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Improvement of gelling properties of porcine blood plasma using microbial transglutaminase

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Abstract

The effect of microbial transglutaminase (*MTGase*) on the texture and water-holding capacity (WHC) of heat-induced gels prepared from porcine blood plasma at pH 5.5 was investigated. Different concentrations of commercial *MTGase* were added to plasma and incubated for several times under specific conditions of temperature and pH. From the results obtained, it can be postulated that enzymatic treatment enhances textural properties and WHC of plasma gels at pH 5.5, especially when incubated with 3% of the commercial product for 3 h at 30 °C and pH 7. This treatment increased by 0.4 N the hardness of gels and reduced by 3% the water released after gel centrifugation with respect to the control samples. SDS–PAGE confirmed that cross-linking took place when *MTGase* was added to plasma solutions. However, the results suggest that the sole addition of *MTGase* was not effective enough to improve the gelling properties of plasma proteins under acidic conditions.

Keywords: Porcine plasma; *MTGase*; Cross-linking; Texture; Water holding capacity

1. Introduction

Salt and/or some phosphates are usually added in order to increase water-binding capacity and also to improve texture of meat products. However, their use is legislated and, nowadays, the demand for salt-reduced food products is increasing. For this reason, alternatives to the use of these ingredients are being progressively considered. Blood plasma from industrial abattoirs is commonly used in the meat industry (Caldironi & Ockerman, 1982). Its heat-induced gel-forming ability is one of the most valuable functional properties for its use as a food ingredient in cooked meat products (Ni & Hayakawa, 2001). Moreover, plasma proteins have been reported as good fat substitutes in reduced-fat meat products (Cofrades, Guerra, Carballo, Fernández-Martín, & Jiménez-Colmenero, 2000). Although plasma can be added to different food products over a wide pH range, its gelling properties decrease as pH is reduced due to protein denaturation (Parés, Saguer, Saurina, Suñol, & Carretero, 1998; Saguer, Fort, Mompió, & Carretero, 2004). The improvement of the gelling properties of plasma at acidic conditions could be very interesting in order to enhance textural properties and to reduce the syneresis of meat products, especially when considering that pH 5.5–6.5 are the typical pH values of these kind of products.

It is known that blood plasma is a complex mixture with over 100 different proteins being albumin (60%), globlulins (35%) and fibrinogen (4%) the most important ones, with serum albumin being the main protein implicated in the formation of heat-induced gels. Certain factors such as calcium and clotting-related enzymes – which can be reactivated when calcium is added – could enhance gelation and gel strength of plasma-containing foods (Hickson, Dill, Morgan, Suter, & Carpenter, 1980; O'Riordan, Kinsella, Mulvihill, & Morrissey, 1989a; O'Riordan, Morrissey, Kinsella, & Mulvihill, 1989b; Wismer-Pedersen, 1979). However Chang, Hwang, and Chjang (1999) observed that the role of calcium in the gel formation of plasma is complex, with high or low calcium levels proba-

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bly leading to either the denaturation of plasma proteins and clotting-related enzymes or the insufficient activation of these enzymes. Calcium can also promote excessive protein–protein interaction, resulting in aggregation rates faster than the protein unfolding capacity (Casper, Wendorff, & Thomas, 1999). Moreover, the presence of calcium can modify the solubility of other proteins which are present in the food product. Other alternatives should thus be considered.

Transglutaminase (*TGase*) is frequently used to improve functional properties of food proteins. The enzyme catalyses the incorporation of primary amines into certain proteins through acyltransfer between the γ-carboxyamide of glutaminyl residues into the protein and various primary amines. In the absence of amine substrates, *TGase* catalyses the deamidation of glutamine residues using water molecules as acyl acceptors. The cross-linking through ε-(γ-Glu)Lys covalent bonds can be intermolecular and intramolecular and causes significant changes in physical properties of protein-rich foods, especially in those with high content of lysine and glutamine. Transglutaminase is directly related to nature; several *TGases* could be used, each one showing its own properties. However, the most interesting could be that from *Streptoverticillum mobaraense*. It is the only one that has become commercially available until now.

The main characteristics of the microbial transglutaminase from *Streptoverticillum mobaraense* (*MTGase*) are being a Ca-independent enzyme (Ando et al., 1989) on the one hand and on the other to present a relatively low substrate specificity (de Jong, Wijngaards, Boumans, Koppelman, & Hessing, 2001), thus reacting with a larger number of substrate proteins than plasma and erythrocyte *TGases* from mammalian blood. Moreover, the reaction when BSA is used as substrate is more intense with *MTGase* than with plasma or erythrocyte *TGase* (de Jong & Koppelman, 2002). Plasma proteins are excellent sources of lysine (Duarte, Carvalho-Simoes, & Sgarbieri, 1999; Ramos-Clamont, Fernández-Michel, Carrillo-Vargas, Martínez-Calderón, & Vázquez-Moreno, 2003; Young, Lewis, Landmann, & Dill, 1973). However, the cross-linking rate also depends on the accessibility of reactive lysine and glutaminyl residues. Globular proteins are poor substrates due to their compact structures, and this limits the accessibility of transglutaminase to target residues (Motoki, Nio, & Takinami, 1984; Traoré & Meunier, 1992). These proteins, though, could become more susceptible to polymerization following partial unfolding by adjusting temperature and times of incubation during enzymatic treatments.

Our goal was the improvement of the gelling properties at pH 5.5 of porcine plasma using *MTGase*. Particularly, this work aimed on determining not only the effects of enzyme concentration but also the incubation time at the selected temperature on textural properties and water holding capacity of heat-induced plasma gels. At the same time, the participation of the maltodextrine present in the commercial enzymatic complex and the incubation temperature effect on these properties will be elucidated.

2. Materials and methods

2.1. Porcine blood plasma

Samples of porcine plasma came from an industrial slaughterhouse. Blood had been previously collected during the slaughter and mixed immediately with a sodium citrate solution (1% w/v) in order to prevent coagulation, and subsequently centrifuged. Once in the laboratory, the plasma was recentrifuged for 15 min at 2530*g* and 5–10 °C (SORVALL RC 5C Plus, Dupont, Newtown, USA) in order to eliminate cellular residues.

2.2. Physico-chemical parameters

Moisture and protein contents and pH of plasma samples were determined according to the Association of Official Analytical Chemists methods (AOAC, 1980).

2.3. Effect of MTGase concentration and incubation time

Microbial transglutaminase preparation (ACTIVA®, Ajinomoto Co., Inc. Transglutaminase, Japan) was supplied as a mixture containing 99% maltodextrine and 1% microbial transglutaminase. The transglutaminase activity of the commercial product was approximately 100 units g^{-1} and the enzyme solution gave a single band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS–PAGE). In the present study, the enzyme concentration is reported as the commercial product concentration and referred as % MTG.

Seven hundred milliliters of porcine plasma was separated in seven aliquots. Three *MTGase* concentrations (1%, 3% and 5% MTG w/v) and two reaction times (1 and 3 h) were assayed at pH 7 and 30 °C. After *MTGase* reaction, the plasma was adjusted to pH 5.5 with HCl 3N and subsequently submitted to the gelation treatment. Non-treated plasma adjusted to pH 5.5 was also included to be used as control. This experiment was repeated three times with plasma collected on different days.

2.4. Effect of heat treatment and maltodextrine

Five hundred milliliters of porcine plasma was separated in five aliquots, each one being submitted to one of the following treatments: (1) neither enzymatic nor heat treatment; (2) incubation at 30 °C for 1 h without enzyme, (3) incubation at 30 °C for 3 h without enzyme; (4) incubation at 30 °C for 3 h with MTG (3% w/v); (5) incubation at 30 °C for 3 h with MTG (3% w/v) previously inactivated by heating at 90 °C for 20 min (Ruíz-Carrascal & Regenstein, 2002). All aliquots were also adjusted to pH 5.5 before gelation. This experiment was also repeated three times with plasma collected on different days.

2.5. Preparation of heat-induced gels

Heat-induced gels were prepared by introducing 100 ml of plasma solutions into synthetic gut (Wienie-pak® 2350/84, Teepak LLC, Belgium) and heated at 80 °C for 45 min, completely submerged in a heated water bath. After that, they were immediately cooled to 20–25 °C in a water bath and kept under refrigerated conditions overnight (16 h). Samples were tempered at room temperature (30 min) before being used for water holding capacity and texture analyses.

2.6. Texture analysis

Hardness, springiness and cohesiveness of protein gels were determined from gel portions (14 mm length and 8 mm dia) by performing Texture Profile Analysis (TPA) test by using a TA XT2 texturometer (Stable Micro Systems, Surrey, UK). Samples were compressed twice with an aluminium cylindrical probe (50 mm dia) until 30% deformation was reached. The uniaxial compression rate and the recuperating time after first compression cycle were 1 mm s^{-1} and 2 s, respectively. Hardness, springiness and cohesiveness where calculated from TPA curve. For each gel, measurements were taken from three different portions.

2.7. Water-holding capacity (WHC)

A technique combining filtration and centrifugation based on the procedure proposed by Parés et al. (1998) was used. Gel cylinders (14 mm length and 8 mm dia) were placed into bags made of GORE-TEX® filter membrane with a pore diameter of 0.45 μm (W.L. Gore y Asociados S.L., Barcelona, Spain). The bags were suspended inside centrifuge tubes, centrifuged at 4000*g* for 10 min at 15 °C, and the weight of released water was measured. The results are reported as percentage (w/w) of water released after centrifugation. Three replicates were performed for each sample.

2.8. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

To detect protein changes induced by the treatments, samples were analyzed by SDS–PAGE Phastsystem electrophoresis (Pharmacia LKB · Phast System; Uppsala, Sweden), after reduction with β-mercaptoethanol, in Phastgel homogeneous 12.5% polyacrylamide gels (Amershan Bioscience Europe Gmbh; Uppsala, Sweden). Protein solutions were initially prepared by mixing 200 μl of sample with 800 μl of distilled water; after that, this solution was newly diluted (1:4) using buffer Tris/HCl 10 mM, EDTA 1 mM and pH 8.0. Then, SDS and β-mercaptoetanol at a concentration of 2.5% and 5%, respectively, relative to sample volume were added. The mixture was incubated for 5 min at 100 °C. Bromophenol blue was added to indicate the electrophoretic front. The gel electrophoresis was conducted at a constant voltage (250 V for 30 min). The gels were treated with 2.5% gluteraldehyde in distilled water and then stained with 0.1% Coomassie Brilliant blue G-250 in methanol:acetic acid:distilled water (3:1:6). The destaining was performed in methanol:acetic acid, 30% and 10%, respectively. After that, gel was dipped in 10% acetic acid containing 10% glycerol for 5 min. Bench-Mark™ Protein Ladder with molecular weights from 20 to 220 kDa, purchased from Invitrogen (California, USA), was used as molecular weight standards.

2.9. Experimental design and statistical analysis

Sampling days were considered as blocks in a randomized complete block design. The statistical analyses were carried out with the Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, USA). In all cases, data were submitted to ANOVA using the general linear model procedure (Proc GLM) and the significance level for all tests was $\alpha = 0.05$. When possible, a polynomial contrast was applied if a significant effect was obtained in order to get a comparison among the levels of a quantitative factor. This contrast assumes that the levels of the factor are regularly spaced and that the sample size for the levels is equal. In other cases, the Tukey–Kramer test with less significant differences at $P \leqslant 0.05$ was used to compare sample means. In any case, the homogeneity of variances was assessed using the Barlett's test before ANOVA.

3. Results and discussion

3.1. Physico-chemical parameters

The moisture content of porcine blood plasma was found to be $90.8 \pm 0.2\%$, a value which is very similar to those cited in the literature (Parés, 1998; Ranken, 1980). The protein content ($6.06 \pm 0.14\%$) was slightly lower than that reported by other authors (Cheftel, Cuq, & Lorient, 1985; Parés, 1998) yet the differences in the blood collection system and centrifugation conditions at the slaughterhouse could partially explain these results. On the other hand, pH showed a considerable dispersion range, comprising values from 7.28 to 7.76, in some cases indicating acidemia or alkalosis, which could be partially attributed to physiological effects associated to slaughter stress.

3.2. Effect of MTGase treatment on gelling properties of plasma gels at pH 5.5

Since no information about incubation conditions of *MTGase* in plasma was available before this work, several preliminary experiments were carried out in our laboratory in order to establish temperature and pH conditions during enzymatic incubation. It is known that maximal activity of *MTGase* from *Streptoverticillium mobaraense* takes place at pH 7 and at 50–60 °C, with optimal reaction times ranging from 30 min to 1 h at these temperatures (Ajinomoto Co., Inc.), and showing a relative activity lower than 50% at

30 °C (Seguro, Nio, & Motoki, 1996). However, our trials showed on the one hand that the enzymatic treatments at temperatures higher than 30 °C had very negative effects on textural properties of plasma proteins (Fort, Mompió, Dávila, & Saguer, 2004). The incubation temperature during enzymatic treatment is important not only attending to the optimal conditions for enzyme activity and the susceptibility of substrate to the enzyme attack; but also taking into account the possible effects on protein structure and those due to the activity of other enzymes present in plasma that could alter protein functionality. On the other hand, it was also observed that incubation pH had not effects on plasma gel texture in the value range from 5.5 to 7 (Fort et al., 2004). These results partially disagree with the ones reported by other authors who maintained that this parameter sharply affects *TGase* activity (Sakamoto, Kumazawa, & Motoki, 1994). The effects on porcine plasma gelling properties at pH 5.5 of microbial transglutaminase at different concentrations and several incubation periods at 30 °C and pH 7 before gelation were thereby determined, and a non-treated sample was included in each experiment (Fig. 1).

The statistical analysis indicated that gels from enzyme-treated samples were overall significantly harder than those from control samples ($P < 0.05$). Hardness values obtained for plasma gels of control samples are consistent with those reported in previous studies (Saguer et al., 2004). When only enzymatic treatments were compared, a significant effect of concentration on hardness was detected ($P < 0.05$), with the quadratic term in the polynomial contrast of concentration being significant ($P < 0.05$) and showing evidence for the hump between lower and higher concentrations. These results agree with several studies evidencing that the use of *MTGase* above the optimum concentration can have on the one hand a detrimental effect on textural properties of gels (Asagami, Ogiwara, Wakameda, & Noguchi, 1995; Imm, Lian, & Lee, 2000; Jiang, Hsieh, Ho, & Chung, 2000; Kuraishi et al., 1997; Sakamoto et al., 1994; Tsai, Lin, & Jiang, 1996). It has been hypothesized that an excess of cross-linking covalent bonds would inhibit uniform development of the protein network (Kuraishi et al., 1997). On the other hand, not only *MTGase* concentration but also incubation time significantly affected gel hardness ($P < 0.05$), this parameter being higher when enzymatic treatment was applied for 3 h. Although no higher incubation times were considered in this study, previous trials which were carried out in our laboratory including periods of incubation until 5 h (data not shown) indicated that, at the same tested enzyme concentrations, the saturation of the response was achieved in the time range from 2 to 3 h.

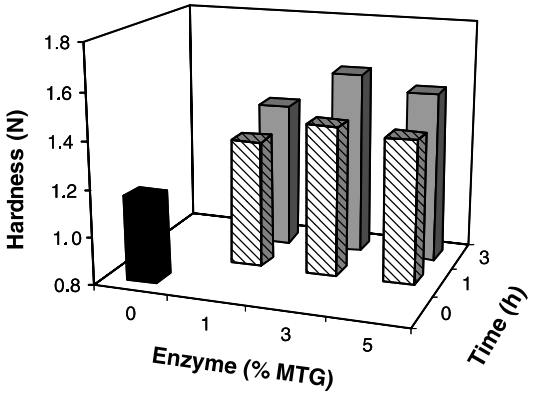

Fig. 1. Hardness of heat-induced plasma gels at pH 5.5 as influenced by *MTGase* concentration, expressed as % of commercial product (MTG), and incubation time at 30 °C and pH 7 previously to gelation.

Incubation with microbial transglutaminase also caused a significant increase of springiness of plasma gels at pH 5.5 with respect to control samples ($P < 0.05$) (Table 1). However, no significant differences depending on *MTGase* concentration or incubation time were found ($P > 0.05$). In all samples values of springiness higher than 0.9 were obtained, indicating a high elasticity, with relatively small increases because of the enzymatic treatment. Contrasting to this, enzymatic treatments did not significantly affect cohesiveness gel ($P > 0.05$), being the values for treated-samples essentially the same as for the controls.

The effects of the *MTGase* concentration and reaction times on water losses after centrifugation of plasma gels are shown in Fig. 2. The statistical analysis indicated that WHC, expressed as released water after centrifugation of acid gels prepared from *MTGase*-treated plasma, was significantly lower than that from the control samples ($P < 0.05$), which could result from increased protein-water interactions. However, no significant ($P > 0.05$) differences among the assayed enzymatic treatments were detected for this parameter, though the minor losses were obtained when treated with 3% MTG for 3 h. In this case, the reduction in water loss was around 3% with respect to control samples. Other authors observed that *MTGase* improved not only gel hardness but also WHC (Imm et al., 2000). The best treatment seems largely to be 3% MTG for 3 h. This enzyme concentration is equivalent to 52.5 enzymatic activity units, a relatively high value compared to those ones found in the literature. Even so, disparate values are reported, depending basically on substrate characteristics and its concentration: from 0.1 to 1 units of enzymatic activity per gram of surimi (Jiang et al., 2000; Ramírez,

Table 1
Springiness and cohesiveness of plasma gels at pH 5.5 as a function of the *MTGase* concentration, expressed as % of commercial product (MTG), and the incubation time at 30 °C and pH 7

MTG (% p/v)	Incubation time (h)	Springiness	Cohesiveness
0 (control)	0	0.90 ± 0.01 a	0.54 ± 0.01
1	1	0.92 ± 0.01 b	0.55 ± 0.01
1	3	0.92 ± 0.01 b	0.55 ± 0.01
3	1	0.93 ± 0.01 b	0.55 ± 0.01
3	3	0.93 ± 0.01 b	0.55 ± 0.01
5	1	0.92 ± 0.02 b	0.54 ± 0.00
5	3	0.93 ± 0.01 b	0.55 ± 0.00

Minor letters indicate significant differences between treatments according to the Tukey–Kramer test.

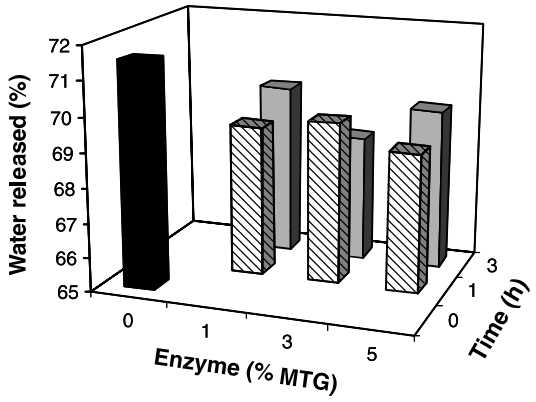

Fig. 2. Water released after centrifugation of heat-induced plasma gels at pH 5.5 as influenced by *MTGase* concentration, expressed as % of commercial product (MTG), and incubation time at 30 °C and pH 7 previously to gelation.

Santos, Morales, Morrissey, & Vazquez, 2000; Ramírez, Uresti, Téllez, & Vázquez, 2002); 1.4 units per gram of protein in beef gels (Pietrasik & Li-Chan, 2002a); 3–6 units per gram of protein working with batter pork (Pietrasik & Li-Chan, 2002b); 10 units per gram of protein to improve gelling properties of oat globulin (Siu, Ma, Mock, & Mine, 2002); and 20 units per gram of protein to cross-link milk proteins (Faergemand & Murray, 1998).

In order to demonstrate the participation of *MTGase* in the improvement of gelling properties, enzymatic reaction products for different enzyme concentrations and incubation times at 30 °C were analysed by SDS polyacrylamide gel electrophoresis and compared to the electrophoretic pattern of the control samples (Fig. 3). The results confirmed that intermolecular cross-linking has occurred. As concentration of *MTGase* or incubation time increased, some protein bands (A_1, A_2, A_3, A_4), whose molecular weight were roughly 50, 88, 139 and >220 kDa, respectively, progressively decreased or disappeared. In addition, the formation of high molecular weight polymers was detected in the form of bands both in the stacking gel (B), which could not enter the separation gel, and those ones which did not penetrated the running electrophoresis gel (D). Consequently, the molecular weight of these new polymers was higher than 250 kDa. Moreover, a band of 29 kDa was also evidenced (E) when plasma was treated with *MTGase*, which could result from the cross-linked small peptides.

It has been shown that *MTGase* cannot attack native serum albumin when it is in the native state due to its compact globular structure, though under determined conditions it is able to form polymers with other substrates structurally and thermodynamically compatible (Han & Damodaran, 1996; Traoré & Meunier, 1992). In general, globular proteins can become more susceptible to polymerization following partial unfolding due to chemical modification, altering pH, treating with reducing agents, and adjusting temperature and times of enzymatic incubation (Aboumahmoud & Savello, 1990; de Jong et al., 2001; Faergemand, Otte, & Qvist, 1997; Ikura, Goto, Yoshikawa, Sasaki, & Chiba, 1984; Nonaka et al., 1989; Traoré & Meunier, 1992). Therefore, it could be expected in the assayed conditions that serum albumin would not participate in the cross-linking reactions. From electrophoretic gels, it also seems clear that at least one monomer with lower (A_1) and three with higher (A_2, A_3 and A_4) molecular weight than serum albumin participated in the cross-linking reactions.

3.3. Effect of thermal treatment and maltodextrine

Besides the obtained evidences for the cross-linking reactions when plasma was treated with *MTGase*, the effects of heat treatment alone on the gelling properties were determined. Fig. 4 shows the effects of maintaining plasma at 30 °C during 1 and 3 h on the hardness and the capacity

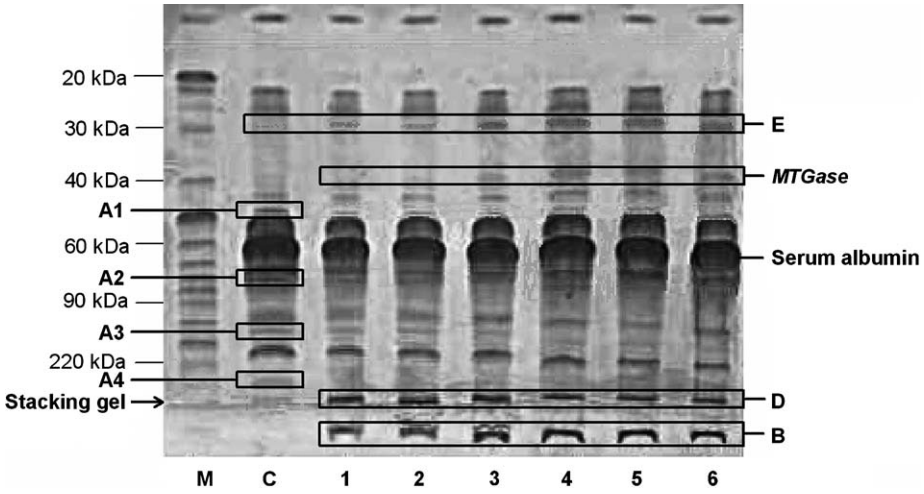

Fig. 3. Changes in SDS–PAGE profiles of plasma solutions incubated with various amounts of *MTGase* for several times at 30 °C and pH 7: (M) molecular markers from 20 to 220 kDa; (C) control; (1) 1% MTG/1 h; (2) 1% MTG/3 h; (3) 3% MTG/1 h; (4) 3% MTG/3 h; (5) 5% MTG/1 h; (6) 5% MTG/3 h.

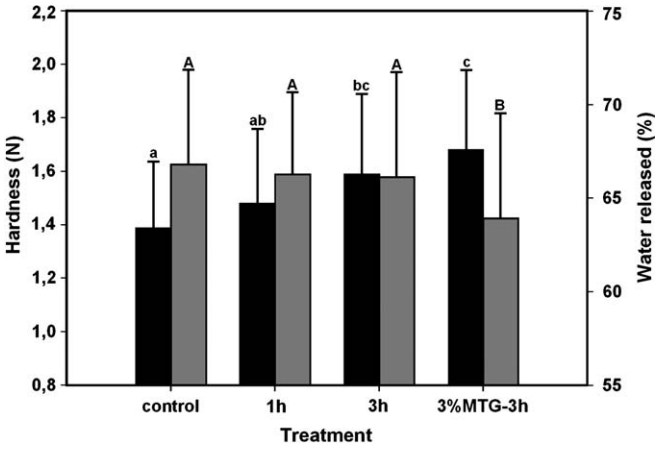

Fig. 4. Effect of heat treatment at 30 °C and pH 7 for 1 or 3 h previous to gelation on hardness (■) and WHC (▒), expressed as water released after centrifugation, of heat-induced plasma gels at pH 5.5. The treatment with 3% MTG for 3 h was also included. Values are means ± SD ($n = 3$). Treatments with the same minor or capital letter are not significantly different ($P < 0.05$) for hardness or water released after centrifugation, respectively, according to the Tukey–Kramer test.

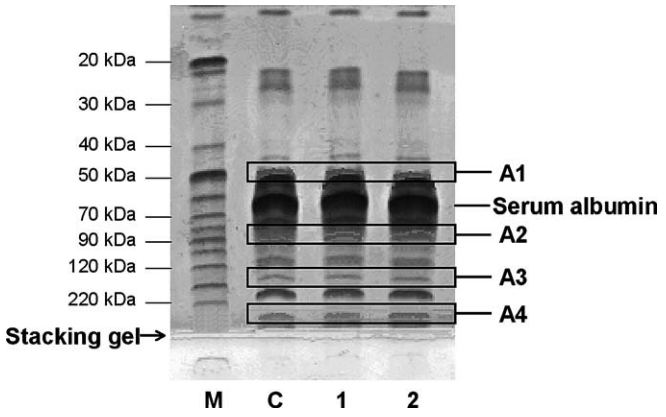

Fig. 5. SDS–PAGE profiles of plasma solutions incubated at 30 °C and pH 7 for several times before gelation: (M) molecular markers from 20 to 220 kDa; (C) control; (1) 1 h; (2) 3 h.

to bind water of plasma gels at pH 5.5. At the same figure, the results corresponding to enzymatic treatment with 3% MTG for 3 h were included. As it can be observed, treatment at 30 °C during 3 h significantly increased hardness as compared to control samples ($P < 0.05$), reaching values not significantly different from those corresponding to the enzymatic treatment ($P < 0.05$). These results indicate that the hardness improvements from *MTGase*-treated plasma gels were not due to enzymatic activity. On the contrary, springiness, cohesiveness (Table 2) and WHC (Fig. 4) were not significantly affected by heat treatment ($P > 0.05$); only the treatment with 3% MTG for 3 h caused a significant decrease of released water and increase of springiness ($P < 0.05$), as in the previous experiment.

In the polyacrylamide gel of plasma solutions treated at 30 °C during 1 and 3 h (Fig. 5) no bands evidencing cross-link reactions were observed, indicating that no polymerization reactions other than disulfide bonds could have taken place; in addition, no other changes in electrophoretic patterns of these plasma solutions with respect to the control samples were detected. Due to the high compositional complexity of plasma, which includes the presence of multiple and variable enzymes, the phenomena responsible for the plasma behavior during heat treatment can not be easily explained. However, results could suggest that, even at conditions close to room temperature, the configuration of the protein macromolecules could be slightly perturbed after several hours. It is possible that minor local unfolding leads internal hydrophobic groups to move outside of the molecule exposing them to the solvent; this way, an increase in new protein surfaces could result in more hydrophobic interactions between molecules. Currently, evidence for the existence of a multiplicity of intermediate stages between the native and unfolded states of proteins is growing (Farrell et al., 2002). Nevertheless, other possibilities such as protein modification as a consequence of the activity of several enzymes that have been found in plasma should also be considered.

Finally, the possible maltodextrine effects on heat-induced plasma gels properties were also determined by incubating the plasma solution with inactivated commercial *MTGase* at the optimal conditions (Ruíz-Carrascal & Regenstein, 2002). The obtained results indicated that the maltodextrine present in the enzymatic complex did not have significant effects on gelling properties ($P > 0.05$), being the values of hardness, springiness, cohesiveness and WHC very similar to those of the control samples.

Table 2
Springiness and cohesiveness of plasma gels at pH 5.5 as a function of the *MTGase* concentration, expressed as % of commercial product (MTG), and the incubation time at 30 °C and pH 7

Treatment	Incubation time (h)	Springiness	Cohesiveness
0 (control)	0	0.91 ± 0.02 a	0.54 ± 0.02
30 °C	1	0.92 ± 0.02 a	0.55 ± 0.02
30 °C	3	0.92 ± 0.01 a	0.55 ± 0.02
3% MTG	3	0.93 ± 0.01 b	0.55 ± 0.02

Minor letters indicate significant differences between treatments according to the Tukey–Kramer test.

4. Conclusions

It can be concluded that gelling properties at acidic conditions of plasma gels can be improved by treatment with *MTGase* under specific conditions, especially because it can contribute to reduce the degree of syneresis. The most effective treatment resulted in incubating plasma solutions with 3% MTG for 3 h at 30 °C and pH 7. At these conditions, the enzymatic treatment caused an increase of around 0.4 N in gel hardness, approximately 30% higher than the control, and a reduction in released water after gel centrifugation of around 3%. It is important to note

that improvements on hardness could be partially related to the thermal treatment associated to the incubation conditions. In general, these results could also suggest that some minor plasma proteins are involved in the improvement of gelling properties of blood plasma. We consider that there are many alternatives to be tested in order to enhance these results, especially through the combination of *MTGase* with a reducing agent or by increasing substrate concentration through plasma ultrafiltration before enzymatic treatment.

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