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Effect of transglutaminase on heat-induced gel properties at acid pH of mixtures of plasma and haemoglobin hydrolysate from porcine blood

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Abstract

The purpose of the present study was to determine the effects of treating protein solutions prepared by mixing different percentages of haemoglobin hydrolysate and plasma from porcine blood with microbial transglutaminase (*MTGase*) on the properties of thermal-induced gels at pH 5.5. Solutions were adjusted to pH 7.0 just before their incubation with different *MTGase* concentrations. After the enzymatic treatment, solutions were lowered to pH 5.5 and submitted to the thermal gelation process. The results indicate an overall better gelling ability of the lowest HbH-content mixtures relative to those containing the highest HbH-content ones, with a clearly different behaviour regarding the *MTGase* effects.

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Keywords: plasma, haemoglobin hydrolysate, heat-induced gelation, acid pH, microbial transglutaminase

1. Introduction

Porcine plasma forms heat-induced gels with excellent texture and water holding capacity (WHC) at physiological pH (7.4). However, these properties are strongly decreased as pH is reduced to acid conditions. This supposes an important handicap because plasma is often used as a techno-functional ingredient in meat products, which use to be acid. As pH approaches the p*I* of serum albumin (4.8), the main plasma protein, unspecific aggregation through hydrophobic interactions is favoured while thiol/disulfide interchange reactions are limited. Both phenomena are responsible for the losses of gel properties. Different alternatives have been considered in the past in order to minimize the effect of pH reduction on plasma gelation properties. Treating plasma with microbial transglutaminase (*MTGase*),

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particularly under high hydrostatic pressure and keeping pressured plasma under refrigeration at least for 2 h before the gelation treatment, has been shown to be effective in improving texture of heat-induced plasma gels at pH 5.5 but without enhancing its WHC, which is related to the poor effects on gel microstructure [1,2]. More recently, this question has been tackled by mixing plasma with haemoglobin hydrolysate (HbH). Some studies revealed that heat-induced gels with enhanced properties can be obtained by mixing proteins and protein hydrolysates from different sources, probably due to modifications in the molecular interactions contributing to the gel formation [3,4] and, consequently, provoking changes in the gel microstructure [5]. Both, protein-peptide binding and/or hydrolysate-promoted precipitation by solution acidification just after adding this last mixture component have been suggested as aggregation-implied mechanisms [6,7,8]; so, they can strongly be affected by pH and ionic strength [5,9]. Moreover, it is also known that the efficacy of mixed solutions in enhancing gel properties depends not only on the specific proteolytic enzyme used but also of the protein hydrolysate:protein ratio [3]. However, thermal gels from HbH-plasma mixtures (3.5-14.3% of plasma proteins replaced by HbH) adjusted to pH 5.5 showed not better but rather similar textural characteristics relative to gels from plasma alone under the same conditions. In addition, more exudative gels were obtained when $\geqslant$10.7% of protein was in the form of HbH [10]. Fan *et al*. [11] have also reported losses in the gel-forming ability of protein and hydrolysate mixtures (both from soy) as the percentage of hydrolysate increased, attributed to a more relevant role of native molecules than peptides in the formation of gel network. In spite of that, mixture gelling properties were as good as those of 100%-native protein solutions after treating the mixtures with transglutaminase (*TGase*) previously to gelation if the ratio between both mixture components was appropriate, which seemed to be related to improvements on gel microstructure. *TGase*-treatment of solutions with a partial replacement of dairy native proteins by zein hydrolysates has also been successfully applied to improve other functional properties [12].

So, the present work aimed to determine whether heat-induced gels at pH 5.5 with good properties can be obtained by treating haemoglobin hydrolysate (HbH)-plasma mixtures with microbial transglutaminase (*MTGase*). This way, solutions with different HbH:plasma ratios were treated with different enzyme concentrations before the thermal gelation and gel texture along with its water holding capacity were determined. SDS-PAGE was used to set the effects of the treatments at molecular level.

2. Materials & Methods

2.1. Porcine plasma and haemoglobin hydrolysate (HbH) samples

Five porcine blood samples were hygienically collected from an industrial abattoir on different days, to get dehydrated plasma (4) and HbH (1). At any case, blood was immediately mixed with sodium citrate solution (1% w/v, final concentration in blood) and kept under refrigeration until it was centrifuged in the laboratory at 2530g at 5 ºC for 15 min (SORVALL RC 5 C Plus, Dupton, Newtown, USA). Plasma and red cell fraction were separated by decantation after centrifugation. Plasma was spray-dried using a Büchi Mini Spray-Dryer B-191 (Büchi Labortechnik AG, Flawil, Switzerland) at an air outlet temperature lower than 80 ºC; feed flow of 670 ml h^{-1}; aspirator flow rate of 60 l h^{-1}; and spray air flow pressure of 5 bar. Red cell fraction was haemolysed using a FPG 7400 high-pressure laboratory valve homogenizer (Stansted Fluid Power Ltd., Essex, UK) under the following conditions: inlet pressure, 5 bar; processing pressure, 10 MPa; inlet and outlet temperature, 15 ºC and 25 ºC, respectively, to obtain haemoglobin solution. Then, it was firstly submitted to enzymatic hydrolysis by trypsin (EC 3.4.21.4) at pH 7.5 and 40 ºC for 2 h and subsequently by pepsin (EC 3.4.23.1) at pH 3 and 40 ºC for 1 h. The hydrolysate was obtained as the supernatant after centrifugation at 20 090 g and 15-20 ºC for 30 min. It was immediately frozen at -80 ºC and then freeze-dried in a VIRTIS Unitop SQ (Virtis, Gardiner, NY, USA) at -15 and 15 ºC for the primary and secondary drying stages, respectively. Contents of water (ISO R-1442), protein