Pressure/Heat Combinations on Pork Meat Batters: Protein Thermal Behavior and Product Rheological Properties

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Common formulation batters were prepared by pork meat comminution and processed for 30 min by pressure/thermal combinations of 200 and 400 MPa with five temperatures from 10 to 70 °C. Processing performance was monitored by determination of pH, cooking loss, and three textural parameters to assess the quality of pressurized versus unpressurized samples. Protein thermal denaturation was determined by differential scanning calorimetry to evaluate the unfolding effects of processing. Net effects were pressure−temperature interdependent, pressure denaturation being particularly effective at nondenaturing temperatures. Pressure suppressed subsequent thermal denaturation at unfolding temperatures, preventing batter proteins from denaturing to an extent dependent on the magnitude of both pressure and temperature processing parameters. Pressurized batters needed higher temperatures to overcome pressure effects and regain total protein thermal denaturation. Pressure/thermal treatments impaired meat gelling at normal cooking temperatures, leading to undesirable effects on the pressurized batters. The extent of native protein remnant after pressure/heat processing explained the apparently erratic physical behavior of the pressurized batters.

Keywords: *High pressure/thermal treatments; comminuted meats; mechanical properties; texture; protein denaturation; DSC*

INTRODUCTION

There is increasing interest in high-pressure processing of foods because of the generally beneficial effects that this, combined with thermal treatments, can have on the shelf life and quality of the end product (Farr, 1990; Hayashi, 1992). Sodium chloride and phosphates have traditionally been used in the meat industry to promote water binding and reduce cooking loss, but pressure processing at 100−200 MPa seems to allow salt and phosphate reduction in frankfurter-type sausages, particularly low caloric formulations (Mandava et al., 1994). Meat products have been pressurized to produce a general improvement in the gel-forming capabilities of meat proteins and consequent enhancement of the textural properties of processed meat (Macfarlane, 1985; Cheftel and Culioli, 1997). Pressurization prior to thermal treatment enhances the thermal gelation characteristics of meat myofibrillar proteins either isolated or in complex formulations such as meat patties (Macfarlane et al., 1984) or low- and high-fat burgers (Carballo et al., 1997), but no beneficial effects have been detected as a result of pressurization prior to cooking of meat emulsions with different fat contents (Carballo et al., 1996). However, Icard (1995), as cited by Cheftel and Culioli (1997), reported a strong restructuring effect, without any exudation, on finely comminuted bovine meat processed at 450 MPa and 50 °C for 30 min. The sequence and the levels used in the application of high-pressure and thermal treatments seem to be critical in meat processing.

No application of high-pressure/thermal combinations on complex meat formulations such as meat emulsions has been found in the literature, and this is the aim of the present work. The study comprises common meat batter formulations submitted to two levels of hydrostatic pressure (200 and 400 MPa) in combination with five different temperatures (from 10 to 70 °C), the highest being that commonly used in industrial cooking. The corresponding effects on the proccessed products are assessed through their physical behavior by using three mechanical parameters, pH, and water-fat-holding capacity. Protein denaturation derived from combined pressure/thermal processing is monitored through the residual protein nativeness in the processed batters, as determined by differential scanning calorimetry (DSC), to explain the above physical behavior.

MATERIALS AND METHODS

Sample Preparation. Pork meat trimmed of visible extramuscular fat and pork back fat were combined with 2.5% NaCl, 0.18% sodium tripolyphosphate (TPP, food grade), and chilled water to produce a formulation with 22% fat and 12% protein content by comminution at 10 °C. One single batch of about 5.5 kg was prepared for all of the experiments. In the course of the work, a second batter of similar but low-fat formulation (9%) was also prepared for additional DSC determinations.

High-Pressure/Thermal Treatment. The batter was sampled in 50 mL flexible plastic jars (height = 7 cm; diameter = 3 cm) by filling them up (60 ± 1 g) without entrapping air. The jars were hermetically closed with threaded caps and placed in (8 cm × 30 cm) Ultra-Cover latex bags (Amevisa S.A., Madrid, Spain), which were filled with thermostated water (±0.5 °C) at the corresponding temperature. The bags were then placed in the ~2.5 L capacity pressure vessel (length = 30 cm; diameter = 10 cm), which was then refilled with thermostated water as the pressure-transmitting medium, and pressure was applied in a high-pressure pilot unit ACB Model AGIP665 (GEC, Alsthom Nantes, France). Two hydrostatic pressures of 200 and 400 MPa and five different temperatures in steps of 15 °C from 10 to 70 °C were used for batter

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processing. The second batter was treated at both pressures and temperatures of 60, 70, and 80 °C. The holding time for each pressure/temperature combination was previously determined as the time needed for the geometric center of the respective unpressurized sample in its container to attain the selected temperature. Samples were immersed in the controlled (±0.2 °C) water bath, their temperatures were registered by a T-type thermocouple connected to a YEW3087 recorder (Yokogawa Hokushin Electric, Tokyo, Japan), and the equilibration time was obtained for each processing temperature. As these results were alike, a single holding time of 30 min was selected for all treatments. Depressurization was then carried out at the processing temperature. Samples in their containers were rapidly brought to ambient temperature, transferred to a refrigerator, and kept there at 4 °C overnight until analysis. Nonpressurized, heated-only samples were also produced in each case for reference purposes.

Compositional Analysis, pH, and Cooking Loss. Water, protein, and ash contents were determined in triplicate according to common methods (AOAC, 1984); fat content was evaluated by difference. The pH was determined in triplicate on a homogenate of 5 g of batter in 50 mL of distilled water by a Radiometer PHM93 pH-meter (Copenhagen, Denmark) at ambient temperature. Cooking loss was determined in quadruplicate as percent of the weight lost by the processed sample at ambient temperature (removed from the plastic jar and any visible exudate taken off) with respect to the initial weight of the unprocessed material.

Rheological Assessment. This was based on a penetration test carried out in quintuplicate on the samples in their containers once they attained ambient temperature. The test was performed with a 0.5 cm diameter cylindrical stainless steel plunger attached to a 100 N cell connected to the crosshead of a Universal Testing Machine 4501 (Instron Engineering Corp., Canton, MA) governed by a Vectra ES/12 computer (Hewlett-Packard Co., Avondale, PA). Force−deformation curves were obtained at 1.0 cm/min crosshead speed. The rheological parameters measured were the penetration force, apparent elasticity, and gel strength. Penetration force PF (N) was the load required for the sample to rupture. Gel strength GS (mJ) was taken as the work of penetration given by the area swept by the curve from the first contact to the rupture point. Apparent elasticity E_a (N/cm^2) was calculated according to the method of Hickson et al. (1982). This basically involved a counterflow back-extrusion model for the analysis of the elastic step (linear part of the curve), which enables the evaluation of an apparent tensile stress and the corresponding apparent strain.

Thermal Analysis (DSC). The thermal behavior of the samples was determined by means of a calibrated Perkin-Elmer differential scanning calorimeter DSC7 (Norwalk, CT). The samples, of around 15 mg (± 0.002 mg) weighed by an electronic balance Perkin-Elmer AD4, were capsulated in aluminum pans and then hermetically sealed. Three to five samples of each class were scanned from 5 to 90 °C at 10 °C/min under dry nitrogen purge of 30 mL/min, and a second heating was occasionally run to check that all proteins had already been irreversibly denatured in the first. After DSC evaluation, every capsulated sample, with a pinhole made in the cover, was desiccated at 105 °C for water content determination. Temperatures t (°C) and enthalpies (area under the DSC trace and a straight baseline between the integration limits) ΔH (J/g) were within 1 °C and 10%, respectively.

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

RESULTS

The percent analysis of the two batters was as follows: moisture, 62.0 (75.1); fat, 22.3 (9.2); protein, 12.6 (12.4); ash, 3.2 (3.3). Figures in parentheses refer to the low-fat formulation.

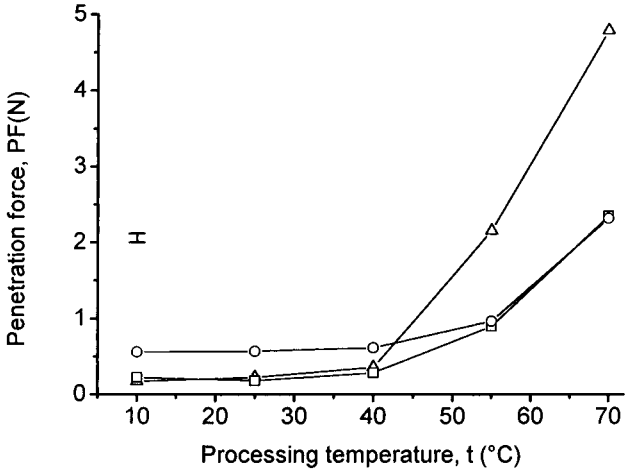

Figure 1. Penetration force as a function of processing temperature for unpressurized (△) and pressurized meat batters at 200 (□) and 400 MPa (○). Vertical bar indicates confidence interval at $P < 0.05$.

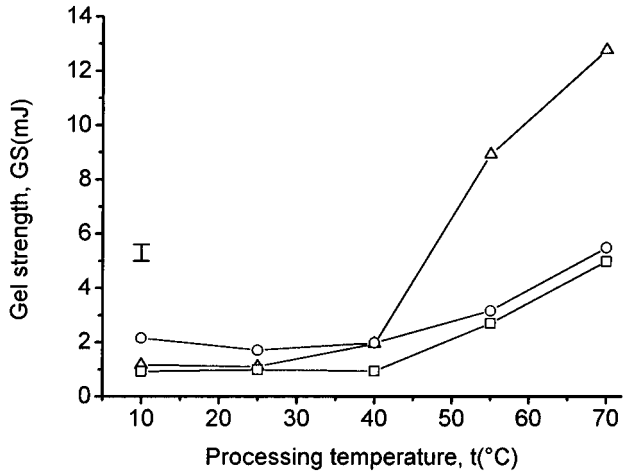

Figure 2. Gel strength as a function of processing temperature for unpressurized (△) and pressurized meat batters at 200 (□) and 400 MPa (○). Vertical bar indicates confidence interval at $P < 0.05$.

pH and Cooking Loss. The three series of batters exhibited a slight increase in pH as the processing temperature rose. The unpressurized samples showed an increment of ~0.13, from 6.51 to 6.64, at the limit temperatures of 10 and 70 °C respectively. The 400 MPa samples presented a significantly higher initial pH (6.59) and an insignificantly lower final value (6.63) than the control. The 200 MPa batters presented pH values that were lower at the lower temperatures and higher at the higher temperatures than in the corresponding 400 MPa samples. Differences between the two pressurized batter series were not significant, but the crossing patterns were clearly apparent.

Samples subjected to the three lower temperatures were too sticky for handling and could not be assessed for cooking loss. In the unpressurized batters results were significantly higher at higher temperatures: 1.7% at 55 °C and 3.2% at 70 °C. In the pressurized samples, exudation was very low in all cases: <0.3%, without significant differences.

Texture. Penetration force, gel strength, and apparent elasticity for the three batter series are presented in Figures 1, 2, and 3, respectively. In all cases the diagrams can be divided at the 40 °C mark into two well-defined zones: while at the lower temperatures the

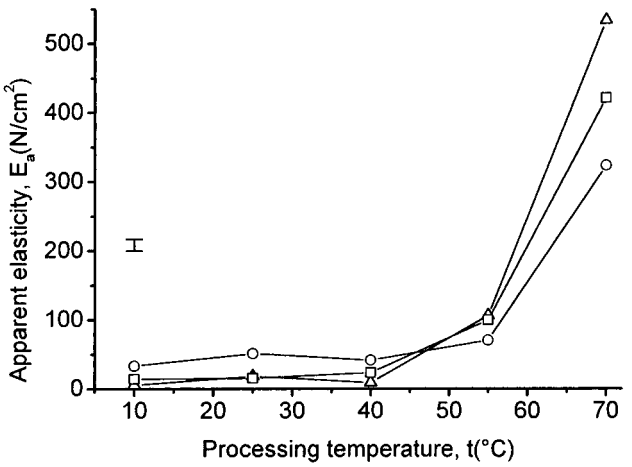

Figure 3. Apparent elasticity as a function of processing temperature for unpressurized (△) and pressurized meat batters at 200 (□) and 400 MPa (○). Vertical bar indicates confidence interval at $P < 0.05$.

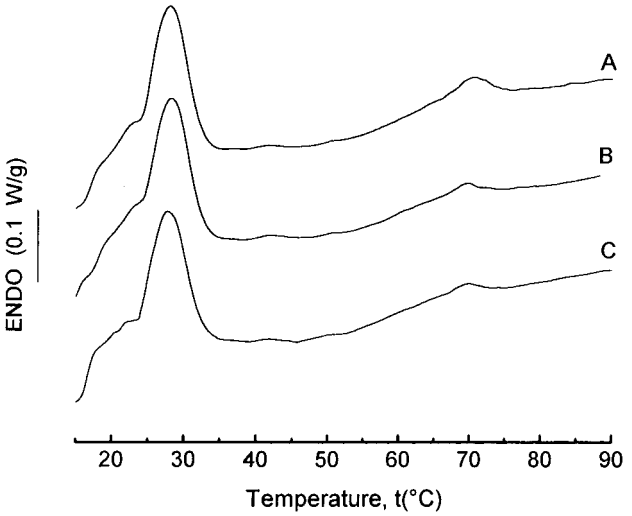

Figure 4. DSC profiles of unpressurized (A) meat batter and meat batters pressurized at 200 (B) and 400 MPa (C) processed at 55 °C.

three parameters remained practically independent of the thermal treatment, they exhibited an appreciable continuous increment at the higher temperatures. At the low-temperature interval, the values of each of the three parameters were not significantly different within any given batter series. The 200 MPa series was generally close to the unpressurized series. The 400 MPa samples presented the statistically significant (in general) highest PF, GS, and E_a values with respect to the unpressurized samples. At the high-temperature interval, the pressurized batters showed crosswise patterns, and the unpressurized samples exhibited the fastest and highest increase with temperature, attaining the significantly highest values in all three parameters (PF, GS, and E_a).

Thermal Behavior. Typical DSC traces of the batters subjected to 200 and 400 MPa at 55 °C are shown in Figure 4 along with the corresponding unpressurized sample, as an example of the high-pressure/temperature effects. The ordinate scale is normalized to dry matter for comparison. The curves record two endothermic zones separated at about the 45 °C mark: the melting of the fat component and then the thermal denaturation of the still native proteins left over after the corresponding batter treatment.

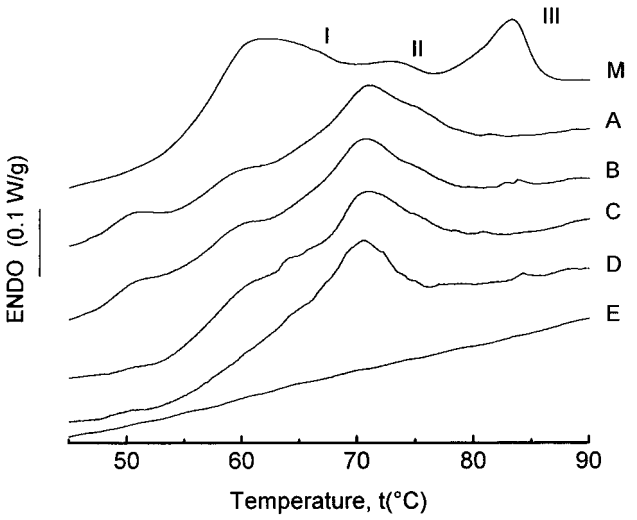

Figure 5. DSC profiles of unpressurized samples: raw minced meat (M) and meat batters processed at 10 (A), 25 (B), 40 (C), 55 (D), and 70 °C (E).

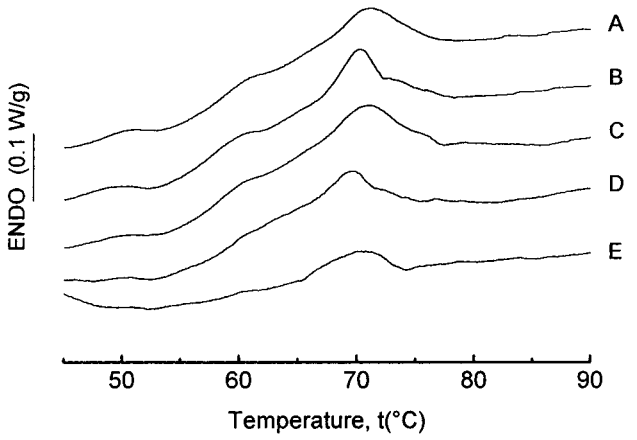

Figure 6. DSC profiles of pressurized meat batters at 200 MPa and 10 (A), 25 (B), 40 (C), 55 (D), and 70 °C (E).

The melting characteristics of pork back fat are already known (Barreto et al., 1996) and are not relevant for this study; any influence of the pressure on the solidification/melting behavior of the fat is practically lost after pressure release and cold storage. DSC traces mainly consisted of a big endothermal peak centered at 28 °C with an associated melting enthalpy of 19.6 J/g (fat), the pork back fat then being ~77% liquid. The important fact, however, was the excellent reproducibility exhibited by fat melting, as an indirect index of the high degree of homogeneity attained for the batters and the goodness of the DSC sampling. This allowed the assumption of nominal bulk batter composition (33.2 and 49.8% protein on dry basis, respectively, for the first and second formulations) for the DSC capsulated samples, which in turn enabled quantification of the effects recorded and direct comparison among samples. This is illustrated by Figures 5, 6, and 7, which show the DSC thermal behavior of the protein matrix of the three series of samples. Ordinate scales are normalized to protein content, and the respective areas directly determine the heats of thermal denaturation observed by the still native meat proteins fraction remaining in the corresponding batter after processing.

Raw minced pork meat (Figure 5M) showed the typical trace with three main transition regions (Wright et al., 1977) due to the thermal effects largely associated with denaturation of light meromyosin (I), heavy mer-

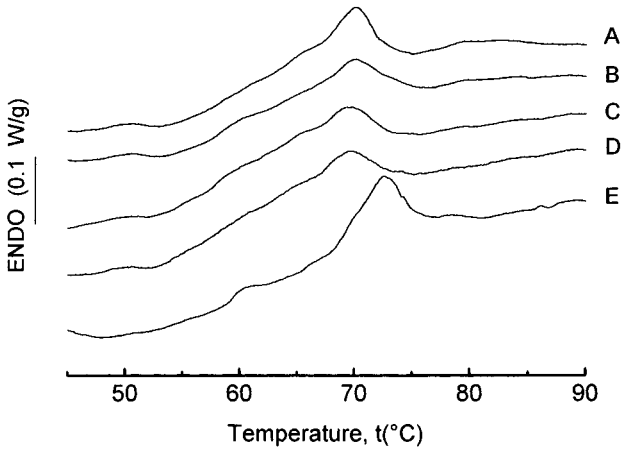

Figure 7. DSC profiles of pressurized meat batters at 400 MPa and 10 (A), 25 (B), 40 (C), 55 (D), and 70 °C (E).

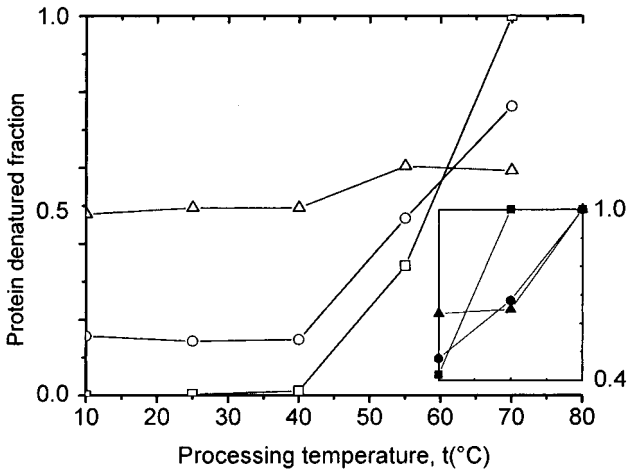

Figure 8. Protein denatured fraction as a function of processing temperatures for unpressurized (□) and pressurized meat batters at 200 (○) and 400 MPa (△). Low-fat batter data (solid symbols) are shown in the inset.

omyosin, collagen and sarcoplasmic proteins (II), and actin (III) (Stabursvik and Martens, 1980). The average thermal data obtained were as follows: 54.5 °C for onset of the first endotherm; 61 (I), 65 (II), and 73 °C(III) for maximum temperatures of the three main endotherms; 14.5 J/g (dry matter) for the enthalpy of denaturation, taken from 45 to 88 °C, with 64.5 (I), 16 (II), and 19.5% (III) distribution. DSC pattern and data compare well with literature values for porcine muscles (Quinn et al., 1980; Stabursvik et al., 1984; Xiong et al., 1987). Figure 5A corresponds to batter treated for 30 min at 10 °C (identical to the raw batter) and shows the dramatic effects undergone by minced meat in its comminution and mixing to batter: the first endotherm split into two small peaks at 50.5 and 60 °C, and the main endotherm centered at 71 °C (shoulder at 76 °C); the upper integration temperature decreased by about 3 °C, and the denaturation enthalpy was 10.7 J/g (referred to protein hereafter), about 28% lower than in the original minced meat (essentially due to destabilization of actin, which practically disappears from III). Results appeared to be consistent with the protein degradation caused by mechanical reduction of pork meat in the presence of NaCl (0.42 M) and TPP (5 g/kg of protein-plus-fat) (Quinn et al., 1980; Findlay and Barbut, 1992). DSC traces of unpressurized batters heated at 25 and 40 °C (Figure 5B,C) remained almost identical to that of the previous treatment at 10 °C, with no significant differences in temperatures or denaturation enthalpies (10.6 and 10.5 J/g, respectively). Batter treated at 55 °C underwent effects already visible in the myosin zone (Figure 5D): the integration range decreased considerably (52−82 °C), and the main peak appeared alone at 70 °C; the denaturation enthalphy diminished to 7.5 J/g. Treatment at the highest temperature (70 °C) produced total denaturation of the meat proteins (Figure 5E) in the unpressurized batters. From kinetic studies on different muscle systems (Wagner and Añon, 1985; Findlay et al., 1986) no thermal denaturation of proteins could practically be expected prior to batter treatment at the two highest temperatures.

Batters pressurized and heated at the three lower temperatures behaved similarly to the unpressurized batters in that denaturation enthalpies (from 52 to 80 °C) were not significantly different within a given batter series (9.0, 9.2, and 9.1 J/g for the 200 MPa series, Figure 6A−C), but the higher the pressure, the lower were the DSC denaturation enthalpies recorded (5.6, 5.1, and 5.4 J/g for the 400 MPa series, Figure 7A−C). Also, in treatment at 55 °C both pressurized batters showed significantly reduced enthalpies in the range 52−75 °C (5.7 and 4.2 J/g, Figures 6D and 7D, respectively). Surprisingly, treatment at 70 °C did not yield a "flat" DSC trace for the corresponding pressurized batters. New thermal events were recorded instead, showing 2.6 J/g for the 200 MPa sample (nearly half of the 200 MPa/55 °C), mainly in the central region (71 °C) but extending over 51−81 °C (Figure 6E). These results were further enhanced at 400 MPa (Figure 7E), notably at about 60 °C (light meromyosin) and 66 °C (pressure stabilization of the native, hydrogen-bonded helical structure of collagen) and mainly with a new main peak centered at 73 °C (actin); chemical studies are in progress for protein allocation to these DSC profiles. The associated enthalpy (4.4 J/g) was 80% of the initial in the series, not significantly higher than the previous one (400 MPa/55 °C) but significantly higher than the corresponding result at lower pressure (200 MPa/70 °C).

Pressurized batters should thus need higher processing temperatures for total protein denaturation. To verify this peculiar thermal behavior and look for any influence of the fat phase, additional experiments were carried out on the low-fat formulation. Apart from minor quantitative differences, the general phenomena were essentially reproduced in that large DSC enthalpies of thermal denaturation were again recorded in both pressurized batters treated at 70 °C, the result being greater at the higher pressure. Both pressurized batters treated in combination with 80 °C presented a "flat" DSC trace, as did the unpressurized batter treated at 70 °C. Denaturation enthalpies for the three series of low-fat batters treated at 60, 70, and 80 °C were, respectively, 6.2, 0, and 0 J/g (unpressurized); 5.6, 3.4, and 0 J/g (200 MPa); and 3.9, 3.8, and 0 J/g (400 MPa).

These DSC data for enthalpies of thermal denaturation are directly related to the protein fraction of the batter surviving in native condition from the corresponding process, since it is determined on the batters after processing. The biggest DSC denaturation enthalpy recorded will thus correspond to the lowest denaturing process. Data were normalized to the denaturation enthalpy of the initial raw meat batter (100% native, arbitrarily) and subtracted from 1 to calculate the fraction of protein denatured by batter processing, as shown in Figure 8. These data are then

a relative index denoting the denaturing character of a process.

DISCUSSION

While pressure transmission is uniform and immediate in its application, heat transmission by conduction is nonisotropic and a rather slow process; hence, by kinetics, heat effects on protein denaturation should be modulated by prior pressure effects in the present experimentation. After 30 min pressure/temperature treatments of the comminuted pork meat system, the net effect on protein denaturation clearly falls into two main behavior patterns (Figure 8), depending on whether the processing temperature is or is not in itself denaturing. Thus, in processing at nondenaturing temperatures (t < 40 °C) pressure causes protein denaturation in direct relation to pressure level (very important at 400 MPa), independent of the temperature. At temperatures of thermal denaturation (t > 50 °C) the net effect of the pressure/temperature combinations was interdependent with both processing parameters. In treatments at 55 °C protein denaturation reached higher levels in both pressurized batters than in the unpressurized sample; both pressure and heat seemed to contribute to protein denaturation at 200 MPa, but pressure started to partially offset heat effects at 400 MPa. Despite complete protein denaturation in the heated-only batter, thermal denaturation in pressurized batters treated at 70 °C was counteracted by prior pressure effects since an important fraction of proteins, increasing with pressure, was preserved from unfolding in both pressurized batters. Pressure driving became predominant and overcame thermal driving, so that protein thermal denaturation was substantially hindered at 200 MPa and almost entirely inhibited at 400 MPa. Thus, pressurized high- and low-fat batters required higher temperatures (80 °C) than unpressurized samples (70 °C) for total protein denaturation (Figure 8, inset). Globular proteins in solution (chymotrypsinogen, metmyoglobin, ribonuclease A) are known to denature under pressure (p) with an elliptical temperature (t) dependence (Gekko, 1991). According to this author, a reasonable explanation for this kind of behavior may be found in the type of analytical form that the equation of state ($p-V-t$) of a protein presents (which can be derived by simulation from the temperature dependence of the isothermal compressibility of the protein, as the author illustrates for lysozyme). Therefore, the slope of the curve in the $p-t$ diagram [related to the change in the partial specific volume (V) experienced by the protein in the native−denatured transition] will invert its sign from negative ($\Delta V > 0$) to positive ($\Delta V < 0$), passing through a zero slope ($\Delta V = 0$) at a critical $p-t$(maximal) coordinate. Pure mechanical and thermal driving forces may be thus antagonistic in the protein denaturation by combined pressure/heat treatments (in contrast to the additive roles that pressure and heat may exhibit in separated processing steps). In the $\Delta V < 0$ domain, only conformational transitions associated with volume reduction will be permitted in the subsequent heating. Thermal driving forces may overcome pressure effects by increasing temperature (moving to the $\Delta V > 0$ domain) and transitions involving increasing volume changes will also occur. Pressure and thermal driving forces may eventually cancel each other ($\Delta V = 0$), and only transitions with nil volume change will be permitted; that is, no additional unfolding transition will occur. The chemical and physical complexity of the batters is far away from the above model systems, but it seems reasonable that the unfolding reactions induced by pressure/temperature in the batter protein matrices could be expected also to follow such elliptical profiles. The denaturing pictures displayed by pressurized batters in Figure 8 are entirely compatible with such a scheme.

Comparison of Figures 1−3 with Figure 8 suggests strong correlations between every one of the rheological parameters and the protein denaturation degree of the batters (including the crossover patterns exhibited by the three mechanical parameters). At the lower temperatures of treatment, PF and GS are directly related to the protein denatured fraction within the pressurized batters, but unpressurized batters show an intermediate behavior. E_a is directly related in all three cases. It therefore seems that the protein functionality induced by high-pressure/low-temperature treatments is different from that induced by heat [also observed by Icard (1995) in bovine meat batters with or without NaCl] and involves some structuralization, yielding soft and sticky aggregates (which could be of interest in meat paste conditioning). At the higher temperatures of treatment all three rheological parameters are directly related to the protein denatured fraction, the highest scores occurring in the unpressurized, heated-only batters, particularly at 70 °C (current industrial cooking temperature of meat sausages). Strong restructuring effects are observed in both pressurized batters, yielding smoothly textured gels with no exudation, which may be of potential application [also reported by Icard (1995) for finely comminuted bovine meat processed at 450 MPa and 50 °C for 30 min]. From a technological viewpoint, however, the high-pressure/high-temperature combinations induced gels with unsatisfactory rheology with respect to heat-induced gels. DSC revealed that comminution in the presence of NaCl and TPP produces a meat system suitable for low-temperature gelation (70 °C), endowing the unpressurized, heated-only gels with appropriate water- and fat-holding and textural properties.

In conclusion, DSC demonstrated for the first time that, depending on both pressure and temperature, comminuted meat pressurization may efficiently preserve protein from subsequent thermal denaturation in combined pressure/heat treatments. The extent of protein nativeness persisting in the pressurized batters may explain their physical behavior. Complete unfolding by heating alone at appropriate temperature involved the protein functionality for optimal batter gelation.

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