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Pressure-assisted gelation of chemically modified poultry meat batters

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Abstract

Changes were induced in the characteristics of poultry meat protein using specific chemical modifiers to investigate the effect of pressurization, prior to heating, on gelation, texture and thermal behaviour of meat batters. Values of hardness and chewiness were higher in cooked meat batters treated with urea than in a salt-only sample, but cohesiveness was similar. The β-mercaptoethanol treatment produced a heat-induced gel with very similar properties to the salt-only gel. The rheological behaviours of salt-only and β-mercaptoethanol samples were very similar, but storage modulus values were higher in samples with urea, which accelerated gelation. The pressure-induced reduction of differences in the textural properties of meat batters suggests that hydrophobic interactions play an important role in heat-induced gelation. Differential scanning calorimetry showed that urea clearly destabilized chicken meat batters, while β-mercaptoethanol had very little influence on their thermal behaviour. Pressurization tended to equalize batters and final cooking definitively equalized them.

Keywords: Poultry meat; Thermal gelation; High pressure; Chemical agents; Dynamic rheology; Differential scanning calorimetry

1. Introduction

Several kinds of molecular interaction are involved in thermal gelation of meat proteins, influencing the properties of the matrix (Cofrades & Jiménez-Colmenero, 1998; Gordon & Barbut, 1992; Nakai & Li-Chan, 1988; O'Neill, Mulvihill, & Morrissey, 1994), and hence also influencing numerous characteristics of the final quality of products. This is extremely important for comminuted meat processing.

There is growing interest in the application of high pressure to meat systems, among other reasons because of its effect on protein functional properties. Such behaviour is connected with conformational changes induced in pressurized meat proteins, which affect the type of molecular associations that take place during thermal gelation (Cheftel & Culioli, 1997; Fernández-Martín, Fernández, Carballo, & Jiménez-Colmenero, 1997; Jiménez-Colmenero, Cofrades, Carballo, Fernández, & Fernández-Martín, 1998).

There have been many studies aimed at understanding the molecular forces implicated in the formation and properties of gel networks. Many of these approach such analysis on the basis of selective destruction of protein interactions in gels to investigate different parameters associated with environmental conditions, composition or processing (Cofrades & Jiménez-Colmenero, 1998). Protein fractions obtained by solubilization have been examined by electrophoresis to determine what selective myofibrillar protein interactions may occur during thermal processing of meat protein. However, there are limited reports about how the formation of the protein matrix is influenced by the presence of chemical agents able to selectively alter protein structure by targeting specific residues, and hence the functional properties of meat batters. This approach has been suggested as a means of clarifying the effects of the various forces involved in meat batter formation, although most of the existing information is derived from work with isolated proteins (Gordon & Barbut, 1992). The effects of several of these agents have been studied, for example in rabbit actomyosin gels (O'Neil et al., 1994), sardine kamaboko gels (Roussel & Cheftel, 1990), finely comminuted lean beef mixes (Barbut & Mittall, 1992), cooked meat

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emulsion from chicken protein/pork back fat (Gordon & Barbut, 1992) and mechanically recovered poultry meat (MRPM) model systems (Day, Kerry, O'Connor, & Buckley, 1998). This is a promising approach, but we are unaware of any studies that attempt to make use of the information furnished on controlled modification of meat protein, using selected chemical agents to analyse the effect of pressure treatment on meat systems.

The objective of this paper was to analyse how the changes in the characteristics of the meat raw material, induced by the use of specific chemical modifiers, influence the effect of pressurization on thermal gelation of meat batters. This study should add to the existing information about conformational changes in protein resulting from pressurization prior to heating, the influence on the formation of gel protein structures, and their consequences for the characteristics of final cooked meat products.

2. Materials and methods

2.1. Preparation of meat batters

Fresh chicken breast was obtained from a local meat market. The meat was trimmed to remove visible fat and connective tissue, preground through a 3 mm plate (FTS111, Van Dall SRL. Milano, Italy) to obtain a homogeneous mass, vacuum-packaged and kept frozen at (−20 °C) prior to use, which took place within 2 weeks.

Prior to each experiment, the meat was thawed overnight at 0–2 °C. Sufficient amounts of meat and water and 1.5% NaCl were combined to obtain three different meat batters. One of the NaCl batters was used as a control (sample S) and the other two were treated with two different chemical agents: one with 4.5% urea (sample U) and the other with 0.25% β-mercaptoethanol (sample M). Meat protein content was adjusted to 15% in all formulations; the concentrations of chemical agents were chosen according to Gordon and Barbut (1992). The batters were prepared as follows: raw meat was homogenized and ground for 60 s in a chilled cutter (2°C; Stephan Universal Machine UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany). Water, NaCl and the appropriate chemical agent were then added and the mixture homogenized again chilled under vacuum (2°C, 610 mm Hg) up to an aggregate chopping time of 5 min. The temperature of the batters remained below 10°C at all times.

2.2. Pressure and thermal treatment

The meat batters were placed in flexible plastic jars (diam. = 3.3 cm, ht. = 6.7 cm) containing 60 ± 1 g sample. Each jar was filled, taking special care to avoid air trapping, hermetically sealed and placed in a 8×30 cm Ultra-Cover™ latex bag (Amevisa S.A., Madrid, Spain). Each meat batter was divided into two parts, each of which was subjected to a different treatment. Half of the jars were pressurized (325 MPa, 30 min) using water at 10°C as the pressurizing medium. Pressurizing was performed in an ACB model AGIP N° 665 high pressure pilot unit (GEC, Alsthom, Nantes, France) as described by Carballo, Fernández, and Jiménez-Colmenero (1996). Both the pressurized (P) samples (after pressure release) and the other half of the jars containing non-pressurized samples were then subjected to the same thermal treatment (H): i.e. heating in a water bath at 70°C for 30 min. The heating conditions required to attain a temperature of 70°C were determined beforehand by inserting thermocouples connected to a temperature recorder (Yokogawa Hokushin Electric YEW, Mod. 3087, Tokyo, Japan) at the thermal centre of the samples. After thermal treatment, samples were chilled in iced water and stored for 18 h at 0–4 °C for analysis.

2.3. Proximate analysis and pH

Moisture and ash of the raw meat and unheated samples were determined (AOAC, 1984) in triplicate. Protein content was measured in triplicate by a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St Joseph, MI). Fat content was evaluated by difference. The pH of the raw batters was determined in triplicate using a pH meter (Radiometer PHM 93, Copenhagen, Denmark) on a homogenate of 5 g raw sample in 50 ml distilled water.

2.4. Texture

Texture profile analysis (TPA) of processed samples was performed in a Universal Testing Machine (Model 4501 Instron Engineering Corp., Canton, MA) as described by Bourne (1978). Five cores (diam. = 3.3 cm, ht. = 2.0 cm) were axially compressed to 40% of their original height. Force–time deformation curves were derived with a 5 kN load cell applied at a crosshead speed of 50 mm/min. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; chewiness (Cw) = Hd×Ch×Sp (N×mm).

2.5. Dynamic rheological measurement

Rheological changes in unheated meat batters (non-pressurized and pressurized samples) during thermal gelation were analysed using a Bohlin CSR rheometer

(Bohlin Instruments, Inc., Cranbury, NJ) operating in the small-amplitude oscillatory mode. After equilibration at the initial temperature (10°C), thermal gelation was induced by heating samples from 10 to 70°C at 1°C/min using a Bohlin temperature control unit. Samples were sheared at a fixed frequency of 1.0 Hz with a strain of 0.02. The gap between the plates was set at 1 mm. The sample perimeter was covered with a thin layer of silicon oil to prevent dehydration. The storage modulus (G′) data were collected every minute during shearing measurements. Each measurement was the mean of two replicates.

2.6. Differential scanning calorimetry (DSC)

Thermal behaviour of the different batters, raw and processed, was determined by differential scanning calorimetry as described elsewhere (Fernández-Martín et al., 1997). A previously calibrated Perkin–Elmer DSC7/TAC7DX/PC was used. Heating curves (5–100°C) were recorded (10°C min^{-1}) in triplicate on every kind of sample. Temperatures (t, °C) and enthalpies of transition (ΔH, J/g referred to dry matter) are given within 0.3 and 3%, respectively.

2.7. Statistical analysis

One-way analysis of variance by an F test and least squares differences by Statgraphics 5.0 (STSC Inc., Rockville, MD) were used for comparison of mean values and to identify significant differences ($P<0.05$) among treatments.

3. Results and discussion

3.1. Protein and pH

Protein content of meat batters (14.3–14.9%) was similar in all samples and very close to the target. Like other authors (Barbut & Mittal, 1992), we observed no changes ($P>0.05$) in pH of raw meat batters due to the addition of the chemical agents: 5.95, 5.89 and 5.97 for S, U and M, respectively.

3.2. Nonpressurized samples

The presence of chemical agents in raw meat batters produces some changes in specific residues in meat protein structures, which may influence the type of interactions that take place during thermal gelation, and hence the characteristics of the end product.

Chemical agents affected all TPA parameters except for Sp (Table 1). Cooked meat batters treated with urea were harder and chewier ($P<0.05$), although Ch was similar ($P>0.05$) to the sample with only 1.5% NaCl (Table 1). It has been found that urea destroys hydrogen and electrostatic bonds and increases the availability of hydrophobic interactions which may be involved in protein–protein bonding (Gordon & Barbut, 1992; Whiting, 1987). The increased exposure of hydrophobic protein residues favours aggregation, which on cooking leads to stronger, more elastic and highly cohesive gel structures (Gordon & Barbut, 1992, 1995; Nakai and Li-Chan, 1988). However, it has also been suggested that the destabilizing effect of urea on hydrogen bonds and hydrophobic interaction produces softer gel networks (O'Neill et al., 1994; Roussel & Cheftel, 1990). In fact, the literature contains conflicting reports regarding the effect of urea on TPA parameters (Barbut & Mittal, 1992; Day et al., 1998; Gordon & Barbut, 1992; O'Neill et al., 1994; Roussel & Cheftel, 1990; Whiting, 1987). It is not easy to explain the reasons for these discrepancies, but the answer could lie in the fact that the effect of urea appears to be influenced by a number of factors, including the experimental concentration, the species, and the presence of other compounds (Niwa, Kanoh, Osaka, Nakayama, Watabe & Hashimoto, 1989; O'Neill et al., 1994; Roussel & Cheftel, 1990).

Table 1
Texture parameters for the different heated samples

Sample[a]	Hardness (Hd, N)	Springiness (Sp, mm)	Cohesiveness (Ch)	Chewiness (Cw, N×mm)
Non-pressurized				
S/H	29.3 a	7.1 a	0.59 a	129.5 a
U/H	38.2 c	7.1 a	0.59 a	159.2 c
M/H	23.8 a	7.0 a	0.54 c	89.9 d
Pressurized				
S/P-H	45.9 b	7.2 a	0.66 b	218.9 b
U/P-H	48.1 b	7.1 a	0.60 a	204.9 b
M/P- H	51.3 b	7.2 a	0.67 b	248.6 e
S.E.M.	1.4	0.1	0.01	6.7

[a] S, batter with 1.5% NaCl; U, batter with 1.5% NaCl plus 4.5% urea; M, batter with 1.5% NaCl plus 0.25% β-mercaptoethanol; /H, non-pressurized-and-heated; /P-H, pressurized-and-heated. S.E.M., standard error of means. Different letters in the same column indicate significant differences ($P<0.05$).

The β-mercaptoethanol treatment resulted in a heat-induced gel with very similar textural properties to the salt-only sample (Table 1); there were no differences ($P>0.05$) in Hd and Sp and only slight but significant differences in cohesiveness and chewiness. The β-mercaptoethanol treatment produced the lowest values of Ch and Cw of all the samples studied. Comminuted meat with β-mercaptoethanol has been reported to present higher hardness and chewiness but similar cohesiveness values to salt-only samples (Barbut & Mittal, 1992). On the other hand, Gordon and Barbut (1992) reported that β-mercaptoethanol treatment produced a gel that was less hard and less springy than but as

cohesive as the control batter. Whiting (1987) observed that β-mercaptoethanol had no effect on the gel strength of beef/pork meat batters. Gel texture (elasticity and rigidity) of kamaboko gels increases at low concentrations of β-mercaptoethanol (100 mmol/kg fish paste), but at high concentration (150–200 mmol/kg), the improving effect is reduced or cancelled (Roussel & Cheftel, 1990). β-Mercaptoethanol is known to effectively reduce exposed disulfide bonds in proteins (Barbut & Mittal, 1992; Whiting, 1987). Although not a prerequisite for gelation of chicken myosin, the formation of intermolecular SS bonds does contribute to gel network formation (Smyth, Smith, & O'Neill, 1998); on the other hand, with excessive formation of disulphide bonds, the product may be too firm and resistant to deformation (Gordon & Barbut, 1992). It therefore follows that reducing SS bond formation by means of a reducing agent will cause some loss of gel texture properties (Table 1).

Fig. 1a shows the storage modulus as a function of temperature for different non-pressurized samples. The rheological thermogram of the control meat batter presents the features normally seen in minced meat with low added salt (Egelandsdal, Martinsen, & Autio, 1995). The presence of the chemical agents produced some differences in the rheological behaviour of the samples during heating. In earlier stages of heat processing, urea had a greater effect on raw meat batters, which presented higher G′ values up to 45°C (Fig. 1a). Hydrophobic interactions are important in stabilizing the raw batter (Gordon & Barbut, 1992), but no differences have been found in modulus of rigidity below 40°C in comminuted beef mixes (Barbut & Mittal, 1992). Depending on the sample, at around 45–57°C, G′ values fell to a minimum (G′ value lower in S and M treatments than in sample with urea), which occurred at lower temperatures in the urea sample (Fig. 1a). This kind of reduction in storage modulus has been related to some protein modification due to the addition of salt in minced meat (Egelandsdal et al., 1995). Further heating produced a sharp increase of G′, indicating the formation of a stiff elastic matrix structure typical of heat induced protein gels. Rheological behaviour was very similar in S and M samples, but addition of urea accelerated gelation and resulted in the highest G′ values (Fig. 1a). Similar results have been reported by Barbut and Mittal (1992), who also observed that hardness was lowest with urea, that is the opposite of the finding in this experiment (Table 1).

Fig. 2 represents the thermal response of chicken raw meat, derived batters and processed products. Curve C shows the typical DSC trace for breast meat, with several endotherms centred at about 60.5, 66.4, 71.1, 75.5, and 81.2°C, and protein denaturation enthalpy (48–87°C straight baseline) of 15.3 J/g (Kijowski & Mast, 1988; Jiménez-Colmenero et al., 1998). Comminution in

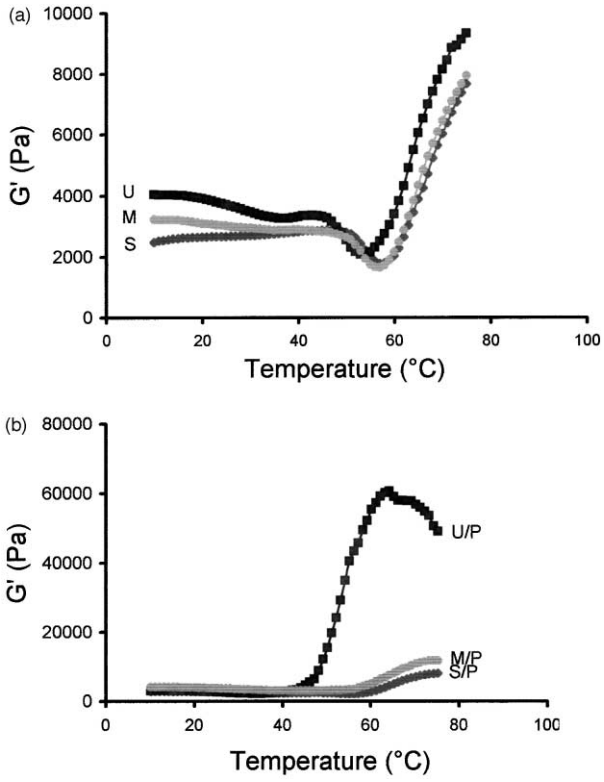

Fig. 1. Changes in storage modulus (G′) during heating (1°C/min) for different samples: (a) non-pressurized, batter with 1.5% NaCl (S), batter with 1.5% NaCl plus 4.5% urea (U) and batter with 1.5% NaCl plus 0.25% β-mercaptoethanol (M); (b) pressurized (325 MPa/30min/10°C), batter with 1.5% NaCl (S/P), batter with 1.5% NaCl plus 4.5% urea (U/P) and batter with 1.5% NaCl plus 0.25% β-mercaptoethanol (M/P).

the presence of 1.5% NaCl caused thermal instability at both extremes of the temperature range as shown in curve S for the corresponding S batter. The myosin (first) major peak was smaller in temperature (57.8°C) and area, and the sharp actin peak was considerably decreased by conversion to a broad signal centred around 74°C. Total denaturation enthalpy decreased to 12.0 J/g, as in a previous report (Jiménez-Colmenero et al., 1998). Cooking S batter, at 70°C/30 min, caused massive denaturation, yielding S/H (residual actin at 79.5°C and 0.4 J/g; S/H curve).

Addition of urea produced further instability (curve U), particularly in relation to the myosin transitions, which shifted downwards in temperature (2.5 and 5° C for the first and second peaks) with a considerable reduction in total enthalpy (8.2 J/g). The decrease in temperatures is consistent with the onset for G′ at a lower temperature in U batter than in control batter S (Fig. 1a). Cooking of U batter yielded an entirely denatured U/H sample, in the sense that its DSC trace presented no thermal transitions (curve U/H). This is consistent with the fact that hardness was significantly highest in U/H (Table 1).

β-Mercaptoethanol, on the other hand, caused very small changes in the original batter (curve M); temperatures fell by very little (around 1°C) and the enthalpy remained practically unvaried (11.5 J/g). It is therefore not surprising that the dynamic rheological behaviours of batters with (M) and without (S) mercaptoethanol were very similar (Fig. 1a). Cooking of M batter yielded M/H (curve M/H) which was very similar (residual actin at 79.5°C, 0.9 J/g) to control S/H. The fact that little lower denaturation was induced may account for the slight (not significant) difference in hardness between M/H and S/H (Table 1).

3.3. Effect of pressurization prior to cooking

Pressure induces some changes in raw meat batter proteins which affect intra and intermolecular interactions (Cheltel & Culioli, 1997). Thus, the properties of processed meat systems depend on how these changes influence subsequent protein denaturation and aggregation induced by heating (Fernández-Martín et al., 1997). Pressurization of meat batters prior to heating modified the characteristics of heat-induced gels (Table 1, Fig. 1b).

With pressurization, the gels presented greater ($P<0.05$) hardness and chewiness, similar ($P>0.05$) springness and, except in urea treatment, greater ($P<0.05$) cohesiveness (Table 1). It has been reported that pressure treatment prior to heating considerably enhances the thermal gelation ability of meat protein, causing an increase in binding strength and Kramer shear force of meat patties (Carballo, Fernández, Carrascosa, Solas, & Jiménez-Colmenero, 1997; Macfarlane, McKenzie, & Turner, 1984). These results indicated that pressurization at non-denaturing temperatures caused some alterations of protein conformation, which favoured protein–protein interaction during heating and hence the formation of stronger gel structures (Table 1; Cofrades, Fernández, Carballo, & Jiménez-Colmenero, 1998). Pressurization increases hydrophobicity and sulfhydryl content, and also denaturation or depolymerization of actin in actomyosin, which enhances the gel-forming capacity of posteriorly heat-induced gels (Ikeuchi, Tanji, Kim, & Suzuki, 1992).

The presence of chemical agents did not substantially alter the pattern of the pressure action, but there were some important aspects. The effect induced by pressure was greatest in the β-mercaptoethanol treatment, which presented the greatest increases of Ch and Cw (Table 1). This meant that the differences in the textural characteristics of the three samples studied were smaller after pressurization; the TPA parameters of pressurized samples were closer to one another than to the non-pressurized samples (Table 1). These results suggest that the effect of pressurization complements the effect of each of the chemical agents in raw meat batter. Urea and pressure appear to affect hydrophobic groups in a similar way; however, following the increase of effective hydrophobicity produced by urea, pressurization can still induce some additional changes leading to increased Hd and Cw (Table 1). In the case of β-mercaptoethanol treatment, although the chemical agent induces the reduction of disulfide bonds, subsequent pressurization of this raw meat system increases the availability of hydrophobic interactions (aside from of the additional changes in sulfhydryl content), which may be involved in protein–protein bonding, thus favouring gels with higher Hd, Ch and Cw (Table 1). This behaviour is consistent with the preponderant role of hydrophobic interactions in gelation processes (Nakai & Li-Chan, 1988). At the same time, the similarity of the textural properties of the three samples suggests that similar gel structures are formed. The reason for this may be that the changes induced by the combined action of the chemical agent and pressurization cause analogous structural alterations (particularly in relation to changes in hydrophobicity), and that during heating these give rise to similar molecular associations and protein gelation processes, so that the gel structures present very similar textural properties (Table 1).

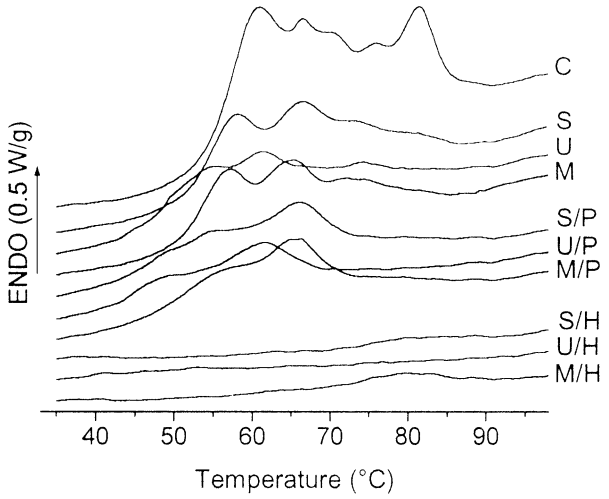

Fig. 2. DSC normalized (dry matter) traces of different samples: C, raw chicken meat; S, batter with 1.5% NaCl; U, batter with 1.5% NaCl plus 4.5% urea; M, batter with 1.5% NaCl plus 0.25% β-mercaptoethanol. /P, pressurized-only products; /H, Heated-only products.

Pressurization induced some changes in the storage modulus (Fig. 1b). In earlier stages of heating (up to 45°C), the behaviour of storage modulus was very similar in all three samples; that is, the differences were smaller than those observed in non-pressurized samples (Fig. 1a). At higher temperatures, there was some variation due to the application of HP, which took two forms. On the one hand, the changes in G′ between 45–57°C were less pronounced in both S/P and M/P samples than in non-pressurized equivalents (S and M; Fig. 1). On the other hand, further heating produced a

greater increase of G′ in the presence of β-mercaptoethanol than in the salt-only sample (Fig. 1b). Pressurization enhanced the effect of urea on rheological behaviour of meat batter, speeding up gelation and giving rise to the highest G′ values (Fig. 1).

Regarding thermal behaviour, curves S/P, U/P, and M/P in Fig. 2 correspond to the DSC traces of the respective pressurized samples from S, U and M batters. They are very similar, obviously reflecting the close visual resemblance of the initial batters themselves. One major variation, induced by pressure, was the disappearance of the actin signal, which is more unstable to pressure. Considerable denaturation of myosin has also been detected in correlation with pressure (325 MPa) at non-denaturing temperature (10°C; Fernández-Martín et al., 1997). Essentially, M/P (peaks at 55.0 and 65.2°C; 6.1 J/g) was close in terms of temperature to the control S/P (55.5 and 65.8°C; 5.2 J/g) but underwent protein denaturation to a relatively smaller degree. This could account for the fact that the G′ value for M/P was only very slightly higher (Fig. 1b). Pressurization of batter with urea gave U/P with a DSC profile also close to the control S/P; however, as in non-pressurised samples, peak temperatures for U/P decreased (45.8 and 61.5°C), particularly for the first myosin transition, and the enthalpy value was lower (4.5 J/g). This is clearly consistent with a much lower onset temperature for the corresponding G′ parameter. Also, the ending of myosin (collagen) denaturation at a lower temperature could be connected with the fact that G′ maximum occurred much earlier (Fig. 1b), at practically the same temperature (61–62°C). U/P presented the lowest protein denaturation enthalpy of all the pressurized samples, meaning that less native-like proteins were left for subsequent thermal denaturation/aggregation. This would lead to a rather less cohesive gel in U/P-H, in consistency with data of Table 1. All these factors may help to explain the reverse trend observed in G′ modulus, which declined at higher temperatures (Fig. 1b). On the other hand, thermal treatment (70°C/30 min) of previously pressurised samples yielded complete protein denaturation in all cases (S/P-H, U/P-H, M/P-H), given that DSC traces were devoid of endothermal events (not shown).

From the standpoint of DSC, we may conclude that, as far as hydrogen-bonding is concerned, urea produced considerable destabilization of chicken breast muscle proteins in the presence of salt. On the other hand, β-mercaptoethanol was practically neutral. Pressurization, in thermally non-denaturing conditions, induced considerable denaturation in all cases, thus reducing the differences among the batters. Urea-treated batters, however, were still the most unstable in terms of thermal transition temperatures and denaturation enthalpy. These patterns of thermal behaviour were consistent with the rheo-dynamics exhibited by the respective batters. Ordinary cooking of non-pressurized batters yielded slightly different functional gels. Where the same thermal treatment was applied to previously pressurized batters, the products were wholly analogous.

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