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Heat-induced gelation of porcine blood plasma proteins as affected by pH

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Abstract

Porcine plasma is a by-product of the meat industry that can be used as a food ingredient. It is a protein mixture, hence its composition can be modified to meet specific functionality requirements. In the present paper, the gelation properties of plasma and its two major fractions (serum and albumin) have been studied at pH 4.5, 6.0 and 7.5. Polyacrylamide gel electrophoresis (SDS–PAGE) revealed that albumin was the constituent that remained soluble to a larger extent during heat-treatments, and that acidic coagulation occurred at pH 4.5, making weak interactions the predominating ones between protein aggregates. Differential scanning calorimetry (DSC) and rheological tests showed that both the thermal stability and the gelation point of protein solutions were lower as pH decreased. The textural properties and water-holding capacities of plasma and albumin gels were more pH-dependent than serum. Albumin gels were the weakest and those of plasma at pH 7.5, the strongest. It has been determined that interactions between protein fractions play a key role in the gelling properties due to synergistic effects. This knowledge should be useful in the engineering of a plasma derivative product designed for specific food requirements, by reformulating its natural composition and enhanced by controlling pH.

Keywords: Porcine blood plasma proteins; Serum; Albumin; Globulins; Fibrinogen; Heat-induced gelation; Rheology; Texture

1. Introduction

The use of blood plasma in food products is of great interest because of its functional properties. Studies on gelling, emulsifying and foaming abilities suggest that plasma could replace some widespread ingredients, such as egg albumen (Howell & Lawrie, 1984; Le Denmat, Anton, & Beaumal, 2000; Oshodi & Ojokan, 1997; Parés & Ledward, 2001; Parés, Saguer, Toldrà, & Carretero, 2000; Raëker & Johnson, 1995; Silva & Silvestre, 2003). The gel-forming ability upon heating is probably the most interesting attribute of plasma, because many food products – i.e., cooked meat products – go through a thermal treatment prior to commercialisation. Under appropriate conditions, a three-dimensional network may be formed, contributing to the development of the internal structure of the foods such as desirable texture characteristics and improved properties like water holding capacity (Hermansson, 1982).

Porcine blood is an important by-product of the meat industry with many potential uses. The typical composition of plasma proteins is as follows: albumin 50–60%, globulins 40–50% and fibrinogen 1–3%. Albumin is a globular protein with a molecular weight of 69 kDa and an isoelectric point (p*I*) around 4.8. Globulins comprise a heterogeneous group of globular proteins, subdivided into α, β and γ fractions, including the immunoglobulins, with molecular weights ranging from few to hundreds kDa and p*I* between 5 and 7. Fibrinogen is a fibrous protein of 340 kDa formed by three pairs of polypeptide units that

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represents around 3% and when it is removed from plasma, the remaining fraction is named serum (Putnam, 1975). These three major constituents have different structures and physical properties that make them behave differently when they are thermally treated (Howell & Lawrie, 1984; Raëker & Johnson, 1995).

The functional properties that blood plasma exhibits are a complex sum of the properties of its individual constituents, the interactions between them and also environmental physicochemical conditions. Previous functionality studies performed on mixtures of proteins including albumin, globulins and fibrinogen gave some synergistic effects (Foegeding, Dayton, & Allen, 1986; Gezimati, Singh, & Creamer, 1996; Howell & Lawrie, 1984; Matsudomi, Oshita, & Kobayashi, 1994; Paulsson, Hegg, & Castberg, 1986). According to Howell and Lawrie (1984), textural characteristics of a cake-type model system formulated with plasma and sugar seem to be governed by albumin: gelation occurred at higher temperatures when the protein profile contained mainly α-globulins and gel strength was lower when fibrinogen was absent. These features suggest that a porcine plasma derivative product could be developed by modifying its natural protein profile, taking advantage of specific interactions between proteins to enhance functionality for particular food requirements.

The aim of this work was to study the behaviour of porcine blood plasma proteins at different pH conditions during heat-induced gelation as a further step towards the understanding of the plasma major constituent's role in this process. Different experimental approaches were used to investigate changes in the physical attributes of heated plasma, serum and albumin samples.

2. Materials and methods

2.1. Blood plasma source

Blood was obtained from an industrial slaughterhouse using sterile bleeding recipients containing sodium citrate as anticoagulant (1% w/v final concentration). Plasma was separated by centrifuging blood at 2520*g* at 4 °C for 15 min (Sorvall RC 5C Plus, DuPont Co., Newtown, CT) and decanting.

2.2. Fractionation

Plasma protein fractions from porcine blood were separated by salting out, using ammonium sulphate as a precipitating agent, following the fractionation process described in Dàvila, Parés, and Howell (2006). All precipitation steps were carried out within an ice bath. Briefly, fibrinogen was precipitated from blood plasma by adding ammonium sulphate to 20% saturation and then removed by centrifugation (10000*g* at 4 °C for 15 min). The supernatant, containing mostly globulins and albumin – namely serum – was recovered. Ammonium sulphate was added to an aliquot of serum up to 60% saturation to precipitate globulins, which were removed by a centrifugation step. The albumin remaining in the supernatant after centrifugation was also precipitated by increasing the ammonium sulphate concentration up to 75% saturation. Albumin precipitates were washed once with a saturated solution of ammonium sulphate (75%) containing 10 mM Tris–HCl and adjusted to pH 7.4, and recovered by centrifugation. Final concentrated albumin precipitates were dissolved in Milli-Q water. These solutions as well as serum and plasma aliquots were exhaustively dialysed against Milli-Q water at 5 °C with a membrane of 12–14 kDa pore diameter (Mediceli International Ltd., London, UK) in order to reduce the content of the precipitating salt. Dialysed solutions were frozen at −80 °C for 24 h and dried in a Virtis Unitop SQ freeze dryer (The Virtis Co., Gardiner, NY) at −15 °C and 15 °C for the primary (sublimation) and the secondary (desorption) drying stages, respectively. Purity of the final protein powders was confirmed by SDS–PAGE electrophoresis and the proximate composition for moisture (ISO R-1442), total protein (ISO R-937), and ash content (ISO R-936) was determined (Table 1). Dried proteins were vacuum-packed and stored in chilled conditions until use.

2.3. Preparation of protein solutions

Experimental solutions at pH 4.5, 6.0 and 7.5 were prepared by dissolving protein powder in an appropriate buffer: trisodium citrate (20 mM) for solutions at pH 4.5 adjusted with citric acid, and NaH_2PO_4 (50 mM) for solutions at pH 6.0 and 7.5 adjusted with NaOH (0.2 M).

2.4. SDS–Polyacrylamide gel electrophoresis (SDS–PAGE)

In order to study aggregation phenomena of plasma solutions (5 wt%) at pH 7.5, 6.0 and 4.5, two types of thermal treatments were applied, (1) a constant heating ramp at 3 °C min^{-1} from 25 to 85 °C and (2) a heating step from 25 to 80 °C at 3 °C min^{-1} followed by an isothermal treatment at 80 °C for 30 min, these regimes representing two typical process conditions used by the food industry. Samples were collected at different temperatures during the heating step and at different times along the isothermal step. Solutions for the electrophoresis analysis contained 50 μL of protein solution in 950 μL of TRIS/EDTA buffer with SDS for nonreducing conditions and 2% β-mercaptoethanol was added to produce reducing conditions. Molecular weights were determined using low molecular

Table 1
Chemical composition of protein powder samples

Fraction	% Protein	% Moisture	% Ashes
Plasma	90.61 ± 1.33	4.62 ± 2.31	2.72 ± 0.38
Serum	91.08 ± 1.13	4.90 ± 1.92	2.46 ± 0.56
Albumin	91.32 ± 1.27	4.99 ± 1.86	1.79 ± 0.20

Values represent means ± standard deviation ($n = 4$).