Journal of Thermal Analysis and Calorimetry, Vol. 87 (2007) 1, 285–290

BIRD MUSCLES UNDER HYDROSTATIC HIGH-PRESSURE/TEMPERATURE COMBINATIONS A DSC evaluation

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Breast muscles from three different birds were subjected to hydrostatic high-pressure (400 MPa)/temperature (10–75°C) combinations, and the denaturation-induced effects on the pressurized proteins monitored by DSC. Comparisons with parallel results from heating-alone processes were established. Actin was the most labile moiety to pressurization and myosin together with sarcoplasmic proteins were next in observing pressure-induced denaturation at low temperatures. Some myosin derivatives (fragments or aggregates) and collagen remained native-like under pressure at any temperature.

As previously reported, pressure and temperature showed interdependent and antagonistic-like effects. Hydrostatic high-pressure caused severe proteins denaturation at non thermal denaturing temperatures. At thermally active conditions, pressure preserved proteins from subsequent thermal denaturation. This last effect was lower than in similar but destructured myosystems (batters) because of the absence of functional salts but presumably also by steric hindrance.

Keywords: *chicken, denaturation-preservation effects, DSC, myofibrillar proteins, ostrich, pressure-temperature treatments, turkey*

Introduction

Food Technology is mainly founded on Thermal Processes inasmuch as food preservation/transformation is conventionally carried out by either applying heat to or extracting it from the foodstuffs. Thermal processes are well established and used by the food industry to supply foods with a normally high score in nutritive and organoleptic attributes. Minimal-Processing is however a recently introduced concept aiming to apply very mild treatments for producing safe foods with physico-chemical, nutritional and organoleptical properties as closer to the original/natural condition/quality as possible. This 'minimal' alteration of foods is being tried to be achieved through application of different physical principia at, initially, room temperature, this being why they have received the 'Non-thermal' qualifying. One of these emerging technologies uses hydrostatic high pressures (HHP) and, in fact, HHP was originally used at room temperature with solely hygienic purposes as a mere mechanical process for killing micro-organisms in foods. However, there is increasing general interest in the application of hydrostatic high pressure to food processing [1–3] and biotechnology [4, 5]. In particular, pressurization of meat products has been studied with different objectives such as hygienic conditioning by inactivation of pathogenic and spoilage micro-organisms at low temperature, or processing of meat batters and meat emulsions to improve myofibrillar protein functionality for proper binding and gelling. Combined pressure/temperature (HHP/*T*) treatments have been reported to accelerate tenderization of whole muscles, and have been shown to be particularly effective in overcoming toughness derived from cold-shortening to enhance meat-eating quality [6, 7]. One major field of application seems to be pressurization at subzero temperatures [8–10] without freezing, in non-frozen storage, or with water/ice phase change in pressure-shift freezing (PSF) or pressure-assisted thawing (PAT). On the other hand, differential scanning calorimetry (DSC) has been shown to be an excellent tool to detect/monitor physical changes experienced by food macro-constituents (proteins, carbohydrates, lipids, water) when the complex food matrices [11] are subjected to processing/storage [12, 13].

Prior studies on the author laboratory have been dealing with meat products form several biological sources as either original muscle structures or mainly, processed by usual comminution with the addition (food grade) of some substances (NaCl, polyphosphates, lard) as meat batters with different formulations (low/high NaCl, presence/absence of polyphosphates, low/high fat). Meat (mostly *Longissimus dorsi M.* and *Semitendinosus M.*) was principally from different mammalians (bovine and ovine ruminants, pork, and their mixtures) [14–20] but also from cold-blood animals like non-fat (blue whiting) and fat (sardine) fish species [21, 22]. HHP/*T* application covered several pressures (100–450 MPa) and temperatures (10–80°C) in combination for processing

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1388–6150/$20.00

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near to or above ambient temperature with holding times between 5–30 min. For below zero processing, a pressure/temperature combination of 200 MPa and –20°C has been used, which corresponded to a monophasic liquid phase domain, close to the water–ice I boundary, in the high-pressure/low-temperature region of the water phase diagram [23, 24]. On the opposite way, pressure-assisted thawed (PAT) on conventionally frozen samples has been carried out by applying 200–150 MPa at the same temperature of 20°C for 5–30 min [25].

Little effort has been directed to pressurization studies on whole muscles from most meat-producing animals [26–28] in addition to own contributions [17, 20, 23–25]. This article summarizes author complementary work on the HHP/*T* processing of structured myosystems from other kind of warm-blood animals like birds. The main objective is to study the protein-denaturating effects induced by HHP/*T* combined treatments in comparison to heating-alone at the same temperatures, as revealed from DSC determinations on the corresponding processed myosystems.

Experimental

Materials

Meat products (~20–25% protein) were (fresh and refrigerated) breast muscle pieces (mainly *Pectoralis major M.* and *Pectoralis minor M.*) from different birds like chicken, turkey, and ostrich from the local market. Samples of intramuscular connective tissue were carefully separated from the three bird muscles in the laboratory.

Methods

Pressure/temperature treatments

Pressure treatments employed an ACB unit (model AGIP665, GEC, Alstom, Nantes, France) of ~2.35 L cylindrical vessel, filled with water as a pressure transmitter and temperature-controlling medium. The unit consists of two hydro-pneumatic pumps for pressurization, and a recirculation heating/cooling system. The pressure and temperature (two stainless steel sheeted *T* type thermocouples, one on the surface and the other at the centre of the meat sample) were recorded digitally (Helios I model from Fluke, Everett, USA) along the process. The muscle pieces were singly vacuum-sealed in polyethylene bags and transferred to a larger vacuum-sealed polyethylene bag that was placed into the pressure vessel. HHP/*T* processing used 400 MPa and several temperatures (10–75°C) in the protein-denaturation range with 30 min holding time.

DSC measurements

Thermal denaturation of the flesh proteins was studied by means of a previously calibrated Perkin-Elmer DSC7/TAC7DX/PC Differential Scanning Calorimeter (The Perkin-Elmer Corporation, Norwalk, USA). Small pieces of sample, free from visible traces of fat and connective tissue, were encapsulated into aluminium pans and hermetically sealed to prevent vaporization losses. At least 4 pans with 15–20 mg meat each (weighed accurately to 0.002 mg by an electronic balance Perkin-Elmer AD4) were used for each individual sample. Connective tissue samples were encapsulated with a proper addition of distilled water (70–80%) to control their thermal denaturation. The samples were scanned at 10°C min^{-1} in the 5–90°C range under dry nitrogen purge of 30 mL min^{-1}. A subsequent rescan was carried out to check for reversibility. Water content of each individually encapsulated (pinhole in the lid) sample was determined by desiccation at 105°C, for thermal data normalization to dry matter content. Temperatures t(°C) and enthalpies of denaturation ΔH(J g^{-1}, dry basis hereinafter) were usually within 0.5 and 6% respectively.

Results and discussion

HHP-induced effects were studied by scanning already pressurized samples since HHP-DSC instruments are not commercially available for in situ thermal analysis [29]. Then DSC data were associated to the thermal denaturation of those proteins remaining native-like after processing. The higher the denaturing character of the process, the lesser the native-like proteins left for the DSC scan and the lower the DSC trace. Typical DSC results from heating-only (a) and pressurization(400 MPa)/heating (b) processes at several temperatures for 30 min on whole breast muscles from chicken, turkey and ostrich are shown in Figs 1–3, respectively, where numerals refer to processing temperatures.

Figure 1a (curve raw) shows a typical DSC pattern of untreated chicken muscle with several peaks at maximum temperatures of around 60.7 (mainly myosin), 66.2, 70.7 and 75.4 (myosin, sarcoplasmic proteins and collagen), and 81.1°C (actin). A mean value of ~18.2 J g^{-1} (dry matter) for total denaturation enthalpy was obtained by straight baseline integration between 45 and 90°C. These results compare well to those reported in the literature [17, 18, 30, 31]. Thermal treatment at the increasing temperatures of 45, 60, and 70°C yielded increasing denaturation effects (Fig. 1a, curves 45, 60, 70) with progressive disappearance of the lower thermostable proteins, starting with myosin but minimally affecting actin even at the

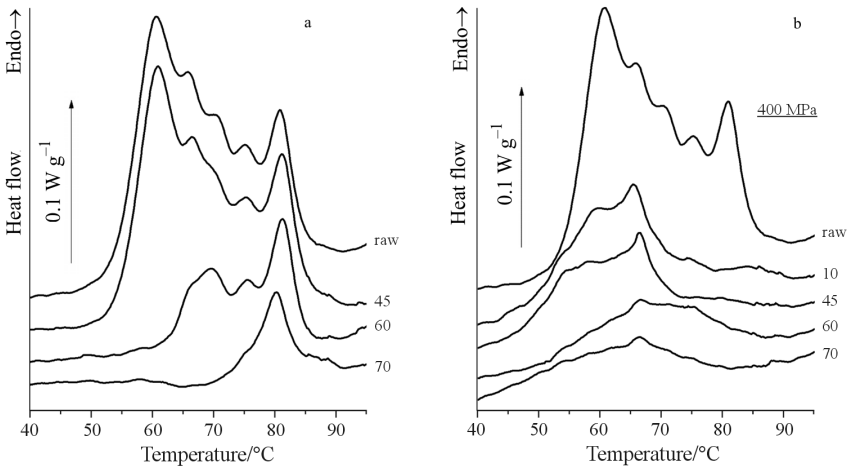

Fig. 1 Effect of different processing conditions on DSC (normalized to dry basis) typical traces on chicken muscle meat: a – unpressurised and b – pressurised samples at 10, 45, 60 and 70°C for 30 min

highest temperature. Figure 1b (curves 10, 45, 60, 70) shows chicken samples subjected to pressurization (400 MPa) at the same temperatures. DSC clearly demonstrated the entirely different behaviour of muscle (mainly myofibrillar) proteins under pressure/heat with respect to heat alone. At the lower temperatures (10 and 45°C), non-denaturing in heating, pressurization induced severe denaturation effects, with the most thermostable actin being the most labile to pressure to the extreme of practically disappearing. It was followed by some myosin domains and sarcoplasmic proteins. The peak at ~67°C of maximum temperature could likely be related to connective tissue (mainly collagen) as commented below. Pressurization at the highest, denaturing temperatures did not yield similar DSC profiles as before since myosin was considerably denatured while some new native-like zones seemed to appear in the middle transitional zone. Discussion of this kind of pressure-induced effects will be done in common for the three muscles.

Figure 2a (curve raw) shows a typical DSC pattern of untreated turkey muscle with big peaks at maximum temperatures of around 60.8 (mainly myosin) and 81.3°C (actin), with less defined intermediate zone (associated to myosin, sarcoplasmic proteins and collagen denaturation) than in chicken. Mean denaturation enthalpy between 50 and 90°C was ~17.3 J g^{-1} (dry matter) close to chicken and similar to whole porcine and bovine muscles. No ΔH data were found having been reported by others. Thermal treatments yielded increasing denaturation effects (Fig. 2a, curves 45, 60, 70) similar to chicken muscle. Figure 2b shows that turkey samples under pressurization at selected temperatures also behaved similarly to chicken.

Figure 3a (curve raw) shows a typical DSC pattern of untreated ostrich muscle with big peaks at maximum temperatures of around 57 (mainly myosin) and 80.9°C (actin), and lowly defined intermediate zone as in turkey. Mean denaturation enthalpy be-

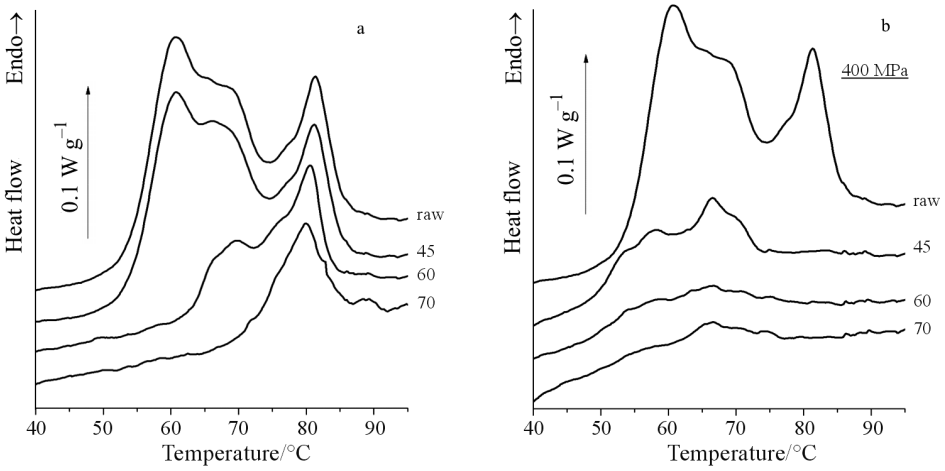

Fig. 2 Effect of different processing conditions on DSC (normalized to dry basis) typical traces on turkey muscle meat: a – unpressurised and b – pressurised samples at 45, 60 and 70°C for 30 min

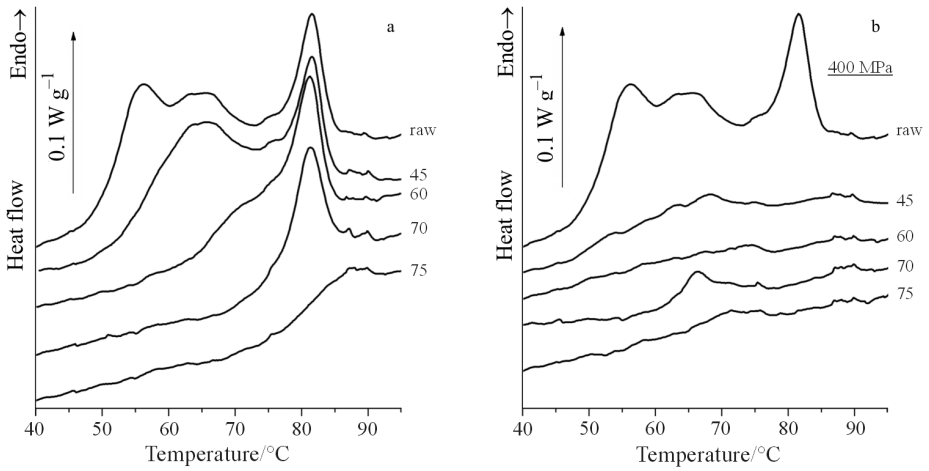

Fig. 3 Effect of different processing conditions on DSC (normalized to dry basis) typical traces on ostrich muscle meat: a – unpressurised and b – pressurised samples at 45, 60, 70 and 75°C for 30 min

tween 40 and 90°C was ~12.9 J g^{-1} (dry matter) considerably smaller (~3/4) than chicken and turkey breast muscles, presumably because first myosin peak appeared considerably smaller and at lower temperature. Consistent with this lower myosin heat stablility, 45°C resulted noticeably denaturing for ostrich breast muscle proteins (Fig. 3a, curve 45). On the opposite, the actin component exhibited somewhat enhanced stability and slightly higher temperatures (Fig. 3a, curve 75) were needed to get similar final denaturing results to those reported for chicken and turkey. Unfortunately quantitative calorimetric data from other authors were not found in a bibliographic screening. Figure 3b presented close resemblance to corresponding cases in chicken and turkey breast muscles.

In order to compare different processing conditions and bird muscles, DSC data were normalized to the denaturation enthalpy of the respective initial raw meat (whole native, arbitrarily) and subtracted from 1 to calculate the fraction of protein denatured (PDF) in each case. PDF was thus taken as a relative index for evaluating the denaturing character of the processing conditions [14], as shown in Fig. 4.

It can be noted the different denaturing character for the involved proteins that a given temperature may have whether was acting as a sole parameter in heating-only processing or associated to pressure in pressurizing/heating combinations. This leading to an entirely different way of protein aggregation-gelling and thus to different processed products with diverse mechanical, rheological or water holding properties. In fact, while heat denaturation for the three muscle proteins was basically (very) low in the temperature range 10–45°C, denaturation obtained by 400 MPa combined with same temperatures was always very high. On the contrary, while heat denaturation evolved very fast in the 45–75°C range, corresponding results in pressurization exhibited a very mild pattern with some reversing tendency. This was particularly noticeable for ostrich breast muscle, this likely indicating a higher temperature for total denaturation than in heat-alone process. Results were species dependent and essentially agree with prior own findings on pressure/heat processing of a wide range of destructurated myosystems (batters) and processing conditions. In these systems, protein denaturation by pressurization at low (non-denaturing) temperatures was directly related to pressure level and holding time, and intensified with increasing salt molarity. At high (denaturing) temperatures, protein denaturation of the pressurized batters lagged with respect to heated-only samples and total protein denaturation was shifted towards higher temperatures. Although evolving similarly in pattern, protein denaturation was quantitatively dependent on meat source. Protein denaturation induced by pressurization directly influenced the texture of the processed matrices, PDF data enabling the interpretation of the apparently erratic rheological behavior of the pressurized samples either at non-denaturing or denaturing thermal conditions. In conclusion, pressure and temperature were interdependent and antagonistic-like effects, as fully demonstrated by DSC. In this kind of pressure/temperature sequential treatments, a general rule was found to be applicable as follows: Depending upon its level, hydrostatic high-pressure caused more or less severe protein denaturation at non-denaturing temperatures, while pressure preserved proteins from subsequent thermal denaturation at thermally active temperatures.

On the other hand, DSC clearly revealed (Figs 1–3) that actin was always the most barolabile myofibrillar moiety in contrast with its highest thermal stability. Sensitivity of myofibrillar proteins to pressurization depends on pressure level and the processing temperature. At the low, non-denaturing temperatures (<40°C, including subzero) and low pressures (<200 MPa), actin is more stable than myosin, espe-

cially at short holding times. Actomyosin is dissociated, myosin filaments disaggregated, myosin molecule depolymerized and degraded (light chains dissociation), this last likely yielding new stable structures appearing as new DSC peaks in the 30–50°C interval [17, 21–27 and references in this last review]. At higher pressures (>200 MPa), like here, G-actin is readily depolymerized and F-actin is the most irreversible denatured moiety. Proteins belonging to the intermediate transitional zone, i.e., some myosin domains together with sarcoplasmic proteins, were next in observing pressure-induced denaturation at low temperatures. Connective tissue (mainly collagen) however remained thermally unaltered (morphological structure changes apart [32]) by pressurization at any temperature as illustrated in Fig. 5.

For the sake of brevity this composition includes only three cases to illustrate different types of collagen and their inertness to pressurization/heating. In presence of excess water collagen generally denatured by heat with a very sharp and symmetric DSC trace but restricted amounts of water may produce bimodal or more complex thermograms, like in the case of starch. We show here the first type of results and, breast chicken connective proteins (collagen) exhibited a maximum temperature of ~66.3°C and ~43.2 J g^{-1} of denaturation enthalpy by heating-alone (Fig. 5, solid line). HHP/*T* treatment practically did not induce modifications and typical results were ~67.2°C and ~41.4 J g^{-1} respectively. Corresponding values for breast turkey connective (collagen) proteins were ~65.0°C and ~23.8 J g^{-1} by heating-alone (Fig. 5, dashed line) and ~66.7°C and ~28.0 J g^{-1} by HHP/*T*. Results for breast ostrich connective (collagen) proteins were ~67.0°C and ~42.5 J g^{-1} by heating-alone and ~69.0°C and ~44.6 J g^{-1} by HHP/*T* (Fig. 5, dotted line). Collagen does not undergo appreciable changes by pressure [33] because its quaternary structure is stabilized by hydrogen-bonding, a very stable structure to high-pressure because of its molar volume positive increment on melting. We have observed it on several myosystems and processing conditions [21–24]. Collagens from different animals present different thermal stabilities because of their environmental conditions (i.e., fish collagens less thermostable than mammalian collagens). Denaturation temperature increases with an increase of amino acid residues, particularly hydroxyproline content, which is considered to play a singular role in the stabilization by hydrogen-bonding (water–bridges included) of the triple-stranded collagen helix. Additionally, native intramuscular connective (mainly collagen) fibres from warm-blood animals have been shown to exhibit denaturation at close temperatures, around 65–70°C, but with two different levels of transitional energy change, nearly twice from each other. Thus intramuscular connective tissues from pork (*Longissimus dorsi M.*) and cow (*Semitendinosus M.*) were very stable to pressurization and/or freezing, with peak denaturation temperatures at ~65°C both cases but ~41 and ~22 J g^{-1} denaturation enthalpy respectively [21, 22]. These figures roughly correspond to present results for connective tissues of chicken and ostrich (Fig. 5, traces chicken, ostrich), and turkey (Fig. 5, trace turkey) breast muscles respectively. The reasons for this enthalpy variation are not quite clear although intermolecular cross-linking may be a main factor. Similarities in the amino acid composition of the acid-soluble and pepsin-solubilized fractions among the specie collagens could likely be the grouping key. Differences in DSC denaturation enthalpies between isinglass and bovine collagen allowed [34] calculations that the thermally labile domain in the molecules were 41 and 66 residues respectively.

Concerning the so-called preservation effects of heating at high, denaturing temperatures under pressurization (Fig. 4), it could likely be related to the formation of some new structures derived from pressure-breakdown of myosin that prior analytical studies of protein solubility and subsequent electrophoresis [15, 35] have pointed to be presumably stabilized by hydrogen-bonding. Worth of remembering is that myosin is a complex molecule presenting different, non-cooperative domains to thermal denaturation [36]. Intra- and intermolecular head-to-head interactions take place upon myosin pressurisation with formation of daisy-wheel shaped oligomers (myosin intact tails extending radially from the heads clamp) [37]. On the other hand, formation of intermolecular antiparallel β-sheet structures has been reported to occur in some simple systems by hydrogen-bonding of partially pressure-unfolded proteins [38]. DSC results on breast bird muscles can likely be compatible with these myosin

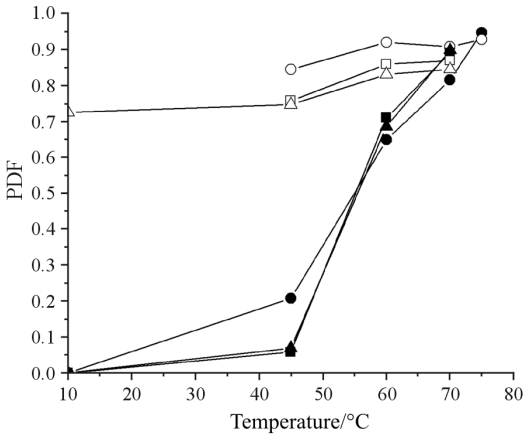

Fig. 4 Protein denatured fraction (PDF) induced by pressure/heat combinations (open symbols) and heating-alone (solid symbols) on the different breast muscle systems as a function of processing temperature: ▲,△ – chicken, ■,□ – turkey and ○,● – ostrich

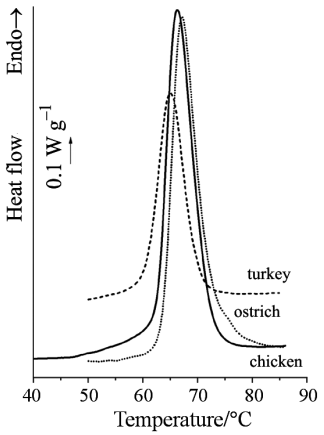

Fig. 5 Effect of different processing conditions for 30 min on DSC (normalized to dry basis) typical traces on the connective tissue from breast muscle meats; (400 MPa/70°C)

aggregates being pressure-preserved in native-like conformation, in essential agreement with own results on destructured myosystems covering a wide span of flesh-food matrices and a broad range of pressure/temperature processing conditions [14–22]. The difference could only be quantitative in the sense that pressurized muscles exhibited a lower and less defined behaviour at the thermal-denaturing temperatures. It is known the key role of some salts added to meat products for the solubilisation and preparation of myofibrillar proteins for their proper thermal denaturation/aggregation and the good water-fat-retention and balanced rigidity/elasticity properties of the meat gel produced. The absence of these batter additives in the whole, intact muscles may likely be a factor in this differential behaviour. Additionally, the ultrastructural condition seems also to play a role in restricting pressure-induced effects by mere steric hindrances.

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DOI: 10.1007/s10973-006-7809-6