate counts from powdered samples were related to the same dry matter, corresponding to the original liquid RBC.

2.5. Colour parameters measurement

The colour parameters CIE L^* (lightness), a^* (redness to greenness), and b^* (yellowness to blueness) of pressurized and non-treated RBC powder were determined using a Minolta CR-300 chroma meter with a CR-A33f glass light-projection tube (Minolta Co. Ltd., Osaka, Japan) calibrated using a reference white ceramic plate. Measurements were made in relation to standard illuminant, D_{65}, at room temperature. Each measurement was performed in triplicate.

2.6. Protein solubility

The solubilities at acid (4.5) and neutral (7) pH of RBC proteins were determined by the method described by Morr et al. (1985). One percent (w/v) solutions of spray-dried RBC were prepared in distilled water, and adjusted to pH 4.5 and 7 with 0.1 N HCl. Solutions were stirred for 1 h on a magnetic stirring plate, avoiding vortex formation. After stirring, aliquots of the solutions were centrifuged at 20,000*g* for 30 min at 15–20 °C. Protein solubility was calculated as the percentage of soluble protein in the supernatant relative to the total protein content in the sample (Kjeldahl procedure; AOAC, 1995). Each determination was done in duplicate.

2.7. Foaming capacity and foam stability measurement

One gramme samples of powdered RBC were dissolved in 200 ml of distilled water (0.5% solution), adjusted to pH 4.5 and 7, and then transferred to a 1000-ml volummetric flask. Solutions were whipped in a Braun Multimix M700 mixer (Braun Española S.A., Barcelona, Spain) with two whisks at maximum speed for 10 min. The flask was placed on a rotational plate during mixing in order to form a homogeneous foam. The foam capacity was determined as the volume (ml) of foam formed after a 1 min rest. The foam stabilities at pH 4.5 and 7 were determined using a gravimetric method. An exactly known quantity of the foam was placed in a dry stainless steel sieve, the dripped liquid was weighed at 15, 30, 45, 60, 90 and 120 min and the foam percentage that remained stable was calculated. Measurements were made in triplicate for each treatment assayed.

2.8. Preparation of the heat-induced gels

Solutions of 17% dry matter of powdered RBC (pressurized and non-treated) in distilled water were prepared, and the pH was adjusted to 4.5 and 7. Solutions were introduced into plastic bags (WP 2350/84, Proveedora Hispano Holandesa S.A., Barcelona, Spain) of 25 mm diameter and 15–20 mm length, and heated in an 80 °C water bath for 30 min. After heating, the resulting gels were cooled in cold water to room temperature (20 °C) and kept overnight at 5 °C prior to the analysis of texture and water-holding capacity.

2.9. Texture analysis

Texture profile analysis (TPA) tests were performed with a TA XT2 texturometer (Stable Microsystems Ltd., Surrey, UK), using a flat cylindrical aluminium probe of 50-mm diameter. Measurements were carried out on three cylindrical samples (25 mm diameter and 15 mm thickness) of RBC gels for each combination (HHP and pH) of treatments assayed. The samples were compressed twice until a 30% strain was attained at a rate of uniaxial compression of 1 $mm\,s^{-1}$. The parameters hardness, adhesiveness and springiness were recorded from the TPA analysis of each individual gel cylinder. The hardness value is the peak force of the first compression of the product and is expressed in Newtons, the

adhesiveness (in $N*s$) is the negative force area, after first compression, that represents the force required to separate the probe from the sample, and the springiness (adimensional) is how well a product physically springs back after it has been deformed during the first compression (Bourne, 2002), and was calculated as the ratio of the height of the sample at the beginning of the second compression cycle to its original height.

2.10. Water-holding capacity analysis

Water-holding capacity of RBC gels was determined using a technique combining centrifugation and filtration, developed by Kocher and Foegeding (1993) with slight modifications proposed by Parés, Saguer, and Carretero (1998a). Cylinders 8-mm diameter and 10-mm length were obtained from RBC gels and introduced into 20×50-mm filtering bags made from Goretex® filter membrane (pore diameter of 0.45 μm) (W.L. Gore y Asociados S.L., Barcelona, Spain) which allowed water to be released without soaking. The bags containing the gel samples were placed in 50 ml plastic centrifuge tubes, suspended at 20 mm from the bottom, and centrifuged at 4000*g* for 10 min at 15–20 °C (Sorvall RC-5C Plus, Dupont Co., Newton, Connecticut, USA). After centrifugation, the weight of released water from gel cylinders was measured. WHC was calculated as the percentage (w/w) of water released with respect to the weight of the sample prior to centrifugation. The determination was carried out in triplicate for each treatment.

2.11. Statistical analyses

This experiment was designed as a completely randomized block with five samples of RBC treated on different days (five replications). Data were analyzed using the SAS® statistical package (SAS® Institute Inc., 1990). Analyses of variance (ANOVA) were applied to investigate the effects of different variables (HHP treatment, pH, and sample) and their corresponding interactions. The Tukey–Kramer HSD test was used to compare means if the interactions were not significant. The significance level for all tests was $P = 95\%$. Data that presented $P < 0.05$ after the Barlett test were transformed: the arcsine transformation (arcsine of square root of [%variable/100]) was applied to protein solubility, WHC, and stable foam percentage whereas microbial counts were transformed into logarithms.

3. Results and discussion

3.1. Contaminating microbiota

Fig. 1 shows the microbial counts of control and pressurized RBC before and after dehydration. The

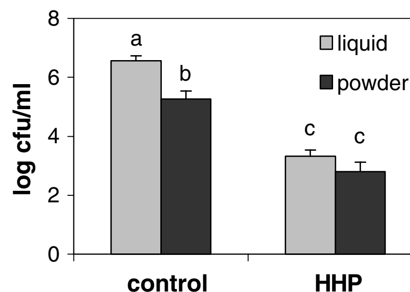

Fig. 1. Microbiological counts from untreated (control) and pressurized red blood cell fraction (RBC) samples before and after spray-drying. Error bars show mean confidence intervals ($P = 95\%$). Different letters indicate significant differences ($P < 0.05$) between treatments.

application of HHP to the liquid RBC and the subsequent spray-drying produced a significant decrease ($P < 0.05$) in the counts of aerobic mesophiles. HHP produced a reduction in the microbial counts of 3.2 logarithmic units. As expected (Toldrà, 2002), spray-drying reduced the microbiological counts of untreated RBC by 1 logarithmic unit and the dehydrated product showed total counts of 10^5 cfu ml^{-1}, which was still too high for food use.

With regard to the pressurized RBC, the application of spray-drying produced a slight (0.5 logarithmic units) but not significant ($P > 0.05$) reduction in contaminating microbiota. This might be due to the fact that the HHP treatment changed the relative percentages of the groups composing the microbial community that can be found in contaminated blood. After pressurization, surviving bacteria were probably less susceptible to thermal damage, thus being more resistant to the reduction effect induced by spray-drying. Nevertheless, the pressurized and spray-dried RBC had final microbiological counts of 2.8 logarithmic units, which is 2.5 units lower than that from spray-dried control RBC.

3.2. Colour CIE $L^* a^* b^*$

The CIE $L^* a^* b^*$ colour parameters of spray-dried RBC (Table 1) reflected the dark red–brown colour due to the oxidation of the heminic iron of the Hb (Fe^{2+}) to metHb (Fe^{3+}) during the spray-drying process (Toldrà, Saguer, Felip, Parés, & Carretero, 2000). Although previously pressurized powdered RBC samples had CIE $L^* a^* b^*$ values that were slightly higher than those of powder from untreated RBC, the influence of HHP treatment was not shown to be significant ($P > 0.05$).

It has been reported that the application of HHP induced changes in myoglobin (Mb) and meat discoloration (Cheah & Ledward, 1997; Cheftel, 1992). Carlez, Veciana-Nogues, and Cheftel (1995) studied the changes produced by HP (from 200 to 350 MPa) on the colour of minced beef and observed an increase in

Table 1
CIE L^* a^* b^* colour values of spray-dried RBC powder obtained from untreated (control) and pressurized red blood cell fraction (RBC)[A]

Colour parameters	Spray-dried pressurized RBC	Spray-dried untreated RBC
L^* (lightness)	38.98 ± 1.51 a	38.60 ± 1.43 a
a^* (redness–greenness)	+21.02 ± 1.48 a	+20.36 ± 1.32 a
b^* (yellowness–blueness)	+14.39 ± 0.75 a	+14.22 ± 0.60 a

Samples with the same letter within the same row are not significantly different ($P > 0.05$).
[A] Means ± confidence intervals ($P = 95\%$, $n = 5$).

the lightness (L^*), which they attributed to the denaturation of the Mb and/or to the displacement or release of the heme group. They also described a loss of redness (decrease of a^*), especially above 400–500 MPa, caused by the partial oxidation of Mb, while the b^* value remained constant. This latter effect does not agree with the results reported by our group in a previous study of liquid RBC (Toldrà et al., 2002) where pressurization produced an increase in both lightness (L^*) and redness ($+a^*$) values.

From the results of the present study, it can be concluded that the oxidative effect on heminic iron induced by spray-drying was dominant, and pressurized samples were as susceptible as control samples to the oxidative darkening process observed in the spray-drying of RBC.

3.3. Protein solubility

Previous studies showed that spray-drying induced changes in the native structure of Hb and, subsequently, a certain degree of denaturation that had a negative effect on the protein solubility of RBC (Toldrà, 2002).

In this study, the protein solubility of spray-dried RBC was significantly affected ($P < 0.05$) by both pressurization and pH (Fig. 2). Solubility at neutral pH (7) was lower than that at acid pH (4.5) in both untreated and pressurized samples. HHP treatment always led to reduced solubility. HHP treatment had a denaturing effect on the Hb, resulting in a noticeable reduction in solubility at pH 7, close to the pI of the Hb (according to Stryer, 1995; 6.9 in oxyhemoglobin and 6.7 in desoxyhemoglobin). Although the decrease in solubility at neutral pH was higher than it was at pH 4.5 in pressurized RBC powder, as has previously been reported for liquid RBC, the interaction between treatment and pH was found not to be significant. In our previous study (Toldrà et al., 2002), HHP treatment produced a loss of Hb solubility of 13% in liquid RBC at pH 7, whereas the reduction was just 1.3% at pH 4.5.

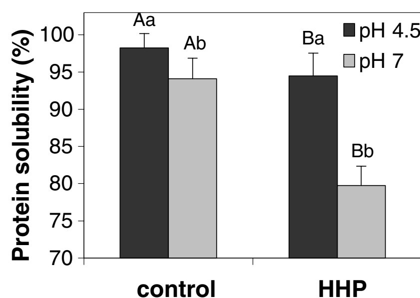

Fig. 2. Protein solubility at pH 4.5 and pH 7 from 1% spray-dried red blood cell fraction (RBC) solutions obtained from untreated (control) and pressurized RBC. Error bars show mean confidence intervals ($P = 95\%$). Different letters indicate significant differences ($P < 0.05$) between treatment (capital letters) and pH (small letters).

Defaye et al. (1995) also reported that pressurisation of bovine metmyoglobin close to the pI led to reduced solubility and the formation of precipitates and suggested that both hydrogen bonds and electrostatic interactions contributed to the formation of the precipitate. At acidic pH, the presence of electric charges in the partially unfolded Hb could avoid the aggregation of molecules and allow the solubility to be maintained. On the other hand, precipitation and loss of solubility are favoured due to the absence of electrical repulsive forces close to the pI.

In this study, both pressurization and spray-drying had denaturant effects on Hb and reduced the protein solubility of RBC under two pH conditions. This did not agree with the results found by Parés et al. (1998b) which showed that spray-drying did not appreciably affect the protein structure of porcine blood plasma, whereas HHP treatment seemed to have a denaturing effect. Control RBC powder had a solubility of 98% at pH 4.5 and 94% at pH 7, whereas the solubilities of pressurized samples were 94% and 80% at acid and neutral pH, respectively. Therefore, HHP might increase the susceptibility of Hb to the heat-induced denaturation that takes place during spray-drying.

3.4. Foam properties

The foaming capacity (FC) of spray-dried RBC from untreated and pressurized samples, depending on the pH, is presented in Fig. 3.

Differences in the volume of foam formed from 0.5% RBC solutions between samples of different pH were shown to be significant ($P < 0.05$). FC was higher at neutral pH as was expected since the FC of proteins usually tends to be maximum at pH values close to their pI (Zayas, 1997). However, Tybor and Dill (1975) observed that globin isolate from bovine hemoglobin showed the greatest foam expansion at pH values

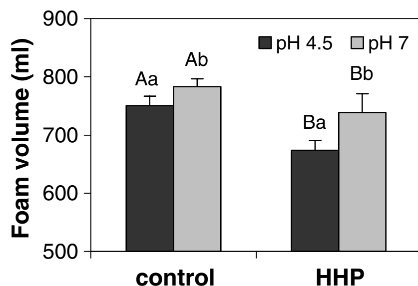

Fig. 3. Foaming capacity (volume of foam) at pH 4.5 and pH 7 of 0.5% spray-dried red blood cell fraction (RBC) solutions obtained from untreated (control) and pressurized RBC. Error bars show mean confidence intervals ($P = 95\%$). Different letters indicate significant differences ($P < 0.05$) between treatment (capital letters) and pH (small letters).

between 5 and 6 whereas, at pH 7.2, it was minimum, possibly due to a functionality modification resulting from the chemical treatment applied to Hb in order to remove the heme group.

Foaming capacity was also significantly affected by the HHP treatment ($P < 0.05$). The denaturant effect of HHP on Hb led to a lower FC of RBC powder at both neutral and acid pH. The foam volumes from pressurized RBC were 7% and 6% lower at pH 4.5 and pH 7, respectively, than that obtained from control RBC.

With regard to foam stability (FS), the evolution of foam for a 2-h period is shown in Fig. 4. The effects of HHP treatment and pH on the percentages of stable foam at 60, 90, and 120 min after whipping are presented in Table 2. Although foam volumes from all samples decreased progressively, analysis of FS data showed that more stable foams were formed at neutral pH than at acidic pH ($P < 0.05$), thus being more noticeable after 30 min of rest. Nevertheless, a percentage of the initial foam volume of RBC at pH 7, from 40% to 50%, in control and HHP samples, respectively, was still present after 2 h rest. The ANOVA test showed that the differences between FS in pressurized and untreated samples were not significant ($P > 0.05$).

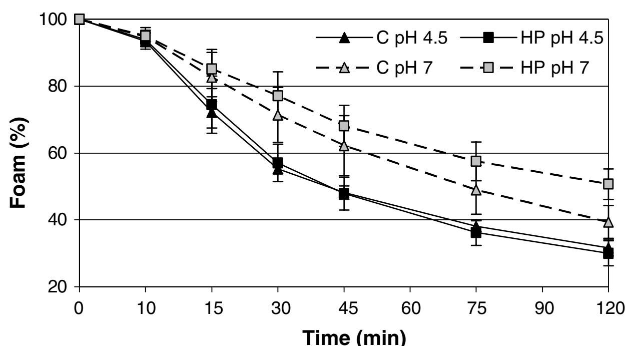

Fig. 4. Foam stability (percentage of foam) at pH 4.5 and pH 7 from 0.5% spray-dried red blood cell fraction (RBC) solutions obtained from untreated (C) and pressurized (HP) RBC. Error bars show mean confidence intervals ($P = 95\%$).

The global effect was that HHP reduced the FC whereas it did not affect the FS of spray-dried RBC. This result is completely different from the effects on blood plasma reported by Parés (1998), who found that a 450 MPa treatment for 15 min at 40 °C reduced the solubility but did not affect the FC and even improved the FS of plasma proteins.

3.5. Texture of heat-induced gels

Although Ranken (1980) reported that globin cannot form a gel but only a thick cream, Hayakawa, Ogawa, and Sato (1982) demonstrated that globin from decolorized bovine hemoglobin can produce a thermally-induced transparent gel. In our study, heat-induced changes to RBC at pH 7 led to a consistent, compact and firm structure, like a sponge, releasing the retained water when compressed but immediately reabsorbing it when decompressed. On the other hand, at pH 4.5, very viscous, cohesive, not very consistent pastes were obtained. Proteins with an elevated number of hydrophobic amino acids, such as Hb and egg albumen, have pH gelling zones depending on the protein concentration (Cheftel, Cuq, & Lorient, 1992). The protein–protein interactions and gel formation are more rapidly produced by increasing the protein concentration as this favours intermolecular interactions. At pH near to the p*I*, the absence of repulsive electric charges leads to the formation of a less expanded and hydrated gel.

The TPA parameters allowed definition of the rheological characteristics of the two kinds of heat-induced gels or pastes obtained from RBC, depending on the pH condition (Fig. 5). The pH 7 gels were harder, less elastic and less adhesive than the pH 4.5 pastes ($P < 0.05$). At

Table 2
Foam percentages at 60, 90, and 120 min of rest, from 0.5% spray-dried red blood cell fraction (RBC) solutions obtained from untreated (control) and pressurized RBC as influenced by pH[A]

Time of rest	Control RBC		Pressurized RBC	
	pH 4.5	pH 7	pH 4.5	pH 7
60 min	48.2 ± 2.0 Aa	62.2 ± 8.9 Ab	47.9 ± 4.9 Aa	68.1 ± 6.1 Ab
90 min	38.1 ± 1.6 Aa	49.0 ± 7.3 Ab	36.2 ± 3.9 Aa	57.5 ± 5.8 Ab
120 min	31.7 ± 2.4 Aa	39.4 ± 4.9 Ab	30.0 ± 3.8 Aa	50.7 ± 4.5 Ab

Different letters within the same row denote significant differences ($P < 0.05$) between treatment (capital letters) and pH (small letters).
[A] Means ± confidence interval ($P = 95\%$, $n = 5$).

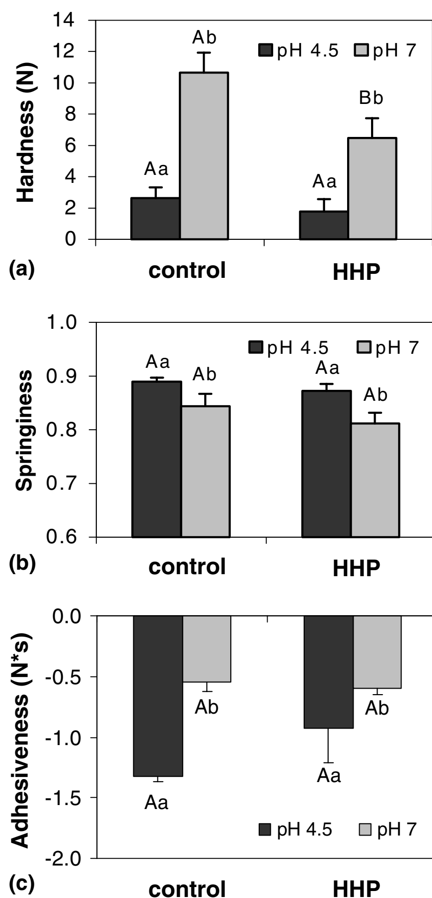

Fig. 5. TPA parameters, at pH 4.5 and pH 7, of gels obtained from spray-dried red blood cell fraction (RBC) untreated (control) and pressurized: (a) hardness; (b) springiness; (c) adhesiveness. Error bars show mean confidence intervals ($P = 95\%$). Different letters indicate significant differences ($P < 0.05$) between treatment (capital letters) and pH (small letters).

the p*I* of the Hb, heating promoted the formation of an elevated number of intermolecular interactions among the denatured Hb chains, leading to a more compact and resistant gel. These results are in agreement with those reported by Parés and Ledward (2001) who found the hardest heat-induced gels at pH near the p*I* of porcine blood plasma proteins.

Pressurization provoked a decrease in all the texture parameters of RBC gels; however, the effects on springiness and adhesiveness were not significant ($P > 0.05$). On the contrary, the hardness of RBC gels differed between pressurized and untreated samples ($P < 0.05$) only at neutral pH. At pH 7, gels from pressurized RBC showed a lower resistance to compression than control RBC gels.

3.6. Water-holding capacity of heat-induced gels

Pressurization did not affect the WHC of heat-induced gels from RBC solutions. The percentages of

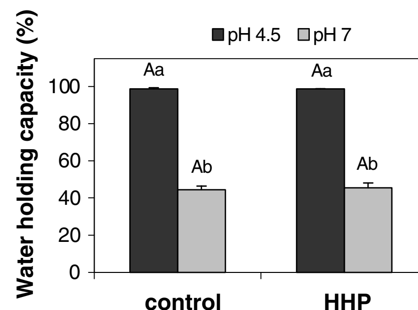

Fig. 6. Water-holding capacity, at pH 4.5 and pH 7, of gels obtained from spray-dried red blood cell fraction (RBC) untreated (control) and pressurized. Error bars show mean confidence intervals ($P = 95\%$). Different letters indicate significant differences ($P < 0.05$) between treatment (capital letters) and pH (small letters).

released water, at pH 4.5 and pH 7, of gels from untreated and pressurized RBC, were not different ($P > 0.05$) (Fig. 6).

On the other hand, WHC was significantly different in samples at different pH values ($P < 0.05$). The acid pastes released approximately 1% of water, whereas the water released was 55% at neutral pH, independent of whether or not the RBC had been pressurized. The WHC values from RBC gels were highly related to their rheological behaviour. Obviously, the protein conformation and electric charges had a direct influence on the WHC of gels. At acid pH, Hb was completely hydrated and thermal denaturation led to the formation of a thick and viscous paste that exhibited an excellent WHC. On the other hand, at neutral pH, a highly consistent gel was formed as a consequence of the aggregation of the denatured Hb molecules in which water was retained by means of capillarity in the interior of the pores of the protein network.

4. Conclusions

The application of HHP treatment (400 MPa, 15 min, at 20 °C) to liquid RBC resulted in several effects on the contaminating microbiota and functional properties of the spray-dried product. A microbial reduction of 3.2 logarithmic units, with respect to the initial contaminating microbiota of the liquid RBC, was achieved. The effects on functionality of spray-dried RBC were diverse, depending on the property. Pressure treatment increased the denaturant effects of spray-drying on RBC since a decrease in protein solubility and foaming capacity was observed in comparison with the non-pressurized samples. However, HHP did not have any negative effects on the colour or the foam stability of RBC.

At neutral pH, hard and consistent gels were formed, in which the water was retained by capillarity, whereas at acidic pH, the pastes were softer, more adhesive and elastic, and with higher water-holding capacities than

those of pH 7 gels. Neither springiness, nor adhesiveness, nor water-holding capacities of heat-induced gels were affected by pressure treatment. On the other hand, only at pH 7 was the hardness of gels decreased by HHP treatment.

These results suggest that HHP treatment, and subsequent spray-drying, might be an appropriate system for preserving RBC.

Acknowledgements

This work was financially supported by the CICYT project of the Spanish government (ALI 99-1055-C02-01). We thank Dr. B. Guamis, director of the Centre Especial de Recerca-Planta de Tecnologia dels Aliments (CERPTA) of the Universitat Autònoma de Barcelona for the use of HHP equipment, Dr. J. Yuste and Mr. J.M. Quevedo for their kind assistance in HHP treatments, and Mrs. A.M. Aymerich for her helpful technical assistance. We also thank Frigorífics Costa Brava (Girona) and W.L Gore y Asociados S.L. (Barcelona) for their kind donation of blood samples and Goretex® filter membrane, respectively.

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