Protein Denaturation and Structural Damage During High-Pressure-Shift Freezing of Porcine and Bovine Muscle

F. FERNÁNDEZ-MARTÍN, L. OTERO, M.T. SOLAS, AND P.D. SANZ

ABSTRACT: Pork and beef muscles were subjected to 200 MPa and −20 °C with or without water freezing. Both tissues responded to the treatment with similar behavior. Protein denaturation was greater when freezing occurred. Pressure-induced cold denaturation was complete for actin and very considerable for myosin and other muscle proteins. Connective proteins remained practically unaltered by pressurization and/or freezing. Structural changes in the muscle at sarcomere levels caused by pressurization were more severe when freezing occurred. Color, drip loss, and textural properties on the pressurized samples also revealed an additional deleterious influence of freezing. Pressurization alone and pressure-shift freezing resulted unsuitable for muscle preservation.

Key Words: high-pressure-shift freezing, muscle ultrastructure, cold denaturation, DSC, TEM

Introduction

THERE IS INCREASING GENERAL INTEREST IN THE APPLICATION of hydrostatic high pressure to food processing (Hayashi 1992; Tauscher 1995; Tonello 1997) and biotechnology (Makita 1992; Mozhaev and others 1994). Pressurization of meat products (Cheftel and Culioli 1997) has been studied with different purposes such as hygienic conditioning by inactivation of pathogenic and spoilage microorganisms at low temperature, or processing of meat batters and meat emulsions to improve myofibrillar protein functionality for proper binding and gelling (Macfarlane 1985). In muscle systems, for example, combined pressure/temperature treatments have been reported to accelerate tenderization of whole muscles (Suzuki and others 1992) and have been shown to be particularly effective in overcoming toughness derived from cold-shortening (Bouton and others 1977) to enhance the eating quality of meat. One of the major fields of application, however, seems to be pressurization at subzero temperatures without freezing, in nonfrozen storage, or with water/ice phase change in pressure freezing or thawing (Kalichevsky and others 1995; Le Bail and others 1997). High-pressure offers the possibility of readily available liquid water at subzero temperatures for long periods of time, thus allowing extension of the self life of certain foods without the need for freezing/thawing operations. This liquid water may experience fast and uniform ice nucleation throughout the sample after sudden depressurization (adiabatic expansion), yielding smaller and more uniformly sized ice crystals than in normal freezing, as illustrated by Otero and others (1997) and Martino and others (1998) in pork muscle. It may then minimize injurious freezing-stress in biological tissues, thus enhancing the quality of the resulting frozen products.

Not many studies are available on potentially useful applications of subzero-temperature treatments under pressure, and most of them concern the processing of vegetables and the texture implications. Egg and soy proteins were recently studied in pressure gelation at different temperatures, including subzero, by Dumoulin and others (1998) who reported that protein functionality was reduced with decreasing temperatures. Fuchigami and others (1998) studied the effect of different combinations in high-pressure-assisted freezing (different ice polymorphs) and thawing on the structure and texture of soybean curd (tofu). According to Cheftel and Culioli (1997), there are very few reports on meat products. Apart from 2 recent contributions on pork muscle (Fernández-Martín and others 1999; Massaux and others 1999), most reports dealt with pressure-assisted thawing of conventionally frozen materials (Murakami and others 1992, 1994, on tuna fish; Zhao and others 1998, on beef) and storage at subzero temperatures without freezing (Deuchi and Hayashi 1992, on raw pork; Ooide and others 1994, on carp and chicken). There is therefore a need for information on how whole muscles react to the combined high-pressure/subzero-temperature treatments particularly regarding protein denaturation and structural changes. This work examines effects of pressure-shift freezing of porcine and bovine muscle tissues compared with conventional freezing process. Pressure/temperature treatments without freezing were also performed to separate freezing from pressure effects.

Materials and Methods

Meat samples

Chilled post-rigor samples of porcine *M. longissimus dorsi* and bovine *M. semitendinosus* were obtained locally (3 to 5 d old). The pieces (typical wideness and thickness values (cm) of 11.5 and 5.5 for pork, and 10.5 and 8.5 for beef, respectively) were cut into about 7-cm lengths and vacuum-sealed in polyethylene bags for processing. Six samples from different animals each of pork and beef were processed individually. Intramuscular connective sheets were trimmed from both meats and subjected to the same treatments.

Pressure/temperature treatments

Pressure treatments employed an ACB unit (model AGIP665, GEC, Alstom, Nantes, France) with a cylindrical vessel of about 2.35-L filled with a mixture of ethylenglycol/water as a pressure transmitter and cooling medium. The unit consists of 2 hydropneumatic pumps for pressurization and a recirculating cooling system. The pressure and temperature (2 stainless-steel sheeted type T thermocouples, 1 on the surface and the other at the center of the meat piece, accuracy > 0.5 °C, response time < 1 s) were

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recorded digitally (Helios I model from Fluke, Everett, Wash., U.S.A.) throughout the process (recording rate of 1 point per s for the 5-min time before and after the expansion, plus 1 point every 15 s for the rest; 1 point/s for whole short-time processes).

A pressure/temperature combination of 200 MPa and −20 °C was used, which corresponds to a monophasic liquid-phase domain, close to the water-ice I boundary, in the phase diagram of water. Large amounts of 0.5 to 0.6 kg meat per batch were processed for several sampling requirements. The samples' core took about 2.5 h to reach the target temperature of −20 °C, surface temperature not surpassing −21 °C. To avoid freezing, samples were brought to ambient temperature before depressurization (recirculating bath at 30 °C). Total pressurization/decompression steps lasted about 3 h. Alternatively, pressure was suddenly released in the other samples while at −20 °C to permit ice I crystallization throughout the sample. Batches of about 40 g (1 piece of about 2 × 2 × 4.5 cm for recording processing parameters and another for testing) were also processed for the only purpose of DSC and TEM evaluations; processing time being then reduced to about 30 min.

Freezing was also carried out at high cooling rates in an air-blast freezing tunnel (Frigoscandia 010, Aga Frigoscandia, Helsingborg, Sweden) working at −40 °C with an air velocity of 5.5 m/s. Once the geometric center of the meat pieces reached the target temperature of −20 °C, they were held for another 30 min; the total process lasting about 3 h. All kinds of frozen samples were allowed to thaw in their bags overnight at 20 °C before evaluation.

Samples and treatments were: F, air-blast freezing; P/NF, pressurized and nonfreezing (200 MPa and −20 °C); and P/F, pressure-shift freezing (200 MPa and −20 °C). Corresponding raw muscle R was used as a control for the 3 processed samples within each of the 6 series per species.

Differential Scanning Calorimetry (DSC)

Thermal denaturation of the muscle proteins was studied by means of a previously calibrated Perkin-Elmer DSC7/TAC7DX/PC Differential Scanning Calorimeter (The Perkin-Elmer Corporation, Norwalk, Conn., U.S.A.). Small pieces of meat, free from visible traces of fat and connective tissue, were encapsulated into aluminum pans and hermetically sealed to prevent vaporization losses. At least 4 pans with 15 to 20 mg meat each (weighed accurately to 0.002 mg by an electronic balance Perkin-Elmer AD4) were used for each individual sample. The samples were scanned at 10 °C/min at 20 to 90 °C under dry nitrogen purge of 30 mL/min. 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 thermal denaturation ΔH (J/g, dry basis hereinafter) were usually within 0.5% and 6%, respectively.

DSC data reflected the thermal denaturation of proteins remaining native-like after processing. Thus, 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. Calorimetric data were normalized with respect to the corresponding untreated (100% native) sample R and the result subtracted from 1 to calculate the protein denatured fraction (PDF, dimensionless) caused by the process (Fernández-Martín and others 1997).

Transmission Electron Microscopy (TEM)

The samples were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2), postfixed in 1% osmium tetroxide, washed, dehydrated with acetone, and finally embedded in Epon 812 (Electron Microscopy Sciences, Fort Washington, Pa., U.S.A.). Thin sections with faces parallel to the myofibril direction were cut and stained with 2% uranyl acetate followed by lead citrate. Electron micrographs ×3000, ×12000, and ×30000 were obtained with a Zeiss Model 902 electron microscope (Carl Zeiss, Oberkochen, Germany).

Drip loss (DL)

This was evaluated as the exudate forced out of thawed samples. Processed cores (5-cm dia and 0.5- to 1-cm thick, weighing about 20 g, shaped before processing) were centrifuged (2000×*g*) 10 min at 0 °C twice, 1 time for each face contacting the centrifuge tube (González-Sanguinetti and others 1985). Three replicates of DL values were determined for each individual sample and expressed as the percentage of liquid phase extracted.

Color measurement

Surface color was evaluated and expressed as (L), (a), and (b) values. Samples of a recent surface from each of 6 processed cores (5 cm dia and 3 cm thick, shaped before processing) were tested at ambient temperature on each individual sample. A Hunter-Lab model D25-9 spectrophotometer (Hunterlab, Reston, Va., U.S.A.), previously calibrated with a white standard, was used.

Mechanical tests

A Universal Testing Machine Instron 1140 (Instron Engineering Corp., Canton, Mass., U.S.A.) was used to obtain force-deformation curves. Eight cylindrical specimens (1.5 cm dia and about 7 cm length cut along the processed muscle) for each individual sample were sheared perpendicular to the myofibers in a Warner-Bratzler (WB1) fixture at 100 mm/min cross-head speed. A round-ended cylindrical 0.5-cm dia stainless-steel plunger was used for penetrometry (P) trials on 3 cores like in DL. In WB tests, a 2nd score (WB2) was taken as the shear force observed at the 1st tip (slope change) in the force-deformation record, according to Bouton and others (1975).

Statistical analysis

Two-way analysis of variance was carried out using Statgraphics package (STSC Inc., Rockville, Md., U.S.A.). Differences between means were resolved by a Duncan test for multiple comparisons. Significance level was $P < 0.05$.

Results

TYPICAL EVOLUTIONS OF CHAMBER PRESSURE AND SAMPLES' center and surface temperatures along the compression and expansion steps are shown in Fig. 1 for a short-time process. Pressure (P) was rapidly raised (about 2.5 min) to 200 ± 5 MPa and maintained there during the pressurization period. The initially rapid descendent surface temperature (St) was arrested and even counterbalanced by the heat of compression of the cooling medium and finally decreased gradually to −21 °C (nonfreezing maximum cooling). Center temperature (Ct) gradually decreased to about −19.5 °C after about 31 min, without spurious freezing signals. Pressure was then suddenly released (EXP). Ct exhibited an instantaneous undercooling (4 to 5 °C) and then heating evolution (Joule effect compensated by the crystallization heat of meat water). Ice crystallization (estimated 25% to 30%) was revealed by a characteristic plateau at about −1.5 °C, in correspondence to the initial freezing point of meat (Fernández-Martín and Sanz 1980). Crystallization progressively vanished, and Ct approached St, decreasing again toward −21 °C. Long-time processes evolved similarly (not shown).

Processing times of about 30 min on small-size samples yielded products that behaved calorimetrically (DSC) and in appearance (TEM) quite closely to the large samples with longer (×5) processing times. Contrary to thermal operations, this is not sur-

prising since, according to different authors (Yamamoto and others 1994; Fernández-Martín and others 2000, among others), increasing duration of exposure to pressure beyond a given holding time is of little repercussion.

DSC

Figure 2 gathers typical DSC traces normalized to dry-matter content. Solid lines refer to samples subjected to long processing times. Dashed lines correspond to short-time samples. No significant differences in relevant temperatures and enthalpies were found between samples with different processing times. Calorimetric data will be then given for long-time samples since the remainder on meat quality required these large specimens. The average value for each of the 4 kinds of samples within a series had a typical standard deviation of 0.4 (0.7 for lowest peaks on R samples) in temperature and 0.3 (0.5 for R) in enthalpy.

Pork muscle data is shown in Fig. 2 (top). Raw, untreated meat (R) exhibited the 3 main endothermic regions of myosin (about 58 °C), myosin plus sarcoplasmic proteins, and collagen (about 64 °C), and actin (about 81 °C) with a total denaturation enthalpy of about 14.4 J/g, which compared well with literature (Wright and others 1977; Stabursvik and Martens 1980; Quinn and others 1980; Xiong and others 1987). Air-blast freezing (F) (30 min holding time once sample center at −20 °C in tunnel at −40 °C) exhibited denaturation enthalpy of 13.7 J/g, only slightly different from the values for raw product. P/NF treatment caused considerable protein denaturation: Endotherm for actin practically disappeared, and myosin and sarcoplasmic proteins were greatly reduced in the DSC trace with denaturation enthalpy of 5.6 J/g. P/F process induced additional protein denaturation reducing denaturation enthalpy to 4.3 J/g.

At > 60% moisture, the DSC trace of untreated connective tissue (C) consisted of a single sharp peak centered about 65 °C (Mcfarlane and Smith 1978; Gekko and Koda 1983; Suzuki and others 1992; Fernández-Martín and others 1999), with an enthalpy change of about 41 J/g. The water content considerably influenced the shape and size of the DSC trace (not shown) both in the temperature shift and double peaks. DSC traces of the treated samples (not shown) were similar in peaks at specific temperatures or peak area.

Figure 2 (bottom) is for beef muscle. DSC trace R is typical for raw beef, with thermal characteristics similar to those reported by other authors (Quinn and others 1980; Wagner and Añón 1985; Parsons and Patterson 1986; Findlay and others 1986; Xiong and others 1987). The characteristic 3 maximum temperatures were at about 58.5, 65 to 70, and 81.5 °C, and enthalpy of denaturation was about 14.3 J/g. The processed beef exhibited enthalpy of denaturation of 13.8, 5.5, and 4.5 J/g for samples F, P/NF, and P/F, respectively.

Connective tissue C was also thermally denatured at about 65 °C (shrinkage temperature for mammalian animal collagen), but the corresponding heat of denaturation was nearly half the value for pork connective tissue, about 22 J/g. Collagen content of *semitendinosus* is less than half that of *longissimus dorsi* muscle. Connective tissue from beef was also very stable to pressurization and/or freezing.

TEM

Short-time pressurized samples exhibited images (not shown) considerably worse than respective long-time samples, particularly in P/F treatment. The reason for this may be traceable to the excision of the small piece (about 2 × 2 × 4.5 cm) of meat used, which required several cuts in the raw muscle. This implied a considerable loss of structural integrity and subsequent alteration of mechanical properties (a smaller sample may behave even worse). Ice was then much more destructive, and big, round crystallization areas appeared throughout a lesser

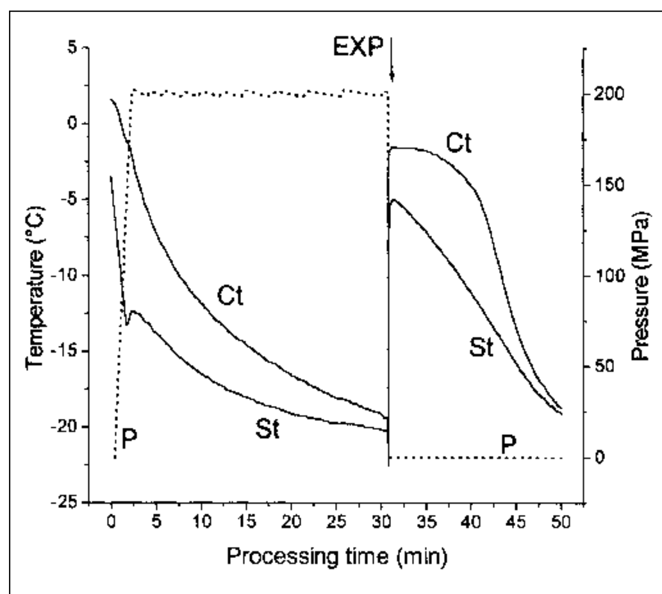

Fig. 1—Typical records of pressure (MPa) and temperature (°C) against time (min) along the compression and expansion (EXP) steps in pressure-shift freezing (P/F). P, Pressure; Ct, Center temperature; St, Surface temperature

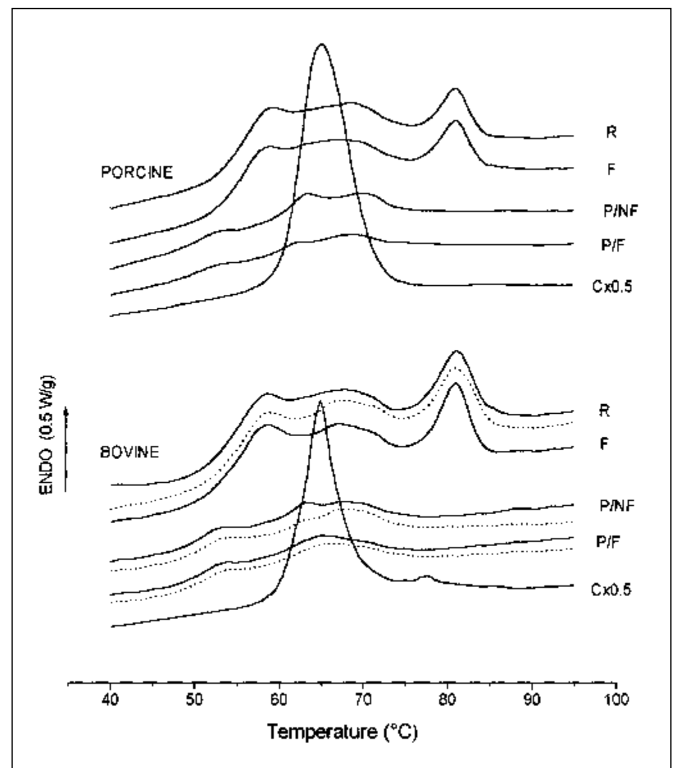

Fig. 2—Normalized (dry matter) DSC traces of porcine *M. Longissimus dorsi* (top) and bovine *M. Semitendinosus* (bottom): R, Raw; F, Frozen; P/NF, Pressurized-Nonfrozen; P/F, Pressurized-Frozen; C, Connective tissue (×0.5, half sensitivity). Solid lines, Long-time processes; Dotted lines, Short-time processes

constraining matrix. Correspondingly to DSC, short-time samples were also considered morphological and structurally similar to the long-time samples, for which the results are given below.

Figure 3 shows (×12000) micrographs of longitudinal sections of F, P/NF, and P/F porcine *longissimus dorsi* muscle. Similar micrographs were displayed by bovine *semitendinosus* muscle samples and are therefore not shown. F-treated muscle exhibits well preserved sarcomeres (A and I bands, M and Z lines clearly differentiated) and myofibrils. In some instances, chains of hollows (rosary beads) that reveal the presence of ice are along myofibrils. The separation near actin I bands was larger than that near the less elastic (more resistant to growing ice) myosin M line. Pressurization alone at 200 MPa and −20 °C caused similar effects to those described by others (Macfarlane and others 1980-81; Suzuki and others 1990, 1992; Ashie and Simpson 1998) in meat treatments with same pressure and ambient temperature. P/NF-treated sample exhibited substantial destruction of sarcomeres (broken A-band with extinction of M-line and H-zone, and loss of define filamentous structure (myosin dissociation); broken I-bands and thickening of Z-line by collapse of the I-band; deposit (tufts) of dense material) and marked fragmentation of myofibrils (by F-actin depolimerization, among others). Sample P/F exhibited further and very intense disintegration due to massive ice crystallization throughout the entire sample: fragmented Z-lines and A-bands embedded in ice. Note that the image (Fig. 3, P/F) does not have a good definition. It may be somewhat blurred, but the main cause was a big compaction of myofilaments by the expansion of ice (broken A-band spanned about 20% more than in P/NF.)

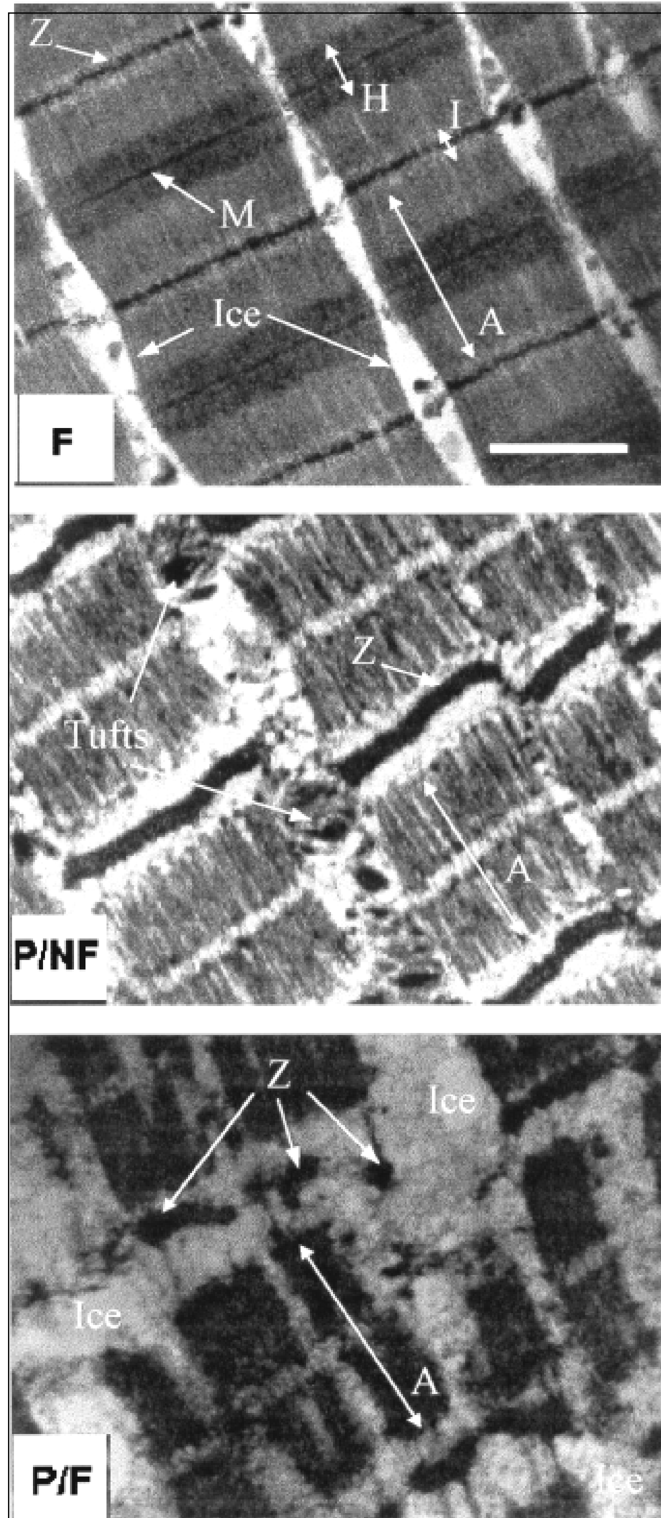

Fig. 3—TEM image of processed samples from porcine *longissimus dorsi* muscle tissue at H12000 magnification (bar = 1 mm). F, Frozen; P/NF, Pressurized-Nonfrozen; P/F, Pressurized-Frozen (Fragmented structural elements embedded in ice). Legends: A, A-band; H, H-zone; I, I-band; M, M-line; Z, Z-line; T, Tufts; Ice, Ice crystals.

Drip loss

Pork and beef exhibited similar DL values, as shown in Table 1. Samples F and P/NF had DL values not significantly different from the corresponding value for R. P/F samples exhibited high DL. Typical standard deviation of the average value for each of the 4 kinds of samples within a series was 1.9.

Color

Pork and beef muscle behaved very similarly, as also shown in Table 1. The appearance of F samples was similar to R samples. P/NF and P/F samples exhibited a cooked appearance, grayish-brown on the outside (Nose and others 1992; Carlez and others 1995). Freshly cut surfaces, particularly pork samples, exhibited a considerable increase in luminosity (L), with the treatments following the order R < F < P/NF < P/F. There were smooth variations in (a) and (b) values. Typical standard deviations were 1.3, 0.7, and 0.6 for the respective average values (L), (a), and (b) of each of the 4 kinds of samples within a series.

Texture

Warner-Blatzler (WB1) values exhibited considerable variability in both kinds of muscle. Variability of WB1 values of raw meat (unknown history) varied largely from sample to sample, much more in pork (about 52%) than in beef (about 26%). F samples exhibited smaller variations of 32% and 19%, respectively. WB1 values of P/F samples had 18% and 6% variability. Processing reduced WB1 variability, with the treatments following the order R < F < P/NF < P/F. Additionally, individual samples properties changed very differently between raw and processed, with either an increase or decrease in WB1 depending upon the raw value. The use of total mean values from all data series to establish comparisons (2-way) among raw and processed samples was therefore of little use (no significant differences among processes). In pork intramuscular fat migrated along fibers to the cut surfaces, particularly suctioned by sudden pressure release. The cored samples used for WB preparations were thus essentially fat free. This meant that pressurized pork muscles (significantly harder than nonpressurized) were not equivalent samples in composition as the corresponding R and F samples. Hence data of Fig. 4 deal only with a single series of samples on beef muscle.

Table 1—Protein denatured fraction (PDF, dimensionless); Drip losses (DL, %); and Tristimulus data on muscles from pork and beef (R, Raw; F, Frozen; P/NP, Pressurized-NonFrozen; P/F, Pressurized-Frozen)

	PDF	DL	Color parameters*		
Samples			L	a	b
R	^{a}0(0)^{a}	^{a}19.7(22.2)^{a}	^{a}38.5(36.6)^{a}	^{a}4.6(12.2)^{a}	^{a}4.8(8.1)^{a}
F^{i}	^{a}0.05(0.03)^{a}	^{a}20.2(25.0)^{a,b}	^{b}44.4(40.9)^{b}	^{b}3.5(9.9)^{b}	^{b}6.3(7.3)^{b}
P/NF	^{b}0.61(0.62)^{b}	^{a}19.0(22.6)^{a}	^{c}64.6(52.2)^{c}	^{a}4.5(10.9)^{b,c}	^{c}8.4(10.5)^{c}
P/F^{i}	^{b}0.70(0.69)^{b}	^{b}31.2(28.1)^{b}	^{c}66.6(53.3)^{c}	^{a}4.5(11.1)^{c}	^{d}9.2(10.1)^{c}
SEM^{j}	0.03	1.7(1.5)	0.4(0.8)	0.2(0.4)	0.2(0.2)

Figures in brackets refer to beef
[i]Data on thawed samples
[j]Standard error of the mean of 6 independent series of samples
*Data on pressurized pork samples affected by compositional changes
Different superscript letters on the same column indicate significant differences (P < 0.05)

Penetration force P values were (likely WB1) not significantly different. WB2 data, however, differentiated F and P/NF samples (not significantly different) from the significantly softer raw R and significantly harder P/F samples. Worth to emphasize is that in handling (cutting for WB sample preparation and subsequent test shearing), these frozen samples P/F lost considerable amounts of liquid phase. These compositional changes helped to explain the contradictory combination of hardest-sample with highest-disintegration (TEM) and invalidated any kind of result on P/F samples.

Discussion

DATA ON THE PROTEIN DENATURED FRACTION (PDF) RESULTing from the process are shown in Table 1. PDF data followed the same pattern in both kinds of meat. Air-blast freezing is recognized as good for meat-quality preservation, and F samples underwent very little protein denaturation in all cases. Pressurization yielded dramatic increments of protein denaturation in both meats. It did not contradict Suzuki and others (1990) who reported no significant differences between the SDS-PAGE (denaturing) electrophoretic pattern of myofibrillar proteins from pressurized and untreated beef muscles. Pressure-shifted freezing caused additional unfolding effects, presumably by sudden solute concentration (ionic force increment) and (ice) mechanical stress. Actin was always the protein most susceptible to pressure-induced denaturation. Myosin and sarcoplasmic proteins were severely affected. Collagen, however, was very stable to pressurization treatments and to freezing, either singly or in combination. Bouton and others (1978) reported pressure-induced stabilization (150 MPa/60 °C) of connective tissue to heat denaturation (about 6º upward shifting for the onset temperature of tendon heat shrinkage), but we never observed significant differences in behavior of processed in contrast to untreated connective tissues (Fernández-Martín and others 1998a, 1999) regardless of sample origin (blue whiting, pork, beef), pressure (100 to 400 MPa), or temperature (−20 to 70 °C) combinations. It coincides with other reports (Macfarlane and others 1980-81; Suzuki and others 1992) that collagen is stable to pressurization because of H-bonded triple α-helix, and only fibril formation is suppressed under high pressure (Gekko and Koda 1983).

Pressure is immediately and isotropically transmitted while heat is slowly and anisotropically transferred. Predominance of pressure-driven over termal-driven mechanisms depends on both pressure and temperature, thus pressure and temperature may induce interdependently antagonistic-like effects on the protein matrix. According to our practical rule (Fernández-Martín and others 1997), pressure induces protein denaturation at nondenaturing temperatures, but proteins are preserved from subsequent thermal denaturation in thermal unfolding conditions. Cold denaturation as induced by pressurization at subzero temperatures was thus consistent with results previously reported on pork- and beef-meat batters (Fernández-Martín and others 1997, 1998b, 1999, 2000) and blue-whiting mince (Fernández-Martín and others 1998a) subjected to pressure (100 to 400 MPa) and nondenaturing thermal conditions (−20 to 40 °C).

The remaining native-like proteins after pressure processing of both muscle types (Fig. 2) appear to be related to different myosin domains at both zones of around 50 to 55 and 60 to 70 °C and to collagen at the last of these (Fernández-Martín and others 2000).

Air-blast frozen muscles F scored quite well in drip loss as compared to the raw muscles R (Table 1). Despite considerable protein denaturation, P/NF samples of pork and beef showed DL values similar to their respective F samples. P/F samples were the worst. It was consistent with the morphological and structural changes revealed by TEM micrographs. Massive ice crystallization (Fig. 3, P/F) disintegrated further the structure severely damaged in the previous pressurization step. Compaction of myofilaments led to the practical destruction of the network that retains water by capillary forces. Thus, unlike samples pressurized alone (Fig. 3, P/NF), water-holding capacity of P/F samples was irreversibly affected.

WB1 and P data (mainly relating to the connective network resistance under large strain, Fig. 4) indicated that pressurization effects on beef muscle were small (not significant), in agreement with the findings of Zhao and others (1998) in pressure-assisted thawing of conventionally frozen beef. Intact stromal proteins, mainly collagen, may mask the effects derived from the pressure-induced denaturation of myofibrillar proteins. WB2 trials (mainly relating to the myofiber resistance at low strain), however, showed that the myofibrillar component of sample toughness increased by processing in all cases (R<F<P/NF< P/F). Anyhow, the clear conclusion derived from textural measurements was that, whatever the initial condition of the muscle, pressure-shift freezing always yielded roughly the same final result, that is, a "typical" product with basically the same attributes reflecting the substantial structural destruction of P/F samples.

Initially, simple geometrical considerations would appear to indicate that cellular (fruits and vegetables) and filamentous

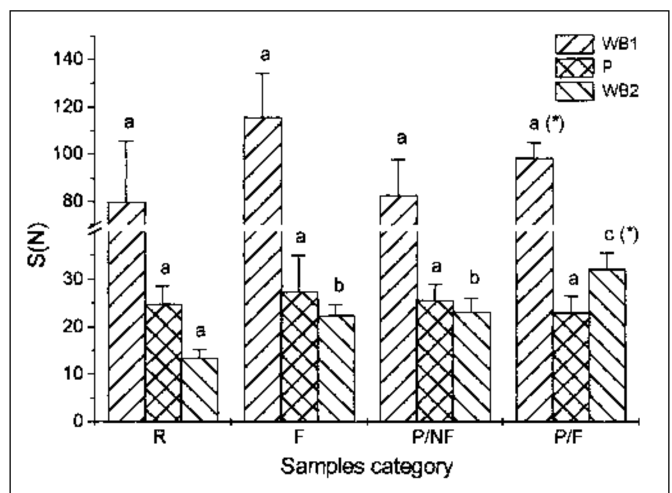

Fig. 4—Strength data S(N) from different texture tests on beef muscle: R, Raw; F, Frozen; P/NF, Pressurized-NonFrozen; P/F, Pressurized-Frozen. WB1, Breaking shear force in Warner-Bratzler test; WB2, Shear force at the first tip in Warner-Bratzler test; P, Penetration force in Penetrometry. Vertical bars indicate standard deviations. Different letters on the same type of bars indicate significant differences (P < 0.05). (*), Data affected by compositional changes.

structures (myosystems) may benefit from flash nucleation, leading to an even distribution of fine ice crystals through the entire bulk sample. It has been then speculated on the properness of pressure-shift freezing for meat preservation. In fact, optical microscopy and SEM did induce such an impression to Otero and others (1997) and Martino and others (1998) since muscle fibers remained unaltered. On the other hand, it has been stressed above that handling some quality indexes (texture) on raw and pressurized meats is rather problematic and also could direct to misleading evaluations. Most authors have tried to overcome it by using cooked samples for testing. Cooking involves so strong changes that, in our opinion, may camouflage original differences, probably leading in turn to incorrect evaluations of the noncooked muscles. In any case, TEM did provide definitely the real view on muscle pressure-shift freezing: Pressurization was the critical step. Muscle tissues could not benefit this special nucleation/growth of ice crystals because myofibrils were fragmented and their sarcomere structures disintegrated by previous pressurization. Subsequent ice crystallization all through the severely damaged proteinic matrix caused further ultrastructural destruction.

Previous TEM reports on muscle pressurized at ambient temperature (Macfarlane and others 1980-81; Suzuki and others 1990, 1992; Ashie and Simpson 1998) and this work at subzero are conclusive about the same nature of structural destruction induced despite so separated temperatures. Really, (Fig. 1) the temperature of muscle under pressure evolved between around +5 and −20 °C. Processing temperatures for pressure suppression of ice crystallization (subzero nonfrozen storage) and for pressure-assisted thawing treatments would be roughly within this range. If the 3 processes were closely similar in processing parameters, they would likely suffer from identical drawbacks.

On the other hand, myofibrillar proteins were severely (cold) denatured, inasmuch as pressure was combined with nondenaturing temperatures (Fernández-Martín and others 1997). This unfolding effect has been reported (Fernández-Martín and others 1997, 1998a, 1999, 2000) on several meat systems pressurized (100 to 400 MPa) at different temperatures ranging −20 to 40 °C. Likely in TEM, the absence or presence of any water phase change (no matter the transition direction) would be of little DSC concern. Any kind of myosystem, whether structured (for example, human organs for transplanting) or otherwise (patty, paste, batter), processed by these pressure/temperature combinations would display a serious protein-unfolding drawback.

According to the authors cited herein, ultrastructural damage is intensified with pressure increase. Protein unfolding (our reports) at a given temperature also increases as pressure level does. Hydrostatic pressures around 50 MPa could then deserve consideration concerning subzero muscle treatments if a limited loss of structural and conformational integrity would not be a serious inconvenience.

Conclusions

PRESSURIZATION WAS THE DECISIVE STEP. MUSCLE TISSUES WERE structurally affected at a double level: The typical sarcomere organization was severely damaged, and concomitantly, the constituent myofibrillar proteins were considerably unfolded. Hydrostatic pressurization at subzero temperatures (200 MPa and −20 °C) either without or (even more) with pressure-shift freezing was very injurious and clearly unsuitable for muscle preservation.

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Authors Fernández-Martín, Otero, and Sanz are with the Departamento de Ingeniería, Instituto del Frio (CSIC), Ciudad Universitaria, E-28040 Madrid, Spain. Author Solas is with the Departamento de Biología Celular, Fac. C. Biológicas, Universidad Complutense de Madrid, Ciudad Universitaria, E-28040 Madrid, Spain. Direct correspondence to F. Fernández-Martín (E-mail: fermarfer@if.csic.es).