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Synergistic action of transglutaminase and high pressure on chicken meat and egg gels in absence of phosphates

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Abstract

The effects of simultaneous application of microbial transglutaminase (MTGase) and high pressure (HP) (500, 700 and 900 MPa/40 °C/30 min), only pressure under the same conditions or heat (75 °C/30 min) were investigated on chicken batters with the addition of egg components and without phosphates. MTGase gels (700 and 900 MPa) showed marked increases in textural parameters compared to gels without enzyme (NE) or those obtained by heat. The addition of enzyme did not show differences between gels obtained at 700–900 MPa; however, gels obtained at 500 MPa were darker and more reddish than those obtained by heat. MTGase gels were more homogeneous and compact. Thermal analysis revealed that pressure levels above 700 MPa caused as much denaturing as did heat. The microstructure and texture of MTGase gels suggest that a higher amount and heterogeneity of crosslinks was produced when meat and egg proteins were treated in the presence of MTGase under specific conditions of pressure.

Keywords: Microbial transglutaminase; High pressure; Chicken meat; Egg components; Gelation

1. Introduction

The growing consumer demand for healthier products has stimulated the development of low-fat meats and meat products, which include some factors associated with raw materials, reformulation of products and technological processes. Consumer demand for processed poultry meat has increased, due to its low-fat content; however, sausages made from chicken meat do not provide desirable gel strength and the use of additives traditionally employed in the meat industry, such as salt and phosphates, is being reduced for health reasons.

Sodium chloride plays a key role in the solubility of myofibrillar proteins required for subsequent denaturation and aggregation to give proper water–fat retention and acceptable textural properties of meat gels. Phosphates act on muscle proteins by increasing pH and ionic strength, which augment the solubility of myofibrillar proteins through actomyosin dissociation and depolymerization of thick and thin filaments. Partial replacement of NaCl by phosphates in low sodium formulations has been effectively used in the meat industry, reducing the negative effect of lower salt levels and improving sensorial and technological properties (Fernández Martín, Cofrades, Carballo, & Jiménez-Colmenero, 2002). Phosphate usage is limited to 0.5% in countries such as the USA and Canada and totally prohibited in Germany for meat products. This limitation is mainly imposed to restrict water addition; however, a level above 0.5% can also cause off-flavours, reported by consumers as metallic and soapy flavours (Barbut, 2002).

In recent years, high pressure (HP) has been increasingly investigated as a method of altering the functional properties of macromolecules and is a powerful tool for the study of proteins and modulation of enzymatic activity (Mozhaev, Heremans, Frank, Masson, & Balny, 1996). This process causes a series of physicochemical changes

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in meat proteins, from solubility to aggregation, depending on processing conditions and system characteristics (Chapleau, Mangavel, Compoint, & de Lamballerie-Anton, 2004), improving meat binding properties and partially compensating for NaCl reduction (de Lamballerie-Anton, Taylor, & Culioli, 2002, Chap. 16). Several studies have demonstrated that meat gels obtained by HP are generally softer than are heat-induced gels (Jiménez Colmenero, 2002), so, some non-meat proteins or hydrocolloids have been added to increase the gel strength.

On the other hand, the enzyme transglutaminase (TGase; EC 2.3.2.13) can catalyse the formation of intra and inter molecular ε-(γ-glutamyl)lysine crosslinks between food proteins, which have unique effects on protein properties, gelation capability, thermal stability and water-holding capacity (Kuraishi, Yamazaki, & Susa, 2001), depending on the macromolecular structure of each substrate. Nonaka, Ito, Sawa, Motoki, and Nio (1997) and Lauber, Noack, Klostermeyer, and Henle (2001) found that MTGase was capable to create crosslinks under pressure and Lee and Park (2002) observed that a 60% of initial MTGase activity was maintained, even after pressurization at 600 MPa/60 min, indicating that the enzyme was pressure-resistant.

Dried egg white has been used as a fat substitute due to the elevated crude protein concentration, the higher gel strength and its ability to stabilize batters. However, ovalbumin has proved to be a poor substrate for MTGase because of its compact structure (Sakamoto, Kumazawa, & Motoki, 1994) but egg yolk is a better one. As for heat-denaturation, HP induces changes in egg white functionality, such as foaming and gelling, and affects the structural properties. Smith, Galazka, Wellner, and Sumner (2000) observed irreversible changes in the β structure of the protein above 400 MPa consistent with previous reports about the α-helix of the ovalbumin, which was reduced slightly by HP treatment. The pressure causes the protein hydrophobic core to become more exposed and thus more readily available for the MTGase action.

In a previous study (Trespalacios & Pla, 2007), customary levels (0.3%) of sodium tripolyphosphates and NaCl (1.5%) were used to obtain low-fat and low-salt chicken gels. Synergistic effects on texture and expressible moisture were observed when HP and MTGase were applied simultaneously; therefore the possibility of reducing, even more, the salt content and eliminating the phosphates was considered.

There are few studies regarding low-fat and low-salt meat gels without phosphates produced by HP or with MTGase at atmospheric pressure; moreover, there are no reports on the application of HP and MTGase combined on meat systems. However, it is necessary to thoroughly investigate the simultaneous application of MTGase and HP, at higher levels of pressure, on meat products to better understand the mechanism involved. The objective of this study was to evaluate the simultaneous effects of MTGase and HP (500, 700 and 900 MPa) on chicken meat and egg batters without phosphates and with a low content of sodium chloride.

2. Materials and methods

2.1. Materials

Fresh chicken legs and eggs were purchased from a local market (Corporación Alimentaria Guissona, S.A., Guissona, Spain). Dehydrated egg white was kindly supplied by Degussa Texturant Systems (Barcelona, Spain) and Transglutaminase Activa™ WM (Ajinomoto Co. Inc., Tokyo, Japan) by Impex Química, S.A. (Barcelona, Spain). The commercial product contains 99% maltodextrins and 1% MTGase with an activity of 100 units/g. According to Ajinomoto's specifications, one unit is the amount of the enzyme that catalyses the formation of 1 μmol of hydroxamic acid/min at 37 °C. In the present study, the enzyme concentration is reported as the commercial product concentration and referred to as % MTGase. Nojax® cellulose casing (22 mm diameter) and polyvinylidene casing (55 mm diameter) were obtained from Viskase Companies, Inc. (Willowbrook, USA) and Krehalon Industrie B.V. (Deventer, Holland). All chemicals for the proximate analysis, pH and microstructure were from Panreac Química, S.A. (Barcelona, Spain).

2.2. Preparation of low-fat and low-salt chicken gels

Skinless, boneless chicken leg (thigh and drumsticks) 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 homogenized with 10% fresh egg yolk, 10% dehydrated egg white and cold water (30%) in a homogenizer Mod. UMC 5 (Stephan Machinery GmbH & Co., Hameln, Germany) at 1800 rpm for 12 min in 80% vacuum. The final temperature of the batters never exceeded 12 °C. Samples with enzyme were treated with 0.3% w/w of the commercial MTGase preparation. Immediately after this, the batters were stuffed into cellulose or polyvinylidene casings by means of a sausage filler Mod. TWF-6 (Dick GmbH, Deizisau, Germany). 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 processed under the same conditions.

2.3. High pressure and thermal treatments

A discontinuous high pressure pilot unit "Food-Lab" Food Processor Model S-FL-850-9W (Stansted Fluid Power, Ltd., Essex, UK) was used for processing at 500, 700 and 900 MPa at 40 °C for 30 min. The times needed to achieve the pressure treatment were 120, 170 and

285 s, respectively, and the decompression time was from 30 to 60 s. The pressure chamber and the water inside were held at 40 °C by circulating hot water through a coil around the walls of this chamber. Finally, all samples were heated in a water bath to assure an internal temperature of 75 °C for 5 min, in order to inactivate the added enzyme, and then cooled in water. For the heat treatment, samples were heated at 75 °C for 30 min in a water bath and then cooled (Thermometer 638 Pt, Crison Instruments, S.A., Barcelona, Spain). Samples without enzyme were treated under the same pressure and heat inactivation conditions. All treated samples were stored at 4 °C prior to their analysis. These experiments were performed twice.

2.4. Yield determination

Gel samples were tempered to 20 °C and removed from the casing, blotted dry with a paper towel and weighed for yield determination, which was expressed as a percentage by calculating as the weight of sample treated by heat or pressure divided by the weight of the non-treated sample.

2.5. Proximate analysis and pH

Moisture, ash and total nitrogen of raw meat, batters and treated samples were quantified in triplicate (Association of Official Analytical Chemists, 1990, Chap. 39). Fat content was estimated by difference. A conversion factor of 6.25 was used for protein content. The pH was measured in triplicate on a homogenate of 5 g of sample in 50 ml distilled water at 20 °C, using a portable pHmeter Mod. 507-05 (Crison Instruments, S.A., Barcelona, Spain).

2.6. 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) and then weighed (W_f). This determination was carried out in quadruplicate. Expressible moisture (E_m) was expressed as the ratio of moisture lost after centrifugation to the initial gel sample weight:

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

2.7. Instrumental colour analysis

The colour of 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. Measurements were done with reference to the illuminant *Fcw* and the 10° standard observer.

2.8. Instrumental texture analysis

The textural characteristics of gels (22 mm diameter) were analysed according to the texture profile analysis (TPA) and cutting force using a TA-XT2 Texture Analyser (Stable Micro Systems, Haslemere, UK) with a 25 kg load cell (±1 g). Six cylindrical replicates were cut (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). Cutting force was measured with a probe HDP/BSK in six cylindrical replicates.

2.9. 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. After rinsing and draining, sections were mounted in a non-fluorescent observation media between two glass slides. The images were obtained with a 10X objective with aperture 0.4. Samples were excited at 568 nm with a Kr/Ar laser.

2.10. Thermal analysis

The chicken meat, egg yolk and untreated batter were subjected to differential scanning calorimetry (DSC). The dried egg white and lyophilized gels (10–15 mg) were scanned after dialyzing against deionized water at 4 °C for 24 h and temperature of 20 °C. DSC was performed on a Mettler Toledo Module Type DSC 821_e/700 (Mettler Toledo GmbH, Germany). Samples were weighed in aluminium pans (40 μl) and then sealed with a crimper. Deionized water was used as reference. For temperature (T) and enthalpy (ΔH) calibration, two standards were used: gallium ($T = 29.8$ °C, $\Delta H = 80.22$ J/g) and indium ($T = 156.6$ °C, $\Delta H = 28.46$ J/g). The heating conditions of samples were in the range 20–100 °C, at a heating rate of 10 °C/min, except for chicken meat samples, which were in the range 20–90 °C (Kijowski & Mast, 1988). The Star^e System V 6.1 software was used to obtain the plots of heat flow versus temperature and to calculate the temperature of extrapolated onset (T_o), temperature of maximum transition (T_m) and ΔH of at least three replicates.

2.11. Statistical analysis

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 $P \leqslant 0.05$. Differences between variables and treatments were determined using a studentized maximum modulus (GT2) test.

3. Results and discussion

3.1. Proximate analysis and pH

Proximate analyses and pH of raw chicken leg meat, batters and gels are shown in Table 1. These results agree with the study on variations in muscle composition of broilers of Xiong, Cantor, Pescatore, Blanchard, and Straw (1993). A low level of lipids was expected since external fat was trimmed off from the meat before mincing, which agrees with Lan et al. (1995) who found less than 4% of lipids in broiler's breast and thigh muscles. An increase in the pH of batters was due to egg white addition.

3.2. Yield and expressible moisture

As shown in Fig. 1, gels without MTGase obtained by pressure (NE) and gels obtained by heat had higher yield values (99–99.8% and 97.7%, respectively) than had gels with MTGase and pressure (94.2–95.4%). Heat generates structures mainly stabilized by disulfide bonds and hydrophobic interactions, while HP promotes hydrogen bonds, which gives better hydration properties than does heat (Angsupanich, Edde, & Ledward, 1999).

Poultry sausages pressurized at 500 MPa for 30 min at 50, 60, 70 or 75 °C, presented lower cooking loss than did those treated at 75 °C for 30 min (Yuste, Mor-Mur, Capellas, Guamis, & Pla, 1999). Fernández Martín et al. (2002) showed that pressurization (400 MPa/70 °C/30 min) of pork batters produced a partial denaturation and both salt-soluble and -insoluble proteins remained native after the treatment.

Enzyme addition increased the expressible moisture. For identical pressure, NE gels showed lower values than did MTGase gels. MTGase gels, obtained at 500 MPa, showed the highest values and were unlike those reported in our previous study (Trespalacios & Pla, 2007) where we found a lower expressible moisture in the pressure MTGase gel, showing a synergistic effect of enzyme and pressure when applied simultaneously. The NaCl reduction from 1.5% to 1.0% and the absence of phosphates in this study decreased the ionic strength and consequently the water-holding properties. However, the expressible moisture was favourably reduced in MTGase gels obtained at 700 and 900 MPa.

Although MTGase gels had lower yield values than had NE or heat gels, they were higher than those reported in low-salt chicken meatballs with pig plasma TGase (0.4–1%) and phosphates (Tseng, Liu, & Chen, 2000) and those of chicken döner kebab with MTGase and sodium caseinate (Kilic, 2003). Pietrasik (2003) reported similar results in beef gels with egg white and phosphates in cooked samples with MTGase.

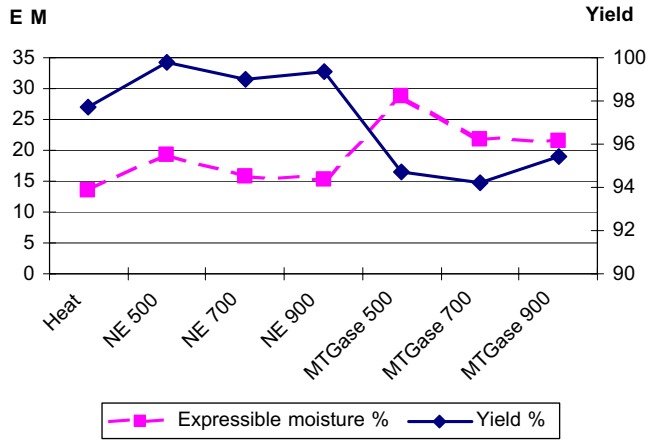

Fig. 1. Expressible moisture (EM) and yield (Y) of chicken meat and egg gels without phosphates; obtained by heat (75 °C/30 min), by pressure (NE) (500 MPa, 700 MPa or 900 MPa) and by pressure and microbial transglutaminase (0.3%) (MTGase).

The results of MTGase addition to meat products at atmospheric pressure have been contradictory. Pietrasik (2003) found that the addition of 0.5% MTGase to beef homogenates decreased the binding properties of gels with egg white and Lee and Park (2003) observed a slight reduction in water-holding of restructured meat with MTGase; however, in cooked pork gels, the increase of NaCl (0–2%) produced gels with better binding properties (Pietrasik & Li-Chan, 2002b). Other studies revealed no significant effect of MTGase (Hammer, 1998; Pietrasik & Li-Chan, 2002a). It is possible that these differences between authors were due to the temperature applied.

Reports concerning the effect of egg proteins on hydration properties of meat gels obtained by heat at atmospheric pressure have also been inconsistent; however, when pressure (400 MPa) was applied at 70 °C, the addition of egg white increased the water-binding of chicken meat batters (Fernández, Cofrades, Solas, Carballo, & Jiménez Colmenero, 1998). There are no studies about the effect of egg proteins mixed with meat proteins at pressure levels above 400 MPa.

Table 1
Proximate analysis and pH of raw chicken meat, chicken meat and egg batters and gels obtained by pressure: without enzyme (NE) and with microbial transglutaminase (MTGase) (0.3%)

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	pH
Raw chicken meat	74.16 ± 0.72 a	1.10 ± 0.00 b	20.8 ± 1.40 b	3.98	6.38
Chicken and egg batters	72.56 ± 0.19 b	2.12 ± 0.01 a	21.1 ± 0.78 a	4.26	6.66
NE gels	71.02 ± 0.00 b	2.17 ± 0.01 b	19.3 ± 0.63 c	7.59	6.82
MTGase gels	68.70 ± 0.14 c	2.09 ± 0.02 a	20.5 ± 0.86 a	8.71	6.81

Different letters in the same column indicate significant differences ($P < 0.05$).

Recent studies have shown that the most pronounced changes in ovalbumin are observed in the pressure range 400–700 MPa, such as an increase in surface hydrophobicity, exposed SH content and susceptibility to enzymatic hydrolysis, while protein solubility, total SH content, denaturation enthalpy and trypsin inhibitory activity all decrease (Van der Plancken, Van Loey, & Hendrickx, 2005a).

We consider that HP at 500 MPa allows MTGase to establish intermolecular crosslinks between ovalbumin and myosin. In our study, the gels obtained at 700 and 900 MPa had better water-binding properties than had those obtained at 500 MPa. The higher expressible moisture at 500 MPa (approx 7%) could be explained by an incomplete network structure between egg and meat proteins, surrounded by non-incorporated liquid. A similar pattern was obtained for the NE pressurized samples (approx 4%).

3.3. Colour

The NE and MTGase samples pressurized at 700 and 900 MPa showed similar L^* values. All samples treated at 500 MPa were darker than were those treated at 700 MPa, 900 MPa and the heat-treated samples (Table 2). This shows that lightening depends mainly on the pressure applied, not on the enzyme. There were no differences in redness or yellowness in NE and MTGase samples treated at 700 and 900 MPa but MTGase samples, treated at 500 MPa, showed the highest a^* and b^* values, while pressurized samples kept the redness better than did heated ones.

It is well known that myoglobin is responsible for the intensity of muscle colour and that the application of HP, even at 5–10 °C, induces drastic changes. The discoloration through pressure-processing may result from a "whitening" effect in the range 200–350 MPa, due to globin denaturation and/or to heme displacement or release, and to the oxidation of ferrous myoglobin to ferric metmyoglobin above 400 MPa (Cheftel & Culioli, 1997). Jung, Ghoul, and de Lamballerie-Anton (2003) found that, in beef meat, the pressure intensity is more significant than is the holding time for redness, total colour difference and metmyoglobin content and that pressure above 300 MPa induces changes in colour parameters, such as a decrease of the total colour difference.

There are no references about the effect of MTGase on meat colour when it is applied simultaneously with HP; however, some studies have been conducted at atmospheric pressure. Tseng et al. (2000) found that addition of 1% pig plasma TGase did not produce significant differences in the colour of low-salt chicken meat balls cooked at 71 °C. Kilic (2003) reported non-significant differences between samples of döner kebab treated with MTGase and sodium caseinate and Lee and Park (2003) observed no changes in restructured meat. Nevertheless, we found an increase in yellowness of MTGase samples treated at 500 MPa, which agrees with Pietrasik (2003), who reported that, while the enzyme addition (0.5%) had no significant influence on the L^* and a^* values of beef gels processed by heat, the presence of egg white increased the b^* parameter.

3.4. Textural properties

Pressured MTGase gels were the hardest and presented the highest chewiness and cutting force of all; moreover, there were no significant differences between gels obtained at 700 and 900 MPa, which registered the highest values. MTGase samples, treated at 700 and 900 MPa, were the most cohesive and resistant to cutting. All gels (NE and MTGase) obtained at 500 MPa had the lowest cohesiveness (Table 3).

In a previous study, pressure treatments at 500 MPa in the presence of MTGase produced more protein crosslinks than did MTGase at 0.1 MPa (Trespalacios & Pla, 2007). Our results suggest that higher pressure levels applied on meat and egg proteins in the presence of MTGase induced a larger number of intra and inter molecular ε-(γ-glutamyl)lysine bonds. It seems that the increase in hardness, chewiness, and cohesiveness is due to the formation of a heterologous complex. Pressurization (300 MPa), prior to setting of turkey pastes with MTGase, induced gelation at 40 or 50 °C but not at 4 °C (Ashie & Lanier, 1999). Chapleau et al. (2004) did not find changes in the secondary structure of myofibrillar proteins after processing from

Table 2
Hunter Lab values of chicken meat and egg gels without phosphates; obtained by heat (75 °C/30 min), by pressure (NE) (500 MPa, 700 MPa or 900 MPa) and by pressure and microbial transglutaminase (0.3%) (MTGase)

Sample	Pressure (MPa)	Temperature (°C)	L^*	a^*	b^*
Heat	0.1	75	77.09 ± 0.33 b	2.64 ± 0.04 d	14.96 ± 0.16 b
NE	500	40	72.35 ± 0.45 c	4.20 ± 0.27 b	15.24 ± 0.15 b
NE	700	40	76.91 ± 0.62 b	3.22 ± 0.24 c	13.58 ± 0.13 c
NE	900	40	78.08 ± 0.35 a	3.16 ± 0.06 c	13.49 ± 0.08 c
MTGase	500	40	74.77 ± 0.28 c	5.14 ± 0.31 a	16.56 ± 0.11 a
MTGase	700	40	78.22 ± 0.15 a	3.25 ± 0.02 c	15.39 ± 0.15 b
MTGase	900	40	78.07 ± 0.17 a	3.21 ± 0.04 c	15.31 ± 0.08 b

L^* = Lightness, a^* = Redness, b^* = Yellowness.
Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3
Textural properties of chicken meat and egg gels without phosphates; obtained by heat (75 °C/30 min), by pressure (NE) (500 MPa, 700 MPa or 900 MPa) and by pressure and microbial transglutaminase (0.3%) (MTGase)

Sample	Pressure (MPa)	Temperature (°C)	Hardness (N)	Springiness (mm)	Cohesiveness (dimensionless)	Chewiness (N mm)	Cutting force (N)
Heat	0.1	75	36.05 ± 0.59 c	6.99 ± 0.24 b	0.538 ± 0.00 cd	135.77 ± 4.09 c	7.26 ± 0.11 bc
NE	500	40	30.48 ± 1.34 d	7.40 ± 0.20 ab	0.515 ± 0.00 f	116.38 ± 5.88 d	5.82 ± 0.43 d
NE	700	40	36.70 ± 2.41 c	7.32 ± 0.19 ab	0.545 ± 0.00 c	146.63 ± 9.65 c	6.03 ± 0.19 c
NE	900	40	36.19 ± 1.39 c	7.25 ± 0.13 ab	0.547 ± 0.00 c	143.61 ± 3.25 c	6.42 ± 0.47 c
MTGase	500	40	44.63 ± 1.45 b	7.57 ± 0.21 a	0.527 ± 0.01 e	178.02 ± 4.68 b	7.98 ± 1.19 b
MTGase	700	40	55.43 ± 4.05 a	7.32 ± 0.08 ab	0.573 ± 0.00 a	232.85 ± 16.39 a	11.5 ± 0.71 a
MTGase	900	40	57.58 ± 2.62 a	7.32 ± 0.14 ab	0.558 ± 0.00 b	235.50 ± 9.29 a	11.4 ± 0.85 a

Different letters in the same column indicate significant differences ($P < 0.05$).

50 to 600 MPa at 20 °C for 10 min; however, there was aggregation at pressures above 300 MPa.

Ovalbumin is stable until 60 °C and remains fairly stable when pressurized at 400 MPa for 30 min, which may be due to the presence of a single disulfide bond (SS), four sulfhydryl (SH) groups and strong non-covalent interactions stabilizing the three dimensional structure (Galazka, Dickinson, & Ledward, 2000). HP induces structural changes on native ovalbumin, exposing glutamyl and lysyl residues, which may be buried inside its tertiary structure, making them accessible to MTGase. However, the lower hardness of gels obtained at 500 MPa suggests that the crosslinks with myosin were insufficient, as a result of incomplete ovalbumin unfolding.

Recently, Menéndez, Rawel, Schwarzenbolz, and Henle (2006) found a 50% MTGase residual activity after 12 min at 600 MPa/40 °C, caused by the destruction of α-helix elements; nevertheless, at atmospheric pressure, the inactivation is achieved after 2 min at 80 °C. Consequently, the notable differences found in hardness and chewiness, between NE and MTGase gels obtained at 700 MPa, indicate that certain enzymatic activity remains at that pressure.

NE gels obtained by HP showed a pattern of texture parameters similar to those with MTGase and pressure (but to a lower extent). In this study, the NE samples treated at 500 MPa were the softest and showed the lowest values for chewiness and cutting force of all samples. However, the NE samples treated at 700 or 900 MPa showed similar values of hardness and chewiness to those treated by heat, indicating that these pressure levels produce a complete gel formation of egg and myofibrillar proteins. It is important to consider that the enzyme inactivation (75 °C/5 min) applied in the process may have consequences for the textural parameters. All pressurized samples (NE and MTGase) had more springiness than had heated ones and those produced at 500 MPa showed the highest values. Van der Plancken, Van Loey, and Hendrickx (2005b) observed a decrease in total SH content in ovalbumin treated under severe conditions (above 70 °C at 0.1 MPa or between 500 and 600 MPa, depending on the temperature). The high degree of exposure of sulfhydryl groups, and subsequent oxidation and sulfhydryl-disulfide bond exchange reactions, resulted in soluble aggregates, which explains why pressure-induced egg white gels are softer and more elastic than are heat-induced ones.

Several authors have studied the effect of TGase on the texture of different materials treated by heat at 0.1 MPa. Meat products, such as low-salt chicken meat-balls (Tseng et al., 2000), chicken döner kebab (Kilic, 2003) or chicken sausages (Muguruma et al., 2003) increased the gel strength. Beef gels exhibited higher hardness, springiness and chewiness when they were treated with TGase; furthermore, the cohesiveness was increased only in samples containing egg albumen (Pietrasik, 2003). MTGase favourably increased hardness and chewiness of pork gels. This increment was bigger for the higher-salt gels and significant linear effects were observed for enzyme concentration (0–0.6%) on hardness and springiness (Pietrasik & Li-Chan, 2002b; Pietrasik & Jarmoluk, 2003). Recently, Kütemeyer, Froeck, Werlein, and Watkinson (2005) demonstrated that the addition of monovalent ions increased the enzymatic activity and the thermal stability of MTGase. The results confirmed that NaCl and KCl had a synergistic effect on the activity, while bivalent ions had only a slight influence ($MgCl_2$) or reduced the activity and thermal stability of the enzyme ($CaCl_2$).

3.5. Microstructure

Confocal microphotographs of pressurized samples, with and without MTGase, are shown in Fig. 2. MTGase gels had a more uniform network structure, which correlates with the firmest texture observed. The NE gels obtained at 500 MPa were coarser, with a loose appearance, and were the most fragile and soft of all. In pressure gels, pore size was greater in NE than in MTGase gels and the pressure intensity caused changes in the gel characteristics. MTGase gels obtained at 700 and 900 MPa showed a more compact structure and smaller pore diameter, with lower expressible moisture than had gels obtained at 500 MPa.

Tseng et al. (2000) and Muguruma et al. (2003) reported that low-salt chicken meat balls and chicken sausages with TGase at atmospheric pressure, had more regular network structures. In a previous study, in pressure meat gels with

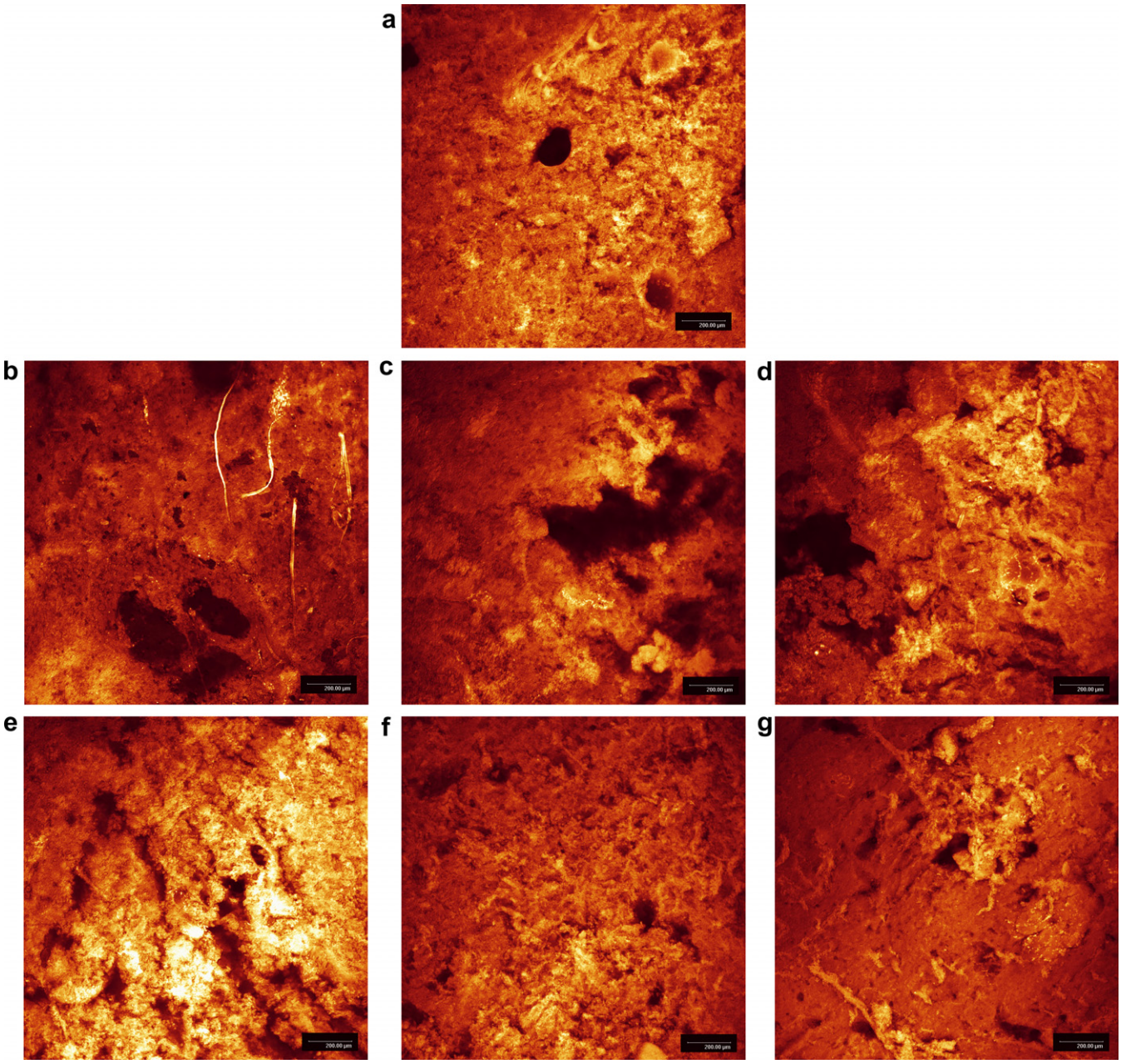

Fig. 2. Microstructure of low-fat and low-salt chicken gels without phosphates: heat gels obtained at 75 °C/30 min (a) or pressure gels (NE) obtained at 500 MPa (b), 700 MPa (c) or 900 MPa (d) and pressure and MTGase gels (MTGase) obtained at 500 MPa (e), 700 MPa (f) or 900 MPa (g). Bar = 200 μm.

phosphates, we also found a more compact and homogeneous structure when MTGase was included in the formulation (Trespalacios & Pla, 2007).

3.6. DSC

Differential scanning calorimetry was used to clarify the role of heat or pressure-induced gelation on texture formation of processed meat products. The thermograms of the raw materials used in the chicken meat batter, egg white and egg yolk (Fig. 3) show several peaks of T_m. The first and last transitions for chicken leg muscle at 59.5 and 84.2 °C were due to myosin and actin and the intermediate peaks are mainly due to sarcoplasmic proteins and connective tissue at 63.4, 65.2 and 68.8 °C with a total ΔH of 14.08 J/g. These results agree with Kijowski and Mast (1988) who reported that thigh chicken meat exhibited three major transitions at 59.6, 65.6 and 75.8 °C. The specific transition temperature depends mainly on muscle type and pH.

The dried egg white showed two main peaks, at 65.8 and 83.16 °C, corresponding to conalbumin and ovalbumin, respectively. The thermogram of fresh egg yolk showed two transitions at 42.1 and 92.4 °C and two endothermic peaks at 28.1 and 84.5 °C; however, when it was lyophilized, only two peaks at 64.2 and 83.2 °C were observed

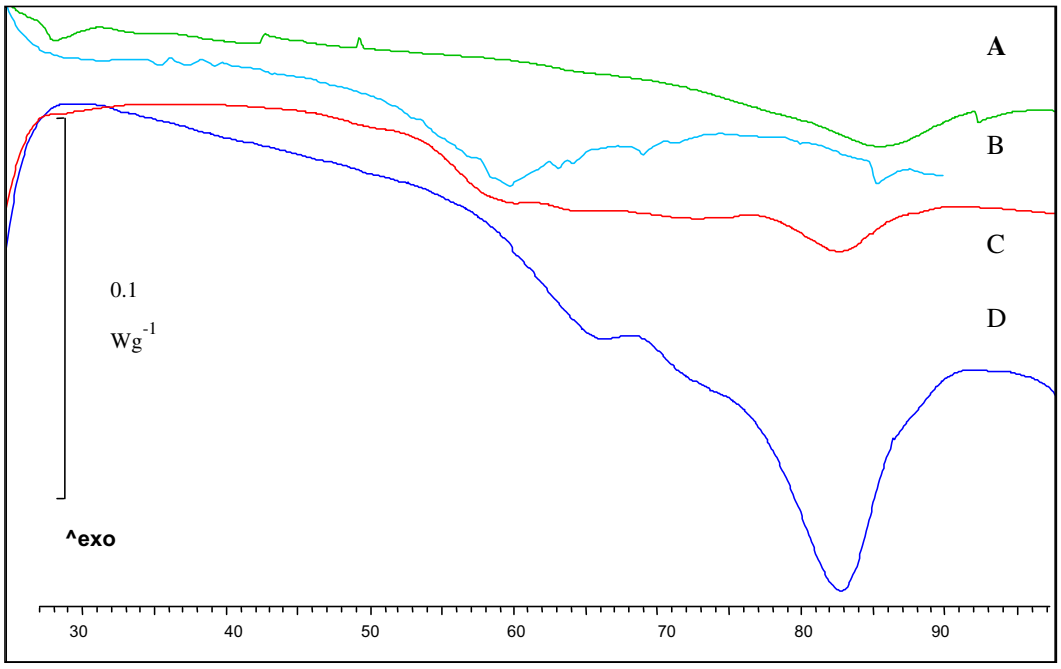

Fig. 3. Thermograms of raw materials and batter: (A) Egg yolk, (B) Chicken meat, (C) Batter before treatments, (D) Egg white.

(curve not shown). Although the chicken batters were inherently complex, they gave characteristic thermograms, which are interpreted in terms of thermal denaturation of the major proteins, with two main peaks at 60.2 and 82.8 °C and total ΔH of 11.48 J/g (35–95 °C, spline baseline).

The heat gel exhibited an almost complete protein denaturation (97.0%) but an endothermic peak at 84.0 °C was observed, with a minimal enthalphy of 0.34 J/g, possibly due to the residual ovalbumin that presented a higher thermal stability. When HP was applied (Fig. 4), NE gels obtained at 500 MPa gave a total ΔH of 4.14 J/g (63.9% of denaturation) with two peaks at 78.1 and 83.4 °C, but MTGase gels showed a transition at 67.5 °C and an endothermic peak at 83.5 °C with a total ΔH of 1.26 J/g (88.5% of denaturation) (range 66–90 °C, spline baseline). There were no differences in T_m corresponding to egg proteins between NE and MTGase gels, but the MTGase addition

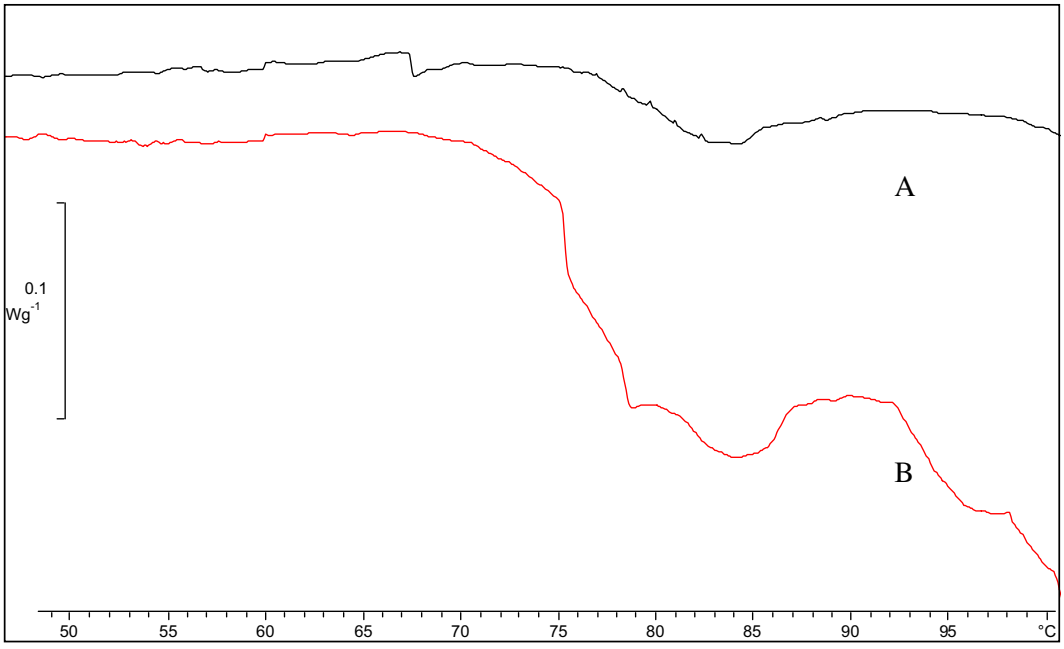

Fig. 4. DSC normalized (dry matter) profiles of chicken meat and egg gels without phosphates; obtained by pressure at 500 MPa: (A) with microbial transglutaminase (0.3%); (B) without enzyme added.

induced an enthalphy difference of near to 70% between them at 500 MPa. This decrease suggests a higher protein denaturation, due mainly to the unfolded ovalbumin, which requires less heat energy to denature completely and to form a larger complex between the myofibrillar and egg proteins mediated by MTGase. It has been reported that application of pressure at non-denaturing temperatures caused some changes of protein conformation, increasing exposure of hydrophobic residues, which favoured protein–protein aggregation interaction and a subsequent heating led to the formation of stronger gel structures (Carballo, Cofrades, Fernández Martín, & Jiménez Colmenero, 2001).

NE and MTGase gels obtained at 700 and 900 MPa gave similar patterns, with loss of 1.0 J/g (more than 91.3% of denaturation) in both cases, suggesting that treatments at these pressure levels were almost as denaturing as was heat treatment. Hayakawa, Linko, and Linko (1996) showed that pressurization of ovalbumin to more than 400 MPa for 15 min induced a protein denaturation dissimilar to that resulting from heating at 80 °C for 10 min; however, denaturation by pressure up to 1000 MPa was found to be more severe than thermal denaturation. Van der Plancken et al. (2005a) reported that no residual denaturation enthalpy could be observed after 20 min at 700 MPa over a temperature range of 10–60 °C.

4. Conclusions

Simultaneous application of MTGase and HP at 40 °C on chicken meat batters with egg proteins, with reduced content of salt and without phosphates, produced gels with substantially more enhanced textural properties than samples subjected only to pressure or those obtained by traditional heat treatment.

The gel obtained at 500 MPa was the most fragile and soft of all. At higher pressures (700–900 MPa), the hardness values were comparable to heat-treated (75 °C/30 min) gels, but these were still softer than those with MTGase and pressure. The increase in cutting force, hardness and chewiness of gels suggests that a heterologous complex of meat and egg proteins was formed when HP processing and MTGase were combined. Although the enzyme and higher pressure at 700 and 900 MPa considerably improved the yield, colour and texture, there were no differences between the two pressure levels. Thermal analysis showed that pressure levels above 700 MPa were as denaturing as was heat treatment; at 500 MPa, 63.9 and 88.5% enthalpy reductions were observed in NE and MTGase gels, respectively.

The synergistic effect of combining enzymatic crosslinking with high pressure treatment at 700 MPa is of interest for food manufacturers, in order to generate high quality products, which exhibit many desirable characteristics that satisfy the consumer's demands, such as low-fat, low-sodium content, phosphate-free and improved sensorial attributes, while assuring the nutritive value and microbiological safety.

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References

Angsupanich, K., Edde, M., & Ledward, D. A. (1999). Effects of high pressure on the myofibrillar proteins of cod and turkey muscle. *Journal of Agricultural and Food Chemistry, 47*(1), 92–99.

Ashie, I. N. A., & Lanier, T. C. (1999). High pressure effects on gelation of surimi and turkey breast muscle enhanced by microbial transglutaminase. *Journal of Food Science, 64*(4), 704–708.

Association of Official Analytical Chemists (1990). Meat and meat products. In K. Helrich (Ed.), *Official methods of analysis* (15th ed., pp. 931–948). Arlington: Association of Official Analytical Chemists.

Barbut, S. (2002). *Poultry products processing: An industry guide*. Boca Raton, FL: CRC Press.

Carballo, J., Cofrades, S., Fernández Martín, F., & Jiménez Colmenero, F. (2001). Pressure-assisted gelation of chemically modified poultry meat batters. *Food Chemistry, 75*(2), 203–209.

Chapleau, N., Mangavel, C., Compoint, J. P., & de Lamballerie-Anton, M. (2004). Effect of high-pressure processing on myofibrillar protein structure. *Journal of the Science of Food and Agriculture, 84*(1), 66–74.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science, 46*(3), 211–236.

de Lamballerie-Anton, M., Taylor, R., & Culioli, J. (2002). High pressure processing of meat. In Josep Kerry, John Kerry, & David Ledward (Eds.), *Meat processing* (pp. 313–331). Cambridge: Woodhead Publishing Limited and CRC Press LLC.

Fernández Martín, F., Cofrades, S., Carballo, J., & Jiménez-Colmenero, F. (2002). Salt and phosphate effects on the gelling process of pressure/heat treated pork batters. *Meat Science, 61*(1), 15–23.

Fernández, P., Cofrades, S., Solas, M. T., Carballo, J., & Jiménez Colmenero, F. (1998). High pressure-cooking of chicken meat batters with starch, egg white, and iota carrageenan. *Journal of Food Science, 63*(2), 267–271.

Galazka, V. B., Dickinson, E., & Ledward, D. A. (2000). Influence of high pressure processing on protein solutions and emulsions. *Current Opinion in Colloid & Interface Science, 5*(3–4), 182–187.

Hammer, G. F. (1998). Microbial transglutaminase and diphosphate in finely comminuted cooked sausage. *Fleischwirtschaft, 78*(11), 1155–1156, 1159–1162, 1186.

Hayakawa, I., Linko, Y. Y., & Linko, P. (1996). Mechanism of high pressure denaturation of proteins. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 29*(8), 756–762.

Jiménez Colmenero, F. (2002). Muscle protein gelation by combined use of high pressure/temperature. *Trends in Food Science & Technology, 13*(1), 22–30.

Jung, S., Ghoul, M., & de Lamballerie-Anton, M. (2003). Influence of high pressure on the color and microbial quality of beef meat. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 36*(6), 625–631.

Kijowski, J. M., & Mast, M. G. (1988). Thermal properties of proteins in chicken broiler tissues. *Journal of Food Science, 53*(2), 363–366.

Kilic, B. (2003). Effect of microbial transglutaminase and sodium caseinate on quality of chicken döner kebab. *Meat Science, 63*(3), 417–421.

Kuraishi, C., Yamazaki, K., & Susa, Y. (2001). Transglutaminase: Its utilization in the food industry. *Food Reviews International, 17*(2), 221–246.

Kütemeyer, C., Froeck, M., Werlein, H. D., & Watkinson, B. M. (2005). The influence of salts and temperature on enzymatic activity of microbial transglutaminase. *Food Control, 16*, 735–737.

Lan, Y. H., Novakofski, J., Mccusker, R. H., Brewer, M. S., Carr, T. R., & Mckeith, F. K. (1995). Thermal gelation of pork, beef, fish, chicken and turkey muscles as affected by heating rate and pH. *Journal of Food Science, 60*(5), 936–940, 945.

Lauber, S., Noack, I., Klostermeyer, H., & Henle, T. (2001). Stability of microbial transglutaminase to high pressure treatment. *European Food Research and Technology, 213*(4–5), 273–276.

Lee, E. Y., & Park, J. (2002). Pressure inactivation kinetics of microbial transglutaminase from Streptoverticillium mobaraense. *Journal of Food Science, 67*(3), 1103–1107.

Lee, E. Y., & Park, J. (2003). Microbial transglutaminase induced cross-linking of a selected comminuted muscle system: Processing conditions for physical properties of restructured meat. *Food Science and Biotechnology, 12*(4), 365–370.

Menéndez, O., Rawel, H., Schwarzenbolz, U., & Henle, T. (2006). Structural changes of microbial transglutaminase during thermal and high pressure treatment. *Journal of Agricultural and Food Chemistry, 54*, 1716–1721.

Mozhaev, V. V., Heremans, K., Frank, J., Masson, P., & Balny, C. (1996). High pressure effects on protein structure and function. *Proteins: Structure Function and Genetics, 24*(1), 81–91.

Muguruma, M., Tsuruoka, K., Katayama, K., Erwanto, Y., Kawahara, S., Yamauchi, K., et al. (2003). Soybean and milk proteins modified by transglutaminase improves chicken sausage texture even at reduced levels of phosphate. *Meat Science, 63*(2), 191–197.

Nonaka, M., Ito, R., Sawa, A., Motoki, M., & Nio, N. (1997). Modification of several proteins by using Ca2+ independent microbial transglutaminase with high-pressure treatment. *Food Hydrocolloids, 11*(3), 351–353.

Pietrasik, Z. (2003). Binding and textural properties of beef gels processed with kappa-carrageenan, egg albumin and microbial transglutaminase. *Meat Science, 63*(3), 317–324.

Pietrasik, Z., & Jarmoluk, A. (2003). Effect of sodium caseinate and kappa-carrageenan on binding and textural properties of pork muscle gels enhanced by microbial transglutaminase addition. *Food Research International, 36*(3), 285–294.

Pietrasik, Z., & Li-Chan, E. C. Y. (2002a). Binding and textural properties of beef gels as affected by protein, kappa-carrageenan and microbial transglutaminase addition. *Food Research International, 35*(1), 91–98.

Pietrasik, Z., & Li-Chan, E. C. Y. (2002b). Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. *Food Research International, 35*(4), 387–396.

Sakamoto, H., Kumazawa, Y., & Motoki, M. (1994). Strength of protein gels prepared with microbial transglutaminase as related to reaction conditions. *Journal of Food Science, 59*(4), 866–871.

Smith, D., Galazka, V. B., Wellner, N., & Sumner, I. G. (2000). High pressure unfolding of ovalbumin. *International Journal of Food Science and Technology, 35*(4), 361–370.

Trespalacios, P., & Pla, R. (2007). Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. *Food Chemistry, 100*(1), 264–272.

Tseng, T. F., Liu, D. C., & Chen, M. T. (2000). Evaluation of transglutaminase on the quality of low-salt chicken meat-balls. *Meat Science, 55*(4), 427–431.

Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2005a). Combined effect of high pressure and temperature on selected properties of egg white proteins. *Innovative Food Science & Emerging Technologies, 6*(1), 11–20.

Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. G. (2005b). Changes in sulfhydryl content of egg white proteins due to heat and pressure treatment. *Journal of Agricultural and Food Chemistry, 53*(14), 5726–5733.

Xiong, Y. L., Cantor, A. H., Pescatore, A. J., Blanchard, S. P., & Straw, M. L. (1993). Variations in muscle chemical-composition, pH, and protein extractability among eight different broiler crosses. *Poultry Science, 72*(3), 583–588.

Yuste, J., Mor-Mur, M., Capellas, M., Guamis, B., & Pla, R. (1999). Mechanically recovered poultry meat sausages manufactured with high hydrostatic pressure. *Poultry Science, 78*(6), 914–921.