FUNCTIONAL PROPERTIES OF SPRAY-DRIED PORCINE RED BLOOD CELLS FRACTION TREATED BY HIGH HYDROSTATIC PRESSURE

M. Toldrà, D. Parés, C. Carretero
Institut de Tecnologia Agroalimentària, Escola Politècnica Superior, Universitat de Girona, Avda. Lluís Santaló s/n, 17071 Girona, Spain.
E-mail: mtoldra@intea.udg.es

Background

Porcine blood is an edible by-product generated in a great volume in industrial abattoirs. The red blood cells fraction (RBC) constitutes the 40% of porcine blood and mainly contains the hemoglobin (Hb). Hemoglobin (90% of the RBC protein) has good functional properties (Tybor *et al.*, 1975; Ranken, 1980; Nakamura *et al.*, 1984; Wismer-Pedersen, 1988). Moreover, this fraction is also an important source of iron (Gorbatov, 1988) that is better absorbed than ferrous salts commonly used in food supplies. These factors make the RBC interesting as a food ingredient.

Previous studies developed in our laboratory (Toldrà, 2002) showed that raw RBC from porcine blood can have a quite high level of microbiological contamination depending on the blood collection system. RBC used in this study had microbial loads around 10^6 cfu·mL^{-1}. Spray drying is the most commonly employed technology to preserve the blood and its fractions. However, in order to improve its microbiological quality it is necessary to find an effective method of decontamination that, applied to the RBC prior to dehydration, can maintain its nutritional, organoleptic and functional quality. Since the RBC and its protein are highly modified or damaged by heat treatments, the use of a non-thermal preservation method as high hydrostatic pressure (HHP) treatment is required.

In a previous work (Toldrà *et al.*, 2002), taking notice of the effects of HHP treatment at several pressure, temperature and time conditions on the microbiological quality of the RBC, we concluded that the most adequate pressurization conditions were 400 MPa at 20ºC for 15 min. This treatment produced a significant improvement of the microbiological quality, did not adversely affect the color, the protein solubility was not seriously affected, and the RBC fraction remained fluid after the treatment.

Objectives

The objectives of this study were to evaluate the functional properties at acid (4.5) and neutral (7) pH of the porcine red blood cells fraction submitted to high hydrostatic pressure treatment and subsequent spray-drying.

Methods

A randomized complete block design experiment with replications was applied on 5 samples of RBC fraction treated on different days but under the same conditions. Each sample was divided into 2 aliquots of about 500 mL, one was used as a non-pressurized control and the other was pressurized before spray drying. Samples were always pressurized and spray dried within 24 hours of the blood collection.

HHP Treatments were performed on a batch isostatic press (Alstom, Nantes, France) at 400 MPa at 20ºC for 15 min. After pressurization, non-treated (control) and pressurized samples were cooled and kept at 5ºC until spray drying.

Spray drying: samples were dehydrated on a lab equipment Büchi Mini Spray Dryer B-191 (Büchi Labortechnik AG, Flawil, Switzerland). Temperature inlet and outlet were 140 and 77ºC respectively.

Color parameters CIE L*a*b* were determined using a Minolta CR-300 chroma meter with a CR-A33f glass light-projection tube (Minolta Co. Ltd., Osaka, Japan).

Protein Solubility was determined by the method described by Morr *et al.* (1985). The solubility was calculated as the percentage of soluble protein content in the supernatant relative to the total protein content in the sample.

Foaming capacity and foam stability: 0.5% solutions of powdered RBC fraction were whipped in a mixer at maximum speed for 10 min. The foam capacity was determined as the volume of foam formed. The foam stability was determined using a gravimetric method calculating the foam percentage that remained stable for 2 hours.

Emulsifying activity: a turbidimetric method based on the technique described by Pearce and Kinsella (1978) was used. Oil-in-water emulsions were obtained by premixing 25% (v/v) commercial corn oil with the aqueous protein solution and homogenized at 12,000 rpm for 1 min with a knife-homogenizer Polytron PT3000 (Kinematica AG, Littau, Switzerland). The absorbance of the diluted emulsion was measured at 500 nm.

Heat-induced gels preparation: solutions at 17% dry matter of powdered RBC were prepared and pH adjusted. Solutions were introduced into plastic bags and heated in a water bath at 80ºC for 30 min, cooled and stored overnight at 5ºC.

Texture profile analysis (TPA) tests were performed with a TA XT2 texturometer (Stable Microsystems Ltd., Surrey, England) using a flat cylindrical aluminum probe of 50-mm diameter. Samples of RBC gels (25 mm diameter and 15 mm thickness) were compressed twice until a 30% of initial height at a compression rate of 1 mm/s.

Water holding capacity (WHC) of RBC gels was determined by a method combining centrifugation and filtration as described by Parés *et al.* (1998). WHC was expressed as the percentage (w/w) of water remaining in the gel after centrifugation.

Results and discussion

Color CIE L*a*b*

The application of the HHP treatment was not found to have significant influence on the color parameters of the spray dried RBC samples ($P>0.05$) although pressurized RBC had CIE L*a*b* color values slightly higher than powder from untreated RBC. Therefore, the oxidative effect on heminic iron induced by spray drying was dominant, and pressurized samples were as much susceptible as control samples to the oxidation of the hem group produced by the dehydration.

Protein solubility

High hydrostatic pressure and pH had significant effects ($P<0.05$) on the protein solubility of spray dried RBC fraction. The solubility at neutral pH (7) was lesser than at acid pH (4.5) in both untreated and pressurized RBC samples. The application of the HHP treatment led to reduced solubility in spray dried RBC. Although the decrease in solubility at neutral pH was higher in pressurized and subsequent spray dried RBC, there was not found a significant interaction between treatment and pH. Pressurization increased the susceptibility of Hb to the denaturant effects of dehydration, especially at pH 7 (p*I*), since after both processes, a decrease in protein solubility at neutral pH was observed.

Foaming and emulsifying properties

Regarding to foaming properties of RBC, the volume of foam formed from powdered RBC solutions was significantly affected ($P<0.05$) by HHP and pH. The denaturant effect of HHP treatment on the Hb led to a lower foaming capacity of RBC powder at neutral and acid pH ($P<0.05$). The foaming capacity was higher at neutral pH due to the fact that the foaming properties of proteins used to be maximum at pH values close to their p*I*. On the other hand, pressurization did not affect the foam stability of spray dried RBC solutions. Neither a negative effect of the application of the HHP on the emulsifying activity of Hb was observed. The maximum emulsifying activity of Hb was reached with a 1.5% of dried RBC at pH 7 and 1% at pH 4.5.

Texture and water holding capacity of heat-induced gels

TPA parameters revealed the rheological characteristics of two kinds of heat-induced gels obtained from powdered RBC solutions depending on the pH condition. At neutral pH, compact, firm and consistent gels were formed, whereas at acid pH, the pastes were very viscous, cohesive, not much consistent, less hard, more elastic and more adhesive than the pH 7 ones. On the other hand, acid pastes had higher water holding capacity than those of pH 7 gels, in which, the water was retained by capillarity in the interior of the protein network pores. The texture and the water holding capacity of heat-induced gels from RBC solutions were not affected by the application of the HHP treatment ($P>0.05$).

Conclusions

The pressure treatment increased the susceptibility of hemoglobin with respect to the denaturant effects of spray drying since a decrease in protein solubility and foaming capacity was observed. However, HHP did not have any negative effects on the foam stability and the emulsifying activity of red blood cells fraction. Neither texture nor water holding capacity of heat-induced gels were affected by pressure treatment. At neutral pH, hard and consistent gels were formed, in which the water was retained by capillarity, whereas at acid pH, the pastes were softer, more adhesive and elastic, and with higher water holding capacity than those of pH 7 gels.

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