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Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels

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Abstract

Low-fat protein gels obtained by pressure are softer than those processed by conventional heat treatment. In this study, microbial transglutaminase (MTGase) (0.3%) was added to chicken batters in order to investigate the combined effect of pressure and enzyme on the functional properties of gels. Batters of meat with egg proteins were treated at 500 MPa for 30 min at 40 °C and then heated at 75 °C for 5 min to inactivate the enzyme. Treated samples showed, under confocal microscopy, a more compact and homogeneous microstructure and exhibited a notable increase in hardness and chewiness as compared to controls that were pressurized but contained no MTGase. They were also harder, more chewy and springy but had a similar cohesiveness and cutting force to those obtained by heat alone.

Keywords: Microbial transglutaminase; High pressure; Chicken meat gels; Texture; Microstructure

1. Introduction

Consumer demand for minimally processed, microbiologically safe, stable food products that are additive-free, has stimulated the interest of food companies in high pressure processing (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998). Growing awareness of the link between diet and health is fast-changing consumer habits, so that there has been increasing request for foods with health-enhancing properties, such as low-fat meat products (Jiménez Colmenero, 2000). In recent years, it has also been recommended that salt intake should be reduced in light of the relationship between high sodium levels and development of arterial hypertension. However, in products with reduced levels of sodium, the functionality of the traditional myosin heat-set matrix may be limited due to low ionic strength, water binding and a decrease in the firmness of meat gels (Smith, 1988; Whiting, 1988).

Many processed meat products that have been traditionally made from pork have high levels of fat. As a result, chicken with fat substitutes is now being used to manufacture emulsified sausages in order to obtain healthier meat products (Jiménez Colmenero, Carballo, & Cofrades, 2001). However, reformulation with fat substitutes can cause a reduction in particle binding, darker product colour, lack of flavour, reduced browning reactions and shorter microbiological shelf-life (Keeton, 1994).

Fisher (1994, chap. IV) indicated that egg proteins help to stabilize batters and may be advantageous in increasing binding properties. Numerous authors have used egg white as a functional ingredient in a number of ground and emulsified meat products to support and ensure the binding properties of meat (Carballo, Barreto, & Jiménez Colmenero, 1995; Carballo, Fernandez, Barreto, Solas, & Colmenero, 1996; Fernández, Cofrades, Solas, Carballo, & Jiménez Colmenero, 1998; Jiménez Colmenero, Barreto, Fernandez, & Carballo, 1996; Pietrasik, 2003; Pietrasik & Li-Chan, 2002a).

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In a previous study, functional characteristics, such as water-holding capacity, texture and colour of low fat chicken gels were modified with the addition of ovalbumen as fat substitute and pressure processing (Trespalacios et al., 2002). Protein gels made under pressure are generally glossier and softer than heat induced gels (Jiménez Colmenero, 2002; Okamoto, Kawamura, & Hayashi, 1990). On the other hand, sausages made from chicken meat obtained by heat (Muguruma et al., 2003) or pressure do not provide desirable gel strength (Yuste, Mor-Mur, Capellas, Guamis, & Pla, 1999).

Transglutaminase (TGase) (EC 2.3.2.13, protein-glutamine γ-glutamyl transferase) is an enzyme that catalyses acyl transfer reactions between the γ-carboxyamide group of glutamine residues and the ε-amino group of lysine in proteins, leading to inter- or intra-molecular cross-linking (De Jong & Koppelman, 2002). Transglutaminases are a widely distributed family of enzymes found in plants, animal tissues and body fluids of mammals, which can modify proteins by means of amine incorporation, cross-linking and deamidation (Motoki & Seguro, 1998). Sakamoto, Kumazawa, Kawajiri, and Motoki (1995) quantitatively analysed ε-(γ-glutamyl)lysine cross-links in 127 different foods and the highest levels were found in fish paste products, processed fish, shellfish, meats, soybeans and raw poultry organs. Thus, TGase cross-linked proteins have been long ingested by man.

The production of TGase for industrial use was made possible by the isolation (Nonaka et al., 1989) and purification (Ando et al., 1989) of a bacterial TGase from a micro organism taxonomically classified as a variant of *Streptoverticillium mobaraense*. Transglutaminases require Ca^{2+} for expression of enzymatic activity; however, microbial transglutaminase (MTGase) is totally independent of Ca^{2+} (Motoki et al., 1990). Such a property is very useful in the modification of the functionality of food proteins, such as casein and myosin, because they are easily precipitated in the presence of Ca^{2+} and become less sensitive to MTGase (Motoki & Seguro, 1998).

Transglutaminase is now widely used in seafood, surimi products, meat products, noodles and pasta, dairy products and baked goods (Kuraishi, Yamazaki, & Susa, 2001). Although the effect of the MTGase is well documented for raw and restructured meats (Kuraishi et al., 1997; Lee & Park, 2003; Nielsen, 1995; Serrano, Cofrades, & Jiménez Colmenero, 2004; Tsao, Kao, Hsieh, & Jiang, 2002), few studies have been reported on characteristics of cooked meat emulsions (Pietrasik, 2003; Pietrasik & Jarmoluk, 2003; Pietrasik & Li-Chan, 2002a; Pietrasik & Li-Chan, 2002b). Only some chicken meat products have been developed (Kilic, 2003; Muguruma et al., 2003; Tseng, Liu, & Chen, 2000).

Nonaka et al. (1989) showed that rabbit myosin was polymerised by a catalytic reaction of the microbial transglutaminase (MTGase), but actine was not affected under the same conditions. On the other hand, globular proteins, such as β-lactoglobulin, α-lactalbumin and ovalbumin, have proven to be poor substrates because of their compact structures, which limit the accessibility of the TGase to the target glutamine and lysine residues (Nio, Motoki, & Takinami, 1985; Sakamoto, Kumazawa, & Motoki, 1994). Furthermore, ovalbumin and conalbumin were found only to be modified by MTGase when a reducing agent like dithiothreitol was used, which is undesirable for food manufacturing (Nonaka et al., 1989). Subsequent investigations have shown that pre-treatment or simultaneous application of high pressure at 400–600 MPa can induce structural changes in the native protein, making it accessible to the acyl binding site of MTGase (Nonaka, Ito, Sawa, Motoki, & Nio, 1997). Other reports of high pressure effects on various biomolecules indicate that this may be a suitable denaturing treatment for enhancing TGase activity (Ashie & Lanier, 1999; Gilleland, Lanier, & Hamann, 1997).

MTGase dissolved in buffer solution exhibits a remarkable stability toward high pressure treatment above 400 MPa at 60 °C (Lauber, Noack, Klostermeyer, & Henle, 2001b). 60% of initial MTGase activity was maintained even after pressurization at 600 MPa for 60 min, indicating that MTGase was pressure-resistant compared to other enzymes (Lee & Park, 2002).

Although many references can be found on the separate application of TGase and pressure to different food products, some hypotheses have been suggested to elucidate the mechanism involved when they are applied together (Ashie & Lanier, 1999; Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2006); there is a lack of knowledge on their combined effects. Thus, the objective of this study was to investigate the simultaneous application of high pressure and MTGase on poultry meat emulsions and to improve the textural characteristics of low-fat and low-salt chicken meat gels added with ovalbumen and egg yolk.

2. Materials and methods

2.1. Preparation of low fat and low salt chicken gels

Fresh chicken thighs and eggs were purchased from a local market (Corporación Alimentaria Guissona, S.A., Guissona, Spain). Skinless, boneless meat was trimmed to remove visible fat and connective tissue, ground twice through 6 and 3 mm plates in a mincer Mod. PC-22 (Sammic, S.L., Azpeitia, Spain), then mixed with NaCl (1.0% w/w total formulation) and left to stand for 18 h at 4 °C. The mixture was homogenised with 10% fresh egg yolk, 10% dehydrated egg white and 0.3% of tripolyphosphates (Degussa Texturant Systems, Barcelona, Spain) and cold water (30%) in a homogeniser Mod. UMC 5, (Stephan Machinery GmbH & Co., Hameln, Germany) at 1800 rpm for 12 min at 80% vacuum. The final temperature of the batters never exceeded 12 °C. Samples with enzyme were added with 0.3% Transglutaminase Activa™ WM (Ajinomoto Co. Inc., Tokio, Japan) which contains 99% maltodextrins and 1% MTGase with an activity of 100 units/g. Immediately after this, the batters were stuffed,

by means of a sausage filler Mod. TWF-6 (Dick GmbH, Deizisau, Germany), into Nojax® cellulose casing (22 mm diameter) (Viskase Companies, Inc., Willowbrook, USA) or polyvinylidene casing (55 mm diameter) (Krehalon Industrie B.V., Deventer, Holland). The samples were vacuum packaged in a Cryovac Corace Packaging VS 26 (Cryovac Europe, Kriens, Switzerland) before treatments. Batters with MTGase were treated no later than 10 min after enzyme was added. Samples without enzyme were produced under the same conditions.

2.2. High pressure and thermal treatments

A discontinuous isostatic press ACB (GEC Alsthom, Nantes, France) was used for HP processing at 500 MPa for 30 min at 40 °C. The time needed to achieve the treatment pressure was ca. 120 s and the decompression time was ca. 30 s. The pressure chamber (22 cm height, 10 cm diameter) and the water inside were held at the indicated temperature, by circulating hot water through a coil around the walls of this chamber. Another batch of samples was treated at 40 °C in a water bath for the same amount of time at atmospheric pressure (0.1 MPa). A third treatment was performed at room temperature (20 °C) at 0.1 MPa. Finally, all samples were heated in a water bath to assure an internal temperature of 75 °C for 5 min to inactivate the added enzyme (Ando et al., 1989) and cooled in water. For the heat treatment, samples were heated to 75 °C (Thermometer 638 Pt, Crison Instruments, S.A., Barcelona, Spain) for 30 min in a water bath and then cooled. Samples without enzyme were treated under the same pressure and heat conditions. All treated samples were stored at 4 °C for their analysis. These experiments were performed twice.

2.3. Proximate analysis and pH

Moisture, ash and total nitrogen of the raw meat, batters and treated samples were quantified in triplicate (Association of Official Analytical Chemists, 1990). Fat content was estimated by difference. A conversion factor of 6.25 was used for protein content. The pH was measured in triplicate using a portable pH meter Mod. 507-05 (Crison Instruments, S.A. Barcelona, Spain) on a homogenate of 5 g sample in 50 ml distilled water at 20 °C. All chemicals were from Panreac (Barcelona, Spain).

2.4. Expressible moisture

Coarsely chopped sample (ca. 1.5 g) (W_i) wrapped with a Whatman No. 1 filter paper was placed in a centrifuge tube and submitted to 4000*g* for 10 min at 20 °C in a centrifuge Mod. J221 (Beckman Coulter, Inc., Fullertone, USA). This determination was carried out in quadruplicate. Expressible moisture (E_m) is expressed as the ratio of moisture lost after centrifugation (W_f) to the initial gel sample weight

$$E_m = (W_i - W_f/W_i)100.$$

2.5. Instrumental colour analysis

The colour of the meat gels was measured using a portable spectrocolorimeter Model 45/0 L Mini Scan XE™ (Hunter Associates Laboratory, Inc., Reston, USA) and expressed as *L* (lightness), *a* (redness), and *b* (yellowness). Six cylindrical replicates (55 mm diameter) were cut to measure the internal colour of gels. Measurements were done with reference to illuminant *Fcw* and the 10° standard observer.

2.6. Instrumental texture analysis

The textural characteristics of gels were analysed according to texture profile analysis (TPA) (Bourne, 1978) and force at cutting using a TA-XT2 Texture Analyser (Stable Micro Systems, Haslemere, UK) with a 25 kg load cell (±1 g). Four cylindrical replicates were cut (22 mm diameter and 20 mm height) and axially compressed twice to 40% of their original height at a crosshead speed of 1 mm/s with an aluminium compression plate P50 (5 cm diameter). Force at cutting was measured with a probe HDP/BSK to six cylindrical replicates across the diameter of the gels (22 mm).

2.7. Confocal scanning laser microscopy

A confocal scanning laser microscope Leica DM IRE 2 (Leica Microsystems, Heidelberg, Germany) was used to observe the microstructure of gels. Gels were cut in slices of 0.5–1.0 mm and soaked in a 0.1% aqueous acridine orange solution (Panreac, Barcelona, Spain) mixed with an equal volume of 1% acetic acid for 5 min to stain the gels (Yiu, 1985). After rinsing and draining, sections were mounted in a non-fluorescent observation medium between two glass slides. The images were obtained with an oil immersion 40× lens with aperture set to 1.3. Samples were excited at 568 nm with a Kr/Ar laser for the observation (Heertje, Vandervlist, Blonk, Hendrickx, & Brakenhoff, 1987).

2.8. Statistical analysis

The data were analysed using the general linear model procedure of The SAS® System for Windows V 8 (SAS Institute Inc., Cary, USA). Level of significance was set for $P < 0.05$. Differences between each variable and treatment were determined using Duncan's multiple range test.

3. Results and discussion

3.1. Composition and pH

The proximate analysis of raw chicken meat was similar to that reported in the bibliography. Results for thigh

chicken meat were: moisture, 74.16% ± 0.72; protein, 19.36% ± 1.99; ash, 1.11% ± 0.06 and fat, 5.37%, which match the values indicated in the study of variations in muscle composition of broilers (Xiong, Cantor, Pescatore, Blanchard, & Straw, 1993). Protein contents in the dried egg white and egg yolk were 88.5% ± 0.95 and 17.5% ± 0.78, respectively. The pH of raw meat (6.65) increased after adding the egg white and the pH of batter with MTGase was slightly more acidic (6.84) than that of samples without MTGase (6.89). Gels without enzyme had 69.11% ± 0.13 moisture and 19.9% ± 1.05 protein while gels with MTGase had 67.7% ± 0.14 moisture and 19.3% ± 1.75 protein.

3.2. Expressible moisture

The addition of enzyme significantly decreased the expressible moisture of samples from each treatment and was significantly higher in the non-pressurised samples, as shown in Table 1. Gels obtained by pressure without enzyme showed lower values than those produced with the enzyme added but without pressure at 20 and 40 °C. The lowest expressible moisture was obtained from the gel with added MTGase and treated by pressure, showing a synergistic effect of enzyme and pressure when they are applied at the same time. The binding properties are strongly influenced by sodium content in processed muscle foods. Higher ionic strength enhances electrostatic repulsions and causes a loosening of the myofibrillar structure, so that there is more space for water to be trapped (Whiting, 1988). Barbut and Mittal (1990) reported that reduced-salt batters were less stable because of lower extraction of salt-soluble proteins. Increased salt levels (from 0% to 2%) have also been reported to reduce the cooking loss (from 28% to 14%) in poultry meat batters (Hongsprabhas & Barbut, 1999).

The effect of pressure on binding properties of meat products depends on various factors, such as animal species, type of muscle, pH and ionic strength, level of fat and protein and treatment conditions, such as pressure, time and temperature. Jiménez Colmenero, Carballo, Fernandez, Barreto, and Solas (1997) reported no differences between the water-binding properties of low-fat and high-fat pork sausages treated at 100 MPa, whereas those treated at 300 MPa showed an increase in total cooked-out fluid and water released, which became more pronounced with a longer pressurisation time. However, poultry sausages pressurised at 500 MPa for 30 min at 50, 60, 70 or 75 °C, presented lower cooking losses than those treated at 75 °C for 30 min (Yuste et al., 1999).

Table 1
Expressible moisture of low-fat and low-salt chicken gels obtained by pressure and microbial TGase (0.3%)[A]

Enzyme	Pressure (MPa)	Temperature (°C)	Expressible moisture (%)
NE	0.1	20	32.5 ± 2.0 b
NE	0.1	40	36.9 ± 2.0 a
NE	500	40	24.2 ± 1.1 d
MTGase	0.1	20	27.6 ± 3.0 c
MTGase	0.1	40	28.4 ± 1.3 c
MTGase	500	40	19.1 ± 2.0 e

NE, no enzyme added; MTGase, microbial transglutaminase (0.3%).
[A] Different letters in the same column indicate significant differences ($P < 0.05$).

The consequence of TGase on binding properties in meat products has been contradictory. Some experiments have shown that proteins in oil-in-water type emulsions can be gelled and that MTGase enhanced the emulsion stability and water uptake ability of chicken batters (Ruiz-Carrascal & Regenstein, 2002). Meanwhile, Pietrasik and Li-Chan (2002b) found that the presence of increasing levels of MTGase (0–0.6%) favourably decreased the cooking loss at different levels of sodium chloride (0–2%) in cooked pork batter gels but that low-salt batters produced gels that had poorer binding properties than gels produced with higher salt.

The addition of 0.5% MTGase, to beef homogenates, significantly decreased expressible moisture, cooking and purge loss from beef gels added with egg white (Pietrasik, 2003). Tseng et al. (2000) also reported that the yield of low salt (1%) chicken meatballs increased with higher levels (0–1%) of crude pig plasma TGase, suggesting that they had better emulsion stability and hydration properties. However, Hammer (1998) did not find a significant effect of MTGase (0.2%) for water-binding properties in finely comminuted sausages. Also, Pietrasik and Jarmoluk (2003) reported that MTGase had no effect on water-binding properties, although it increased the hardness of pork gels at higher levels of sodium caseinate. Cooking and purge losses were not appreciably affected by enzyme presence in beef gels, except when they were formulated with egg white which showed significantly lower purge losses after one week's storage compared to the samples elaborated without MTGase (Pietrasik & Li-Chan, 2002a).

Reports about the effects of egg proteins on hydration properties of meat gels have also been contradictory. Fisher (1994, chap. IV) explained that egg white participates in the formation of a protein network that enhances water-binding capacity and extends freshness of meat products. Conversely, Hammer (1992, chap. 5) reported that egg white plays no part in the structure of gels, while Carballo et al. (1995) did not find any influence on water- and fat-binding properties of pork meat batters. However, when pressure was applied at 70 °C the addition of egg white increased water binding of chicken meat batters (Fernández et al., 1998).

3.3. Colour

The intensity of muscle visual colour is due to its total myoglobin content. When the myoglobin content is kept constant, the colour of comminuted products is mostly influenced by processing parameters: fat content, non-meat ingredients and added or lost water. It is well known that

Table 2
Hunter Lab values of low-fat and low-salt chicken gels obtained by pressure and microbial TGase (0.3%)[A]

Enzyme	Pressure (MPa)	Temperature (°C)	L^*	a^*	b^*
NE	0.1	75	75.0 ± 0.2 a	2.64 ± 0.04 e	15.0 ± 0.2 c
NE	0.1	20	72.0 ± 4.1 b	5.91 ± 1.30 b	16.3 ± 2.2 b
NE	0.1	40	69.7 ± 0.8 c	6.72 ± 0.30 a	17.5 ± 0.6 a
NE	500	40	72.9 ± 1.0 ab	5.58 ± 0.33 bcd	16.5 ± 0.2 b
MTGase	0.1	20	73.7 ± 1.7 ab	4.98 ± 0.37 d	16.9 ± 1.5 ab
MTGase	0.1	40	71.7 ± 1.8 b	5.75 ± 1.01 bc	17.6 ± 0.2 a
MTGase	500	40	74.2 ± 1.7 a	5.11 ± 0.84 cd	17.7 ± 0.1 a

NE, no enzyme added; MTGase, microbial transglutaminase; L^*, lightness; a^*, redness; b^*, yellowness.
[A] Different letters in the same column indicate significant differences ($P < 0.05$).

application of high pressure, even at 5–10 °C, induces drastic changes in the colour of red muscle (Cheftel & Culioli, 1997).

Table 2 shows that there were no differences in lightness of sample obtained at 75 °C and those obtained by pressure with or without MTGase, possibly due to the inactivation step of the enzyme (75 °C for 5 min). However, samples with MTGase treated at 40 °C without pressure were lighter than those were treated without enzyme at same conditions. Non-pressurised samples without enzyme at 20 °C showed the lowest L^* values.

The increase of L^* values or ''whitening'' effect is attributed to the denaturation of the globin moiety of myoglobin. According to Zipp and Kauzmann (1973), denaturation of metmyoglobin under pressure is similar to denaturation caused by heat, acid or urea, and is partly due to the rupture of hydrophobic bonds. They found that, at 550 MPa, the denaturation was complete, rapid and no further changes were seen by spectrophotometry at higher pressures. However, Defaye, Ledward, Macdougall, and Tester (1995) reported that the degree and rate of renaturation of myoglobin solutions subjected to 7.5–8 kbars, was dependent on pH and ionic strength, suggesting that electrostatic forces dominate the protein-protein aggregation and that hydrogen bonds may also stabilise the aggregate. Compared to the standard cooking process, Yuste et al. (1999) found that 500 MPa of pressure, applied at high temperatures (50–75 °C), yielded lighter and more yellow mechanically recovered poultry meat sausages.

At atmospheric pressure, the addition of MTGase decreased the redness of samples treated at 40 and 20 °C. However, there were no significant differences in those samples treated at 500 MPa, with or without enzyme, which were more reddish than the cooked ones. Nielsen, Petersen, and Moller (1995) reported that pork meat samples with 0.4% TGase Factor XIIIa had significant lower a^* values than the controls in raw meat treated at 37 °C for 90 min or 10 °C for 23 h without any other thermal or pressure processing. In addition, gels obtained by heat showed lesser a^* values mainly due to globin denaturation. For example, when minced meat is subjected to pressure above 400 MPa its colour becomes much paler, due to the partial oxidation of ferrous myoglobin into ferric metmyoglobin and with possible globin denaturation (Carlez, Veciana-Nogues, & Cheftel, 1995; Cheftel & Culioli, 1997).

There were no differences in b^* values of samples with or without MTGase treated at the same temperature and atmospheric pressure, however, pressure decreased yellowness in samples without enzyme. Meanwhile, heat treatment decreased the yellowness in relationship to all samples treated at 20 and 40 °C with or without enzyme. Pietrasik (2003) found that MTGase increased b^* of beef gels with egg white processed at 80 °C for 14 min, however, Hammer (1998) observed a linear decrease related to the amount of the enzyme (0–0.2%) in finely comminuted cooked sausages. Both authors reported no differences in L^* and a^*.

Pietrasik and Li-Chan (2002b) also studied the effect of MTGase, salt and heating temperature on the colour of pork gels. While they did not find significant influence of the enzyme, yellowness and redness were inversely proportional to the salt content. Previous reports have shown that TGase had no effect on colour parameters of chicken meat products, such as döner kebabs (Kilic, 2003), low-salt chicken meat balls (Tseng et al., 2000) and pork gels (Pietrasik & Jarmoluk, 2003). No references about the effect on the meat colour were found when MTGase and pressure (500 MPa) were applied.

3.4. Textural properties

Table 3 shows how the enzyme addition notably modified the characteristics of the texture profile analysis in samples processed at 40 °C. However, those treated at ambient temperature did not show significant differences between samples with and without MTGase, due to the short reaction time at that temperature, which it is not the most favourable. According to Ando et al. (1989), the time required for the reaction at 20 °C is 70 and 20 min at 40 °C when there is enough substrate. In restructured meats, several authors have demonstrated the ability of MTGase to bind the meat pieces at chill or mild temperature with reaction times that vary from 2 to 5 h at 5 °C (Kuraishi et al., 1997), 90 min at 37 °C (Nielsen et al., 1995) or 60 min at 40 °C (Tsao et al., 2002).

In this study, the pressurized sample without enzyme was the softest and showed the lowest value for chewiness of all samples. These results concur with those obtained by Hayashi, Kawamura, Nakasa, and Okinaka (1989) and Okamoto et al. (1990), who showed that pressure induced

Table 3
Textural properties of low-fat and low-salt chicken gels obtained by pressure and microbial TGase (0.3%)[A]

Enzyme	Pressure (MPa)	Temperature (°C)	Hardness (N)	Springiness (mm)	Cohesiveness (dimensionless)	Chewiness (N × mm)	Cutting force (N)
NE	0.1	75	35.0 ± 1.7 b	7.01 ± 0.27 d	0.535 ± 0.01 a	131.4 ± 6.5 b	6.98 ± 0.57 a
NE	0.1	20	30.1 ± 0.9 c	7.04 ± 0.10 cd	0.514 ± 0.01 c	108.9 ± 4.8 cd	6.07 ± 0.79 abc
NE	0.1	40	29.6 ± 2.0 c	7.16 ± 0.13 bc	0.518 ± 0.01 bc	110.2 ± 9.5 c	6.07 ± 0.67 abc
NE	500	40	26.8 ± 1.1 d	7.26 ± 0.11 b	0.524 ± 0.01 b	102.0 ± 4.8 d	5.96 ± 0.67 bc
MTGase	0.1	20	31.0 ± 3.0 c	7.18 ± 0.13 bc	0.521 ± 0.01 bc	116.0 ± 10.3 c	4.96 ± 0.66 d
MTGase	0.1	40	33.2 ± 0.6 b	7.29 ± 0.08 b	0.523 ± 0.01 b	127.3 ± 2.1 b	5.17 ± 1.23 cd
MTGase	500	40	42.4 ± 1.7 a	7.48 ± 0.07 a	0.540 ± 0.01 a	171.4 ± 8.2 a	6.81 ± 1.21 ab

NE, no enzyme added; MTGase, microbial transglutaminase.
[A] Different letters in the same column indicate significant differences ($P < 0.05$).

protein gels were softer than heat induced ones but that they also tended toward increase in hardness and decrease in adhesiveness as pressure increased. Yuste et al. (1999) reported that pressure processing at high temperatures yielded less firm, less springy but more cohesive poultry meat sausages compared to standard cooking processing.

Addition of the enzyme at 40 °C increased the hardness and chewiness, due to the formation of ε-(γ-glutamyl)lysine bonds. Nonaka et al. (1989) proved that rabbit myosin was polymerised by MTGase, but actin was not. In experiments by Akamittath and Ball (1992) and Kim et al. (1993), polymerisation of turkey and beef actomyosin was induced by guinea pig liver TGase.

Our results are consistent with findings reported by various authors in cooked meat emulsions and meat products. Finely comminuted sausages containing 0.2% MTGase were harder and firmer than were sausages without enzyme (Hammer, 1998). Pietrasik and Li-Chan (2002a) also reported that beef gel samples with 0.5% MTGase had better textural characteristics than those without enzyme. Microbial TGase favourably increased hardness and chewiness of pork gels, but was not able to improve these parameters in low-salt products to the same levels as in high-salt ones and significant linear effects were observed for enzyme concentration (0–0.6%) on hardness and springiness (Pietrasik & Jarmoluk, 2003; Pietrasik & Li-Chan, 2002b).

Tseng et al. (2000) found that gel strength of low salt chicken meat balls increased with the addition of TGase. Chicken döner kebab also increased in hardness and chewiness with the presence of MTGase (1%) and the values were higher still when sodium caseinate was added (Kilic, 2003). Pietrasik (2003) reported that the addition of MTGase to beef gels induced greater hardness, cohesiveness, springiness and chewiness; moreover, cohesiveness increased only in samples containing egg albumen. In pork gels, springiness was also increased when 2% egg albumen was added (Pietrasik & Li-Chan, 2002a).

It can be seen in Table 3 that the combination of pressure and enzyme rendered samples with higher hardness, springiness, cohesiveness and chewiness values than samples only treated by pressure or with MTGase at atmospheric pressure at 40 °C.

Comparing the cutting force and hardness values of gels obtained at 75 °C and gels obtained by pressure and MTGase, it can be seen that the cutting forces for the two samples were very similar (6.98 and 6.81 N, respectively), while hardness was 34.99 N for heated gels and 42.43 N for the pressurised ones. This fact may be due to the different gelation process: in heat-induced gels, the outer part of gel becomes overcooked but, in the pressure-induced ones, the transmission is instantaneous and isostatic.

Pressurization at 250 or 300 MPa, of muscle proteins at 4 °C, did not induce gelation of turkey pastes. However, pressurization at 40 or 50 °C, prior to setting, increased the strength of turkey gels containing MTGase (Ashie & Lanier, 1999). Lauber, Noack, Klostermeyer, and Henle (2001a) did not detect oligomerization when β-lactoglobulin was incubated with MTGase under atmospheric pressure; however, it was evident when 400 MPa was applied.

Thus, we consider that the increase in hardness, chewiness, cohesiveness and springiness of gels is due to the simultaneous application of 500 MPa and MTGase. This induces structural changes in the native ovalbumin, exposing glutamyl and lysyl residues which may be buried inside their tertiary structure, and making them accessible to the acyl binding site of MTGase, which creates cross links between egg proteins and the myofibrillar proteins of meat.

In Table 3, it is clearly observed that applying MTGase and high pressure at the same time induces dramatic changes in texture. The introduction of cross-links produces structures with better hardness, chewiness and greater springiness compared to conventional heat gels. This behaviour is characteristic of a chemical gel network, whose unbreakable cross-links lead to a very high restoring force at large strains (Dickinson, 1997).

3.5. Microstructure

Enzymes can provide highly specific modifications in the microstructure of a biopolymer, which can result in significant changes in the macroscopic properties. Microphotographs of gels obtained by pressure, with and without enzyme, are shown in Fig. 1. The network of gel without

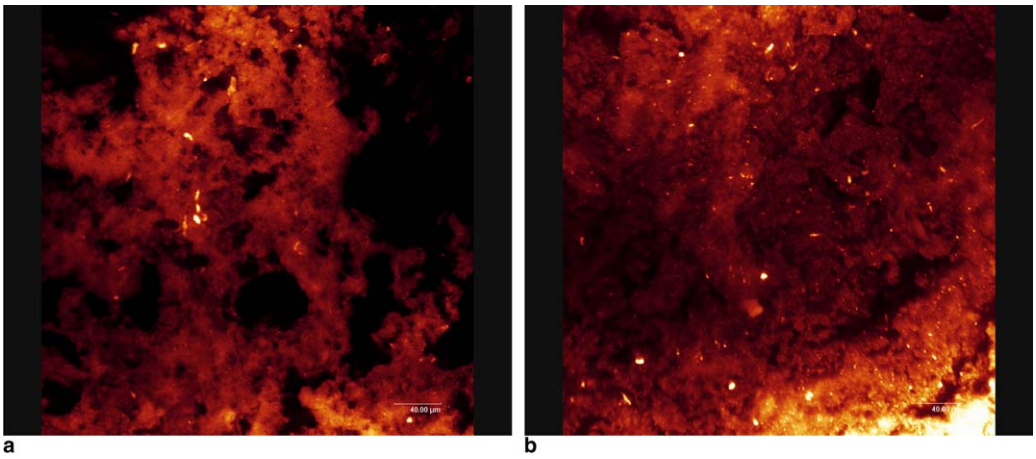

Fig. 1. Microstructure of low-fat low salt gels treated at 500 MPa and 40 °C for 30 min. (a) Without MTGase. (b) With MTGase added. Bar = 40 μm.

MTGase was loose with big, irregular holes; however, a more compact and homogeneous structure was observed when MTGase was present. These observations are coincident with those of texture: samples with MTGase that were simultaneously treated with pressure showed the highest values in overall TPA parameters, possibly due to inter- and intra-molecular ε-(γ-glutamyl)lysine cross-links between egg proteins and myofibrillar proteins. This is consistent with the conclusions of Muguruma et al. (2003) on chicken sausages and with Tseng et al. (2000) who reported that low salt chicken meat balls with TGase had more regular network structures and that higher levels of TGase (1.0%) produced bigger, more complete gel clusters. Siu, Ma, and Mine (2002) Chanyongvorakul, Matsumura, Nonaka, Motoki, and Mori (1995) found large differences in the organization of gel network structures between heat- and TGase-induced gels (oat and soybean), with larger clusters and thicker strands in the latter.

4. Conclusions

Microbial transglutaminase, in combination with high pressure treatment, offers possibilities for cross-linking of myofibrillar proteins with globular proteins, the latter not usually being affected by the enzyme under atmospheric pressure and, consequently, the binding properties, textural parameters, microstructure and colour of low-fat and low-salt chicken gels are enhanced. MTGase and pressure, applied simultaneously, offer a means of synthesizing new heterologous biopolymers valuable for the food industry.

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