Heating of Chicken and Pork Meat Batters under Pressure Conditions: Protein Interactions

Francisco Jiménez-Colmenero,* Susana Cofrades, José Carballo, Paloma Fernández, and Fernando Fernández-Martín

Instituto del Frío (CSIC), Ciudad Universitaria, 28040 Madrid, Spain

Protein molecular interactions involved in the formation of the protein network of pork and chicken gels made by heating (70 °C) under pressure (200 and 400 MPa) were studied. Gel properties were influenced both by pressure treatment and by species. In both species, pressurization favored gels that had better water binding properties but were less hard and chewy. Nondenatured proteins were thermally detected in the batters after all of the combined pressure/heat processes. Heating-under-pressure conditions caused breakdown of myosin molecules, so that salt soluble protein content was higher than in nonpressurized samples.

Keywords: *High pressure−thermal treatments; comminuted meat; protein interaction; electrophoresis; texture; DSC*

INTRODUCTION

There is currently a lot of research on high-pressure treatment of meat and meat products as a possible means of controlling the toughness of meat and improving the gel-forming properties of muscle proteins in processed meat products (Macfarlane, 1985; Suzuki et al., 1990, 1991). Pressure treatment prior to heating has been reported to considerably enhance the thermal gelation ability of meat protein in a model system (Suzuki and Macfarlane, 1984; Ikeuchi et al., 1992a), favoring increased binding strength of meat patties (Macfarlane et al., 1984) and increased Kramer shear force of low- and high-fat burgers (Carballo et al., 1997). However, no advantageous effects of such processing conditions have been detected in meat emulsions (Carballo et al., 1996; Jiménez-Colmenero et al., 1997) or myosin solutions (Yamamoto et al., 1993). Heating-under-pressure treatments applied to pork and chicken meat batters have been found to cause gel/emulsion structures that are weaker (Fernández-Martín et al., 1997) or less hard and chewy (Fernández et al., 1998; Jiménez-Colmenero et al., 1998). Similar findings have been reported for other types of myosystems, Alaska pollack and chum salmon meat gels (Okazaki et al., 1997). The mechanism whereby the product's properties are altered is not clearly understood, but in these cases it seems to be connected with the fact that the pressurization process partially preserves the protein from thermal denaturation during gelation (Fernández-Martín et al., 1997). This effect limits the formation of gel structures and is responsible for the different water binding and textural properties of pressurized and nonpressurized meat emulsions. Heating of meat batters (up to 80 °C) to above the standard cooking temperature (70 °C) in pressure/heat treatments causes complete thermal denaturation of the meat proteins (Fernández-Martín et al., 1997), but it did not make the gels any harder or chewier (Jiménez-Colmenero et al., 1998).

To understand mechanisms of protein denaturation and gelation induced by heating-under-pressure processing, studies are required to analyze the phenomena that occur in the main muscle proteins. Various studies have shown that pressurization causes depolymerization, solubilization, denaturation, and aggregation in myofibrillar proteins (Macfarlane, 1985; Cheftel and Culioli, 1997). It has been reported that pressurization of protein systems induces breakdown of the salt bonds and at least a part of hydrophobic interactions; in contrast, hydrogen bonds appear to be somewhat strengthened under pressure, whereas covalent bonds are much less sensitive to changes in pressure (Cheftel and Culioli, 1997). However, such behavior would appear to be influenced by several factors such as the type of myosystem or the pressurizing conditions (Ikeuchi et al., 1992b; Johnston, 1992; Cheftel and Culioli, 1997).

The objective of the present study was to analyze how heating-under-pressure treatments affect the protein molecular interactions involved in the formation of the protein network of pork and chicken meat gels. Molecular interactions were evaluated on the basis of protein solubility in some solubilizing agents and subsequent electrophoretic analysis. Differential scanning calorimetric (DSC) response, water binding properties, and texture were also studied.

MATERIALS AND METHODS

Preparation of Meat Batters and Pressure Conditions. Fresh chicken breast (pectoralis major and pectoralis minor muscles) and pork (M. biceps femoris, M. semimenbranosus, M. semitendinosus, M. gracilis, and M. adductor) were obtained from a local meat market. Sufficient amounts of meat and water and 1.5% NaCl were combined to formulate two different meat batters. The batters were prepared as follows: raw meat material was homogenized and ground for 60 s in a chilled cutter (2 °C) (Stephan Universal Machine UM5,

* Author to whom correspondence should be addressed (e-mail fjimenez@fresno.csic.es; fax +34 1 549 36 27).

10.1021/jf980354y CCC: $15.00
Published on Web 10/24/1998

Stephan u. Sóhne GmbH & Co., Hameln, Germany). Water and NaCl were then added, and the mixture was homogenized again under chilled vacuum (2 °C, 610 mmHg) until the final chopping temperature of the batters reached 10 °C. This step took 4 min.

Heating (70 °C) under pressure conditions (200 and 400 MPa) was carried out according to the method of Fernández-Martín et al. (1997). For each formulation a nonpressurized control sample (NP) was made by heating under the same conditions as the pressurized samples. After pressurizing, the samples were stored for 18 h at 0−4 °C for analysis.

Proximate Analysis, pH, and Weight Loss. Moisture, protein, fat, and ash of uncooked meat batters and pH and weight loss (WL, as percent fluid released) of heated samples were evaluated according to the procedure of Fernández et al. (1998).

Texture Profile Analysis (TPA). TPA was performed in a Universal Testing Machine (model 4501, Instron Engineering Corp., Canton, MA) as described by Bourne (1978). Five cores (diameter = 3.3 cm, height = 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).

Thermal Analysis (DSC). The thermal behavior of samples was determined by means of a calibrated Perkin-Elmer differential scanning calorimeter DSC7 (Norwalk, CT), according to a procedure described elsewhere (Fernández-Martín et al., 1997). Results are means of three to four values and are reported within ±0.5 °C in temperature and within ±8% in enthalpy.

Selective Protein Solubility. Solubility of cooked meat batters was analyzed to identify types of molecular bonding using agents that cleave or destroy different types of intermolecular bonds: electrostatic (0.6 M NaCl) and hydrogen and hydrophobic (8 M urea) (Kauzmann, 1959; Tsuchiya et al., 1980; Wall and Huebner, 1981). Samples were treated successively with 0.6 M NaCl (solution A) and 0.6 M NaCl plus 8 M urea (solution B). The protocol for each of the samples was as follows: 10 g of gel was homogenized in an Omni-Mixer (ES Homogenizer, OMNI International Inc., Gainsville, VA) for 90 s at 2−4 °C with 50 mL of solution A and then centrifuged (Beckman J2MC, Fullerton, CA) for 30 min at 20000*g* and 4 °C. Once the supernatant was removed, 50 mL of solution A was added to the precipitate, which was stirred for 1 h in a cold room at ~5 °C. The sample was then centrifuged (30 min, 20000*g*, 4 °C), and the two supernatants were mixed and called the salt soluble protein (fraction 1); this was considered to be essentially nondenatured protein (Jiang et al., 1989) taking part in ionic bondings, which had not undergone insolubilization in the heating process (Jiménez-Colmenero et al., 1994).

Fifty milliliters of solution B was added to the precipitate, and this was homogenized (90 s, 2−4 °C), stirred (24 h, 5 °C) in the conditions described above, and then centrifuged (20000*g*, 30 min, 4 °C). The resulting precipitate was then put through the same process again. The combination of the two supernatants was called urea soluble protein (fraction 2); aggregation of this fraction is thought to occur through formation of hydrogen and hydrophobic bonds (Jiang et al., 1989).

Finally, the remaining residue was called insoluble protein (fraction 3). This consisted of proteins that were aggregated by S−S bonding and other covalent bonds, plus insoluble residue composed of meat fiber pieces and connective tissue.

Protein concentration in fractions 1 and 2 was determined according to the Lowry method (Lowry et al., 1951) using specific standard curves for each solution. The results are expressed as the percentage of solubilized protein with respect to total protein of the cooked samples.

Table 1. Proximate Analysis (Percent) of the Chicken and Pork Meat Batters and pH of the Cooked Samples

	moisture[a]	protein[a]	fat	ash	pH
chicken	79.8_a	16.2_a	1.9	2.1	6.1
pork	81.8_b	14.1_b	2.1	2.0	6.1
SEM[b]	0.1	0.2	0.0	0.2	0.0

[a] Values with different letters in the same column are significantly different ($P < 0.05$). [b] SEM, standard error of the mean.

Table 2. Weight Loss and TPA Paramenters of the Different Samples

sample[a]	wt loss (%)	hardness (N)	springiness (mm)	cohesiveness	chewiness (N × mm)
chicken					
NP	2.39_a	48.82_a	6.79_{ab}	0.55_a	183.70_a
200	1.06_b	55.26_b	6.86_b	0.56_a	213.74_b
400	0.54_c	30.22_c	6.44_c	0.50_b	96.95_c
pork					
NP	7.82_d	23.56_d	6.51_{ac}	0.53_{ab}	80.73_d
200	3.02_e	26.60_e	6.66_{abc}	0.51_b	89.80_c
400	0.91_{bc}	8.26_f	5.77_d	0.32_c	15.31_e
SEM[b]	0.13	1.02	0.11	0.01	2.68

[a] Samples: nonpressurized (NP) and pressurized at 200 and 400 MPa. Values with different letters in the same column are significantly different ($P < 0.05$). [b] SEM, standard error of the mean.

Electrophoresis. Protein fractions 1 and 2 from the pressurized and nonpressurized samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) in a Phastsystem horizontal apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden), using 12.5% polyacrylamide gels. The samples were treated according to the method of Hames (1985) (2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue) and then heated for 5 min in a boiling water bath. One-microliter aliquots of the different fractions containing known amounts of protein (ranging from 1 to 2 mg/mL) were applied to the gels.

Electrophoretic conditions were 4 mA/gel, 250 V, and 3 W. The protein bands were stained with Coomassie brilliant blue (PhastGel Blue R, Pharmacia LKB Biotechnology). The molecular weight of the main proteins was calculated by comparing their mobility with a standard high molecular weight protein mix (Pharmacia LKB Biotechnology).

Statistical Analysis. Two-way analyses of variance were performed using a computer statistical package (Statgraphics, STSC Inc., Rockville, MD). The differences of means between pairs were resolved by LSD test to obtain the confidence intervals. Level of significance was set for $P < 0.05$.

RESULTS

Proximate Analysis, pH, and Weight Loss. Moisture content was lower ($P < 0.05$) and protein content higher ($P < 0.05$) in the chicken sample (Table 1). There were no differences ($P > 0.05$) in the fat and ash contents of the chicken and pork meat batters or in the pH of cooked samples. There were some differences in appearance between nonpressurized and pressurized samples; heating under pressure produces gels that are glossier and smoother than gels made by heating alone, which tend to have a more opaque and porous appearance.

In nonpressurized samples, chicken gel had higher ($P < 0.05$) water binding properties than pork gel (Table 2). Pressurizing caused a reduction ($P < 0.05$) of WL in both species which was greater ($P < 0.05$) at 400 MPa than at 200 MPa. The enhancement of water binding properties was proportionally greater in pork than in chicken samples. A similar pattern has been already

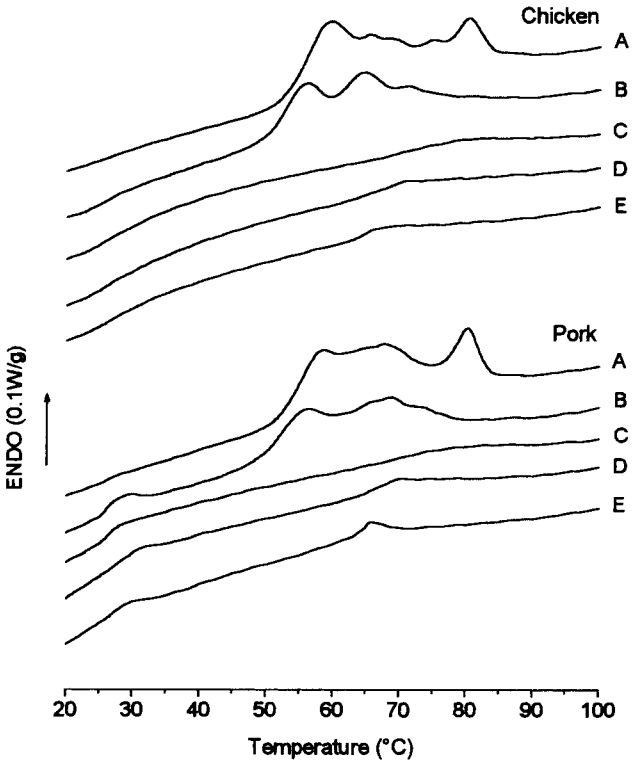

Figure 1. DSC profiles on chicken and pork samples: (A) raw minced meats; (B) raw meat batter; (C−E) meat batter processed at 70 °C as nonpressurized (C), 200 MPa (D), and 400 MPa (E).

described in gel/emulsion systems (Fernández-Martín et al., 1997; Jiménez-Colmenero et al., 1998).

Texture Profile Analysis. There were significant differences in textural properties between species and between heat- and heat-under-pressure-induced gels (Table 2). Hardness and chewiness values were higher ($P < 0.05$) in nonpressurized chicken samples than in nonpressurized pork samples. On the other hand, there were no species-based differences ($P > 0.05$) in springiness and cohesiveness. In chicken and pork samples, pressurization at 200 MPa caused the formation of structures that were harder and chewier ($P < 0.05$) than NP samples but had similar ($P > 0.05$) springiness and cohesiveness values. In both species pressurizing at 400 MPa caused a decrease ($P < 0.05$) in all of the textural parameters considered (Table 2). Similar results have been reported in meat batters from different myosystems such as chum salmon, chicken, and pork (Okazaki et al., 1997; Férnandez et al., 1998; Jiménez-Colmenero et al., 1998).

Thermal Analysis. Thermal behavior of chicken and pork meat samples is shown in Figure 1. Figure 1A is a typical DSC curve of chicken breast meat (Kijowski and Mast, 1988) with several peaks at about 59.3, 65.5, 69.2, 74.6, and 80.5 °C and melting enthalpy of 13.8 J/g (dry bases). The first and last transitions were due to the myosin and actin contributions of the actomyosin complex. The intermediate effects were mainly due to sarcoplasmic and connective proteins. Comminuting with 1.5% NaCl induced lower thermal stability in the batter with around one-third reduction of total transition enthalpy; myosin transition was shifted down ~3.5 °C and the actin signal greatly reduced (Figure 1B, chicken). In nonpressurized sample, heating in strongly denaturing conditions (70 °C/30 min) caused almost complete protein denaturation except for a small residual effect of actin (Figure 1C, chicken). In contrast, noticeable amounts of nondenatured proteins at the middle zone of the DSC curve survived the pressure−heat treatments. Moreover, the higher the pressure applied, the larger were the surviving proteins, as indicated by the figures of 0.8 and 1.4 J/g recorded, respectively, for 200 (Figure 1D, chicken) and 400 MPa (Figure 1E, chicken). This kind of preserving effect of pressure against protein thermal denaturation has been observed before in pork gel/emulsion systems processed by pressure/heat combinations (Fernández-Martín et al., 1997).

The pattern was similar in the case of pork meat. Figure 1A shows a typical DSC curve of fresh pork meat with only three main peaks at about 58.2, 67.6, and 80.3 °C (Wright et al., 1977) and associated enthalpy of 14.8 J/g. The raw batter profile (Figure 1B, pork) was similar to that of chicken, although bigger in area (9.9 J/g) because there was less actin destabilization than before. Consequently, the DSC curve of the nonpressurized cooked batter (Figure 1C, pork) contained slightly more nondenatured actin residue. Once again, pressure protected protein from subsequent thermal denaturation as shown in Figure 1D,E (200 and 400 MPa, pork), with enthalpic effects comparable to those found in chicken meat. The only extra fact recorded was a small endothermic event centered around 30 °C in all samples except the fresh meat. This effect was highest in the raw batter and lowest in the cooked batter and could indicate some protein modification.

DSC scans of salt soluble protein (fraction 1) from nonpressurized pork batter recorded a large peak around 65 °C (assigned to myosin at high ionic strength) and another unknown event at the beginning (around 10 °C). DSC of fraction 1 from pork batter pressurized at 400 MPa recorded only the low-temperature event; there was no signal resembling that of Figure 1E (pork).

Selective Protein Solubility. The protein content in each fraction varied according to the species and the pressure conditions (Figure 2). In nonpressurized samples, the percentage of native proteins (fraction 1) was higher ($P < 0.05$) in pork gels than in chicken gels. There were no appreciable differences ($P > 0.05$) between the species in fractions 2 and 3.

Heating-under-pressure caused an increase ($P < 0.05$) in the proportion of salt soluble protein in both species, which was greater the higher the pressure that was applied (Figure 2). In all cases, protein content of salt soluble protein was greater ($P < 0.05$) in pork than in chicken gels. Solubilization of myofibrillar protein was found during pressurization at 30 °C, although in smaller proportion than has been reported for protein treated at 0 °C (Macfarlane and Mckenzie, 1976).

In chicken samples, pressurization had no clear effect on the urea soluble protein (Figure 2); in pork samples, processing at 400 MPa caused a decrease ($P < 0.05$) in the solubility of proteins involved in the gel network through hydrophobic interactions and hydrogen bridges (fraction 2). It has been found that conformational changes in proteins resulting from pressure induce some breakdown of hydrophobic interactions, although the hydrogen bonds appear to be somewhat strengthened (Cheftel and Culioli, 1997). Pressurization did not clearly influence fraction 3 in either species (Figure 2).

Electrophoresis. The electrophoretic profile of salt soluble proteins and urea soluble proteins varied ac-

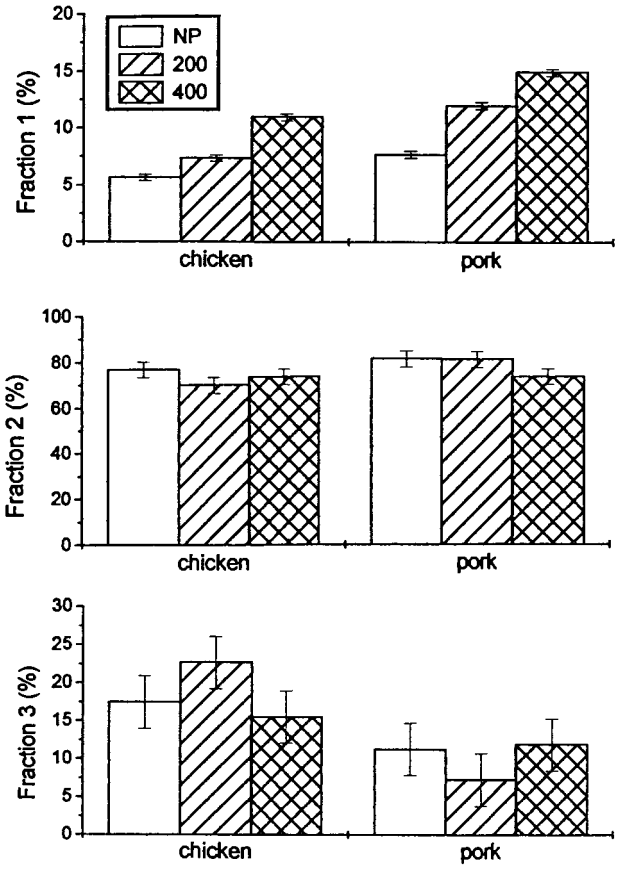

Figure 2. Selective protein solubility of nonpressurized (NP) samples and samples pressurized at 200 and 400 MPa: fraction 1, salt soluble protein; fraction 2, urea soluble protein; fraction 3, insoluble fraction. Vertical bar indicates confidence interval at $P < 0.05$.

cording to the species and the pressurizing conditions (Figure 3). In nonpressurized samples thermal treatment caused protein denaturation and aggregation, so that the amount of myosin heavy chain (MHC) in fraction 1 from both species decreased to undetectable levels. Nevertheless, the electrophoretic band corresponding to actin was detectable, however faintly, which suggests that the actin had undergone less drastic changes. These facts are fully consistent with the DSC traces of Figure 1C (chicken and pork) and are in agreement with previous results (Cofrades and Jiménez-Colmenero, 1998). Also, unlike the chicken samples, in the salt soluble fraction of pork samples there were protein bands of ~67 kDa (Figure 3, lane NP).

In fraction 1, in both species pressurization caused the complete disappearance of actin and the appearance of low molecular weight (MW) proteins in two broad groups: one in the region of 20−30 kDa and the other <11 kDa. More of these proteins appeared at the higher pressurization level. In pork samples processed at 200 MPa, as in the nonpressurized samples, there was a band at 67 kDa (Figure 3, lane 200) that was not visible after pressurizing at 400 MPa (Figure 3, lane 400). The absence of both actin and MHC bands in fraction 1 of all the pressurized batters (Figure 3, lanes 200 and 400) is fully consistent with the corresponding DSC traces of Figure 1D,E (chicken and pork).

The electrophophoretic profiles of urea soluble protein (fraction 2) from chicken and pork gels were similar (Figure 3). MHC and actin were the majority proteins in the nonpressurized samples (Figure 3, lane NP).

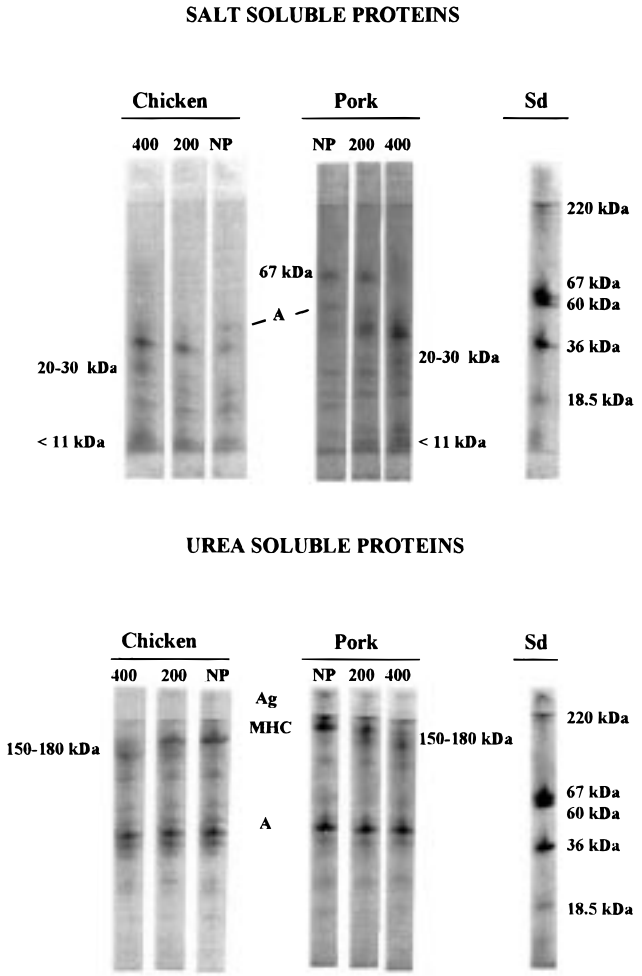

Figure 3. SDS−PAGE of salt soluble protein and urea soluble protein of nonpressurized (NP) samples and samples pressurized at 200 and 400 MPa. MHC, myosin heavy chain; A, actin; Ag, aggregate; Sd, standard protein mix.

Pressurization altered the electrophoretic profile of the urea soluble protein, mainly in the 200−150 kDa zone (lanes 200 and 400). There were no appreciable changes in actin, but pressurization simultaneously caused a decrease in MHC and the appearance of high-MW protein fragments of between 180 and 150 kDa. Both phenomena were more pronounced at higher pressure; at 400 MPa, the presence of MHC was so small as to be almost undetectable. Also, in the application zone between the stacking and resolving gels of either species, polymerized protein was found in the form of large aggregates (Figure 3) that were nonetheless soluble in the solutions.

DISCUSSION

The denaturation and aggregation caused by cooking of the meat batters entailed the virtual disappearance of native protein molecule structures (Figure 1C, chicken and pork) and a decrease of protein solubility in salt solutions (Figure 2). Thermal processes are highly favorable to the formation of hydrophobic interactions and hydrogen bridges and less favorable to disulfide bridges and other covalent bonds (Nakai and Li-Chan, 1988; O'Neill et al., 1994; Cofrades and Jiménez-Colmenero, 1998). This is consistent with the relatively low presence (7−23%) of insoluble protein and the high proportion of protein (75−80%) in the urea soluble

fraction (Figure 2). It is also consistent with the composition of that fraction, largely MHC and actin (Figures 3), the role of which in the formation of the gel network by means of hydrophobic interactions and hydrogen bridges has been reported by other authors (Samejima et al., 1981).

In the experimental conditions, the target pressure was attained uniformly and quickly, whereas the heating process was slower. This indicates that the pressure-induced changes preceded the heating-induced changes that took place at the stipulated pressures. Thus, the combined, sequential action of both kinds of change contributed to the final effect of the processing conditions on the batters.

DSC confirmed results previously described (Fernández-Martín et al., 1997) in that pressure partially preserved protein (more so at the higher pressure level) from subsequent thermal denaturation at cooking temperatures. In the above-cited paper on pork meat emulsions at several temperatures and pressures, it was demonstrated that the rheological behavior (penetration tests) of these pressurized batters was directly related to their nondenatured protein content.

On the other hand, the analytical results indicate that heating-under-pressure treatment favored MHC breakdown, which in both species caused the formation of low- and high-MW protein fragments (Figure 3). On applying heat-under-pressure treatments to myofibrillar proteins, Macfarlane et al. (1986) reported a decrease of myosin and the appearance of a protein fragment of ~150 kDa; its origin was attributed to pressure-accelerated enzymatic breakdown of a higher molecular weight component, possibly myosin or connectin. Increased protease activity on meat protein due to high pressure has been observed during heat-under-pressure (50−60 °C) treatments (Bouton et al., 1977; King et al., 1981; Locker and Wild, 1984; Macfarlane, 1985; Macfarlane et al., 1986), causing breakdown of myofibrillar proteins. Other changes in protein characteristics may also have contributed to the structural breakdown of MHC, because pressure-induced destabilization of protein (e.g., by denaturation) renders them more vulnerable to attack by proteases (Muramoto and Seki, 1989).

Preliminary results have shown that salt soluble fragments from MHC in pressurized pork samples are not responsible for the DSC signal detected in the thermal stability middle zone of the corresponding samples. Thus, although the possible interconection between both kinds of pressure action is to be addressed in a future study, both phenomena (preserving from subsequent thermal protein denaturation and MHC breakdown) seem to occur independently. In fact, thermodynamic considerations cited previously (Fernández-Martín et al., 1997) explain pressure−temperature interdependence in protein denaturation as an aspect of the general behavior of protein molecules.

In any event, both phenomena can help determine sample properties. The presence of low-MW fragments would help explain the increase of salt soluble proteins in the gel network (Figure 2) and the consequent improvement of water binding properties (Table 2). MHC breakdown can also influence the gelation process, possibly by causing the formation of a less complete and ordered three-dimensional gel, which would favor less hard and chewy gel structures (Table 2). The protein denaturation-preserving effect may similarly contribute to sample properties.

Gordon and Barbut (1992) reported that hydrophobic associations favor the formation of harder gel/emulsion structures as measured by TPA. However, the effect of pressurization on texture (Table 2) was not clearly linked to quantitative changes in the proteins involved in the formation of the gel network via hydrophobic interactions and hydrogen bridges (Figure 2). Higher levels of insoluble protein imply that the proteins may be linked by covalent bonds which would be involved in the formation of the network without being broken in the experimental solubilization conditions. In such conditions greater network solubility would mean a weaker structure, something that has been observed in nonpressurized gel/emulsion systems (Cofrades and Jiménez-Colmenero, 1998). Although this is not generally consistent with the textural data found as a function of pressure conditions, it does appear to be linked to species. Chicken samples generally contained lower levels of salt soluble protein and higher levels of insoluble protein than did pork samples (Figure 2), which suggests that more chicken proteins were involved in the formation of the gel network. Differences in the thermal gelation properties of these species have been reported (Lan et al., 1995), namely, that pork meat produces weaker gels with poorer binding properties (Table 2) than chicken meat, although in our experiment the different protein content in the batters of either species could also be a factor (Table 1).

CONCLUSIONS

The gelation processes of chicken and meat batters were different under pressurized and nonpressurized conditions. This behavior appears in principle to be linked to two different effects that pressure produced in these heating-under-pressure treatments. The first is the denaturation-preserving effect on batter proteins with middle thermal stability. The second is myosin molecule breakdown, causing the formation of various molecular fragments that increase the proportion of salt soluble protein. Both effects in conjunction may help to improve water binding properties, but at the same time may limit the gelation process, making the gel matrix less rigid than it is when thermal treatment of meat batters occurs in nonpressurized conditions.

LITERATURE CITED

Bourne, M. C. Texture profile analysis. *Food Technol.* **1978**, *32*, 62−65.

Bouton, P. E.; Ford, A. L.; Harris, P. V.; Macfarlane, J. J.; O'Shea, J. M. Pressure-induced solubilization of myofibrillar proteins. *J. Food Sci.* **1977**, *42*, 132−135.

Carballo, J.; Fernández, P.; Jiménez-Colmenero, F. Texture of uncooked and cooked low- and high-fat meat batters as affected by high hydrostatic pressure. *J. Agric. Food Chem.* **1996**, *44*, 1624−1625.

Carballo, J.; Fernández, P.; Carrascosa, A. V.; Solas, M. T.; Jiménez-Colmenero, F. Characteristics of low-and high fat beef patties: Effect of high hydrostatic pressure. *J. Food Prot.* **1997**, *60*, 48−53.

Cheftel, J. C.; Culioli, J. Effects of high pressure on meat: a review. *Meat Sci.* **1997**, *46*, 211−236.

Cofrades, S.; Jiménez-Colmenero, F. Protein molecular interactions involved in formation of frankfurters: effect of fat level and heating rate. *Meat Sci.* **1998**, *49*, 411−423.

Fernández, P.; Cofrades, S.; Solas, M. T.; Carballo, J.; Jiménez-Colmenero, F. High pressure-cooking of chicken meat batters with starch, egg white and iota carrageenan. *J. Food Sci.* **1998**, *63*, 267−271.

Fernández-Martín F.; Fernández, P.; Carballo, J.; Jiménez-Colmenero, F. Pressure/heat combinations on pork meat batters: Protein thermal behavior and product rheological properties. *J. Agric. Food Chem*. **1997**, *45*, 4440−4445.

Gordon, A.; Barbut, S. Effect of chloride salts on protein extraction and interfacial protein film formation in meat batters. *J. Sci. Food Agric.* **1992**, 58, 227−238.

Hames, B. D. An introduction to polyacrylamide gel electrophoresis. In *Gel Electrophoresis of Proteins. A Practical Approach*; Hames, B. D., Rickwood, D., Eds.; IRL Press: Oxford, U.K., 1985; Chapter 1.

Ikeuchi, Y.; Tanji, H.; Kim, K.; Suziki, A. Dynamic rheological measurements on heat-induced pressurized actomyosin gels. *J. Agric. Food Chem*. **1992a**, *40*, 1751−1755.

Ikeuchi, Y.; Tanji, H.; Kim, K.; Suziki, A. Mechanims of heat-induced gelation of pressurized actomyosin: Pressure-induced changes in actin and myosin in actomyosin. *J. Agric. Food Chem*. **1992b**, *40*, 1756−1761.

Jiang, S. T.; San, P. C.; Japit, L. S. Effect of storage temperatures on the formation of disulfides and denaturation of tilapia hybrid actmyosin (*Tilapia nilotica* × *Tilapia aurea*). *J. Agric. Food Chem*. **1989**, *37*, 633−636.

Jiménez-Colmenero, F.; Careche, M.; Carballo, J.; Cofrades, S. Influence of thermal treatment on gelation of actomyosin from different myosystems. *J. Food Sci*. **1994**, *59*, 211−220.

Jiménez-Colmenero, F.; Carballo, J.; Fernández, P.; Barreto, G.; Solas, M. T. High-pressure-induced changes in the characteristics of low and high-fat sausages. *J. Sci. Food Agric*. **1997**, *75*, 61−66.

Jiménez-Colmenero, F.; Fernández, P.; Carballo, J.; Fernández-Martín, F. High-pressure-cooked low-fat pork and chicken batters as affected by salt levels and cooking temperature. *J. Food Sci*. **1998**, *63*, 656−659.

Johnston, D. E. High pressure. In *The Chemistry of Muscle-Based Food*; Ledward, D. A., Johnston D. E., Knight, M. K., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1992; pp 298−307.

Kauzmann, W. Some factors in the interpretation of protein denaturation. In *Advances in Protein Chemistry*; Academic Press: New York, 1959; Vol. 14, pp 1−64

Kijowski, J. M.; Mast, M. G. Thermal properties of proteins in chicken broiler tissues. *J. Food Sci*. **1988**, *53*, 363−366.

King, N. L.; Kurth, L.; Shorthouse, W. R. Proteolytic degradation of connectin. A high molecular weight myofibrillar protein, during heating of meat. *Meat Sci*. **1981**, *5*, 389−396.

Lan, Y. H.; Novakofski, J.; McCusker, R. H.; Brewer, M. S.; Carr T. R.; McKeith, F. K. Thermal gelation of myofibrils from pork, beef, fish, chicken and turkey. *J. Food Sci*. **1995**, *60*, 941−945.

Locker, R. H.; Wild, D. J. The fate of the large proteins of the myofibril during tenderising treatments. *Meat Sci*. **1984**, *11*, 89−108.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **1951**, *193*, 265−275.

Macfarlane, J. J. High-pressure technology and meat quality. In *Developments in Meat Science−3*; Lawier, R., Ed.; Elsevier Applied Science Publisher: London, U.K., 1985; Chapter 6.

Macfarlane, J. J.; MacKenzie, I. J. Pressure-induced solubilization of myofibrillar proteins. *J. Food Sci*. **1976**, *41*, 1442−1446.

Macfarlane, J. J.; McKenzie, Y. J.; Turner, R. M. Binding of comminuted meat: Effect of high pressure. *Meat Sci*. **1984**, *10*, 307−320.

Macfarlane, J. J.; McKenzie, I. J.; Turner, R. H. Pressure-heat treatment of meat: changes in myofibrillar proteins and ultrastructure. *Meat Sci*. **1986**, *17*, 161−176.

Muramoto, M.; Seki, N. Proteolytic cleavages of carp myosin by several proteases. *Bull. Jpn. Soc. Sci. Fish*. **1989**, *55*, 1605−1613.

Nakai, S.; Li-Chan, E. *Hydrophobic Interactins in Food Systems*; CRC Press: Boca Raton, FL, 1988.

Okazaki, E.; Ueda, T.; Kusaba, S.; Kamimura, Y.; Fukuda, Y.; Arai, K. Effect of heating on pressure-induced gel of chum salmon meat. In *High-Pressure Research in the Biosciences and Biotechnology*; Heremans, K., Ed.; Leuven University Press: Leuven, Belgium, 1997; pp 371−374.

O'Neill, E.; Mulvihill, D. M.; Morrissey, P. A. Molecular forces involved in the formation and stabilization of heat-induced actomyosin gels. *Meat Sci.* **1994**, *36*, 407−421.

Samejima, K.; Ishioroshi, M.; Yasui, T. Relative roles of the heat and tails portions of the molecule in the heat-induced gelation of myosin. *J. Food Sci.* **1981**, *46*, 1412−1418.

Suzuki, T.; Macfarlane, J. J. Modification of the heat-setting characteristics of the myosin by pressure treatment. *Meat Sci*. **1984**, *11*, 263−274.

Suzuki, A.; Suzuki, N.; Ikeuchi, Y.; Saito, M. Effects of high-pressure treatment on the ultrastructure and solubilization of isolated myofibrils. *Agric. Biol. Chem*. **1990**, *55*, 2467−2473.

Suzuki, A.; Watanabe, M.; Iwamura, K.; Ikeuchi, Y.; Saito, M. Effect of high-pressure treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. *Agric. Biol. Chem*. **1991**, *54*, 3085−3091.

Tsuchiya, Y.; Tschiya, T.; Matsumoto, J. The nature of the cross-bridges constituting aggregates of frozen stored carp myosin and actomyosin. In *Advances in Fish Science and Technology*; Jubilee Conference of the Torry Research Station; Connell, J. J., Ed.; Torry Research Station: Aberdeen, Scotland, 1980; pp 434−438.

Wall, J. S.; Huebner, F. R. Adhesion and cohesion. In *Protein Functionality in Foods*; Cherry, J. P., Ed.; ACS Symposium Series 147; American Chemical Society: Washington, DC, 1981; Chapter 6.

Wright, D. J.; Leach, I. B.; Wilding, P. Differential scanning calorimetric studies of muscle and its constituents proteins. *J. Sci. Food Agric*. **1977**, *28*, 557−564.

Yamamoto, K.; Hayashi, S.; Yasui, M. Hydrostatic pressure aggregation of myosin molecules in 0.5M KCl at pH 6.0. *Biosci., Biotechnol., Biochem*. **1993**, *57*, 383−389.

Received for review April 6, 1998. Revised manuscript received July 10, 1998. Accepted August 26, 1998. This research was supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT) under projects ALI97-0684 and ALI97-0759 and by Comunidad de Madrid 06G/053/96.

JF980354Y