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EFFECT OF PRERIGOR PRESSURIZATION ON BOVINE LYSOSOMAL ENZYME ACTIVITY

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Abstract

Longissimus muscle from 8 dairy cows was prerigor pressure (PRP) treated at different pressure levels (0, 34.5, 68.9 and 103.5 MNm^{-2}). β-Glucuronidase (indicator of lysosomal enzymes) activity in the unsedimentable (U) and sedimentable (S) fractions was fluorometrically assayed at 1½, 24 and 168 hr postmortem. At 1½ and 24 hr postmortem, the specific activity of β-Glucuronidase in the U-fraction from PRP treated samples was significantly ($P<0.05$) higher than that in the U-fraction from the control. However, at these times, the sedimentable fraction from the control sample had higher ($P<0.05$) enzyme activity than the sedimentable fraction from the PRP sample. Prerigor pressurization caused a pronounced Z-line degradation. However, there was no Z-line degradation in the control samples, either at 1½ or 24 hr postmortem. Z-line degradation could have been caused by a lysosomal enzyme released early as the result of the pressure treatment.

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KEY WORDS: Prerigor, pressure, bovine, lysosomal enzymes, β-Glucuronidase activity, transmission electron microscope, sodium dodecyl sulfate polyacrylamide gel electrophoresis, Z-line.

Introduction

Tenderness is one of the most important characteristics that render meat acceptable to consumers. Tenderness is a dynamic property, and as such, even originally tender meat can be rendered tough by the postmortem handling e.g. chilling in a holding cooler. Several tenderization techniques have been found to restore tenderness, including prerigor pressurization (PRP) (Elgasim, 1977; Kennick et al., 1980; Macfarlane, 1973). In the past few years PRP has been intensively investigated at our laboratory and its beneficial effect on tenderness has been well documented. Also, several other physico-chemical properties were affected by PRP (Elgasim and Kennick, 1982).

Several investigators have provided evidence for the presence of lysosomes in skeletal muscle (Canonico and Bird, 1970; Ono, 1970; Stagni and Bernard, 1968). Lysosomes are subcellular organelles that house several proteolytic enzymes. Many researchers have reported on the role of lysosomal enzymes in meat tenderness (Moeller et al., 1976; Dutson et al., 1980). Also these studies suggested that unless the lysosomal membrane is disrupted, the lysosomal enzymes are inactive. Disruptive processes including several chemical and physical factors e.g. pH, freezing and thawing, thermal activation and detergent treatment, have been implicated in lysosomal disruption (Sawant et al., 1964).

Ivanov et al. (1960), Ikkai and Ooi (1966) and Joseph and Harrington (1968) concluded that muscle proteins are affected by hydrostatic pressure. Enzymes are proteins and are likely to be affected by pressure treatment. Hydrostatic pressure in the order of 786.6 and 588.6 MNm^{-2} inactivated trypsin and chymotrypsin respectively (Miyagawa and Suzuki, 1963a, b). Also sarcoplasmic reticulum subjected to 150 MNm^{-2} lost its extra Adenosine Triphosphatase (ATPase) activity (Horgan, 1981).

The mechanisms by which pressurization tenderizes meat have not been fully elucidated. In a previous study, Elgasim (1977) hypothesized that lysosomal enzymes are likely to play a role in the tenderness improvement induced by PRP. This study was conducted to see if PRP caused an early release of lysosomal enzymes as monitored by β-Glucuronidase.

Materials and Methods

Sample Preparation and Treatment

Samples were obtained from 8 utility grade dairy cows (approx. wt 480 kg) slaughtered at the Oregon State University Meat Science Laboratory. After slaughter, skinning, evisceration, splitting and washing the carcass, the left side of the carcass was assigned for various pressure treatments, whereas the right side served as the control (CON). The longissimus muscle (from the last lumbar vertebra to the 8th thoracic vertebra) was removed (≈35 min. post slaughter) from the side designated for treatment and pressure treated according to the procedure of Elgasim et al. (1982) except that the pressure was applied at the rate of 2.6 $MNm^{-2}sec^{-1}$. Unless otherwise stated the pressure level used throughout the experiment was 103.5 MNm^{-2}. (1021 atm.)

Four pressure levels, namely 0, 34.5, 68.9 and 103.5 MNm^{-2} were used to study the effect of the pressure level on the activity of β-Glucuronidase. Longissimus muscle between the 6th and 8th thoracic vertebrae was removed from 6 sides designated for the pressure treatment in the study. Each two sides were assigned for 34.5, 68.9 and 103.5 MNm^{-2} pressure treatment, whereas all the muscles from the corresponding sides were used for the 0 (CON) MNm^{-2}. For this part of the study the activity was measured at 1½ hr postmortem in the unsedimentable (U) fraction only.

The pH of the CON and pressure treated samples was measured at 1½, 6, 24 and 168 hr postmortem using a Corning pH meter, Model 125, equipped with a combination glass electrode which was inserted in a freshly made incision each time pH was taken.

Enzyme Extraction

A method similar to that employed by Moeller et al. (1976) was used for the enzyme extraction. At appropriate times postmortem (1½, 24 and 168 hr) samples were removed from each CON and pressure treated muscle, trimmed of external fat and connective tissue and ground in a Hobart meat grinder. Ten grams of the ground meat sample was homogenized in 50 ml homogenizing solution (containing 0.25 M sucrose, 0.02 M KCl and 2.0 mM EDTA, pH 7.0) for 45 sec. in an Osterizer blender. The homogenate was filtered through two layers of cheese cloth and the pH of the filtrate was adjusted to 7.25 to 7.30 with 0.5 M KOH. The filtrate was centrifuged at 105,000 G for 2 hr to give unsedimentable (U) and sedimentable (S) fractions. The U-fraction was filtered through glass wool and the S-fraction was resuspended in 25 ml of the homogenizing solution.

Enzyme Assay

β-Glucuronidase standard and 4-methylumbelliferyl-B-D-glucuronide were obtained from Sigma Chemical (St. Louis, MO). The enzyme activity was measured according to methods described by Moeller et al. (1976).

Electron Microscopy

Observations were made on samples from two animals. Within 1½ hr postmortem, samples were removed from the CON and pressure treated samples and fixed immediately in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 4 hr, then transferred to a fresh phosphate buffer and left overnight. The samples were then fixed in 1% osmium tetroxide (OsO_4) in 0.1 M phosphate buffer, dehydrated in 50, 70, 90 and 100% acetone solutions and stained with a saturated solution of uranyl acetate. Specimens were infiltrated, embedded in Spur's epoxy formulation and cured overnight at 70°C. Silver-grey sections were cut using a diamond knife on a Porter-Blum MT-2 ultramicrotome, stained with Reynold's lead citrate and examined with a Philips EM-300 transmission microscope. At 24 hr postmortem a sample was removed from the CON muscle and similarly prepared for examination with the electron microscope.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Gel electrophoresis of the total extract (unsedimentable fraction + resuspended pellet) and U-fraction was performed following the procedure described by Porzio and Pearson (1977) using 10% gels. The protein concentrations of the two fractions were determined by the biuret method (Gornall et al., 1949).

Statistical Analysis

Since the design of the experiment uses a paired comparison, the data collected for the pH, and enzyme activity in the sedimentable and U-fractions were analyzed by the paired t-test (Steel and Torrie, 1960).

Results and Discussion

β-Glucuronidase activity was determined after treating muscles at 0 (= CON), 34.5, 68.9 and 103.5 MNm^{-2} for 2 min. to determine whether the enzyme activity is pressure dependent. There was a progressive increase in activity with increase in pressure level (Fig. 1), however, the change was observed to be non-linear. The change in activity/pressure unit was higher between 34.5 and 68.9 MNm^{-2}. The cause for this non-linearity was not clear, however, it could be that more enzyme was released but some of it was inactivated. Pressure in the order of 51.8 MNm^{-2} caused an increase in the activity of sarcoplasmic reticulum extra ATPase, however, the activity decreased as the pressure level was increased to 103.5 and 155 MNm^{-2} (Horgan, 1979).

The specific activity of β-Glucuronidase in the U and S-fractions at different postmortem times is shown in Table 1. At 1½ and 24 hr postmortem, the activity in the U-fraction from the PRP sample was significantly ($P<0.05$) higher than its corresponding CON (approximately 23% and 20% increases in free activities at these postmortem times, respectively). At 7 days postmortem, the free activities of both CON and PRP samples were the same ($P>0.05$). Dutson et al. (1980) reported that electrically stimulated ovine samples had higher percent free activity for β-Glucuronidase and cathepsin C. The increase in the activity of β-Glucuronidase in the U-fraction, immediately after the treatment (1½ hours postmortem), was not surprising because, besides other factors, the PRP treatment caused the pH of the sample to be acidic (Fig. 2). At the acidic range observed in this study, Sawant et al. (1964), reported that aryl sulfatase, acid phosphatase and β-Glucuronidase became more available. Divalent

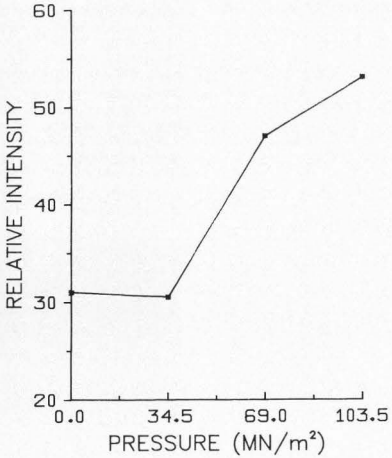

Fig. 1: Changes in the specific activity of β-Glucuronidase (relative intensity) with changes in pressure levels. Temperature (37°C) and duration (2 min.) of pressurization were kept constant. The activity was measured in the U-fraction immediately before and after pressurization (≈1½ hr postmortem). Each point is a mean of 2 samples with 2 observations per sample, except the 1st point, at 0 MNm^{-2}, which is a mean of 6 observations.

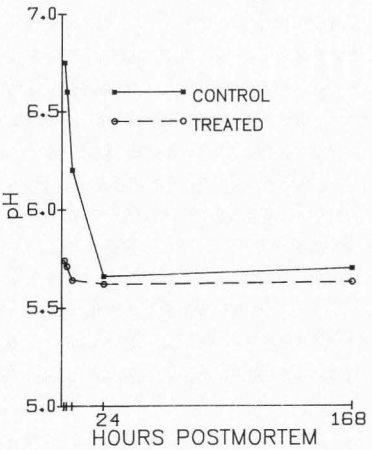

Fig. 2: Changes in the pH of the control (—) and pressure treated (o— —o) longissimus muscle with postmortem times. Conditions of the pressure treatment: 103.5 MNm^{-2}, 37°C, 2 min. duration. Each point is a mean of 8 observations.

cations, especially Ca^{++} and Mg^{++} also enhance the availability of lysosomal enzymes (Sawant et al., 1964). In a previous study using electron microscopy, Elgasim and Kennick (1982), observed that the mitochondrial and sarcoplasmic reticulum systems were both affected by PRP resulting in the release of Ca^{++} in the cell cytoplasm which was evident by the contraction (48% of its on carcass length) observed after PRP treatment (Kennick et al., 1980). However, the effect of pressure on the pH of prerigor muscle cannot be used to explain the differences observed in the β-Glucuronidase activities between PRP and CON samples at 24 and 168 hours postmortem (Table 1). At these times, the pH differences between the two treatments vanished and the samples have the same ultimate pH (Fig. 2).

At this stage our attention was focused on possible molecular changes that could have dictated these differences in the activities of CON and PRP samples. Examination of electrophoretograms (Fig. 3) of the CON and pressure treated samples reveal that the PRP sample from the U-fraction (gel 1) has two bands less than the CON sample (gel 2). The two missing bands were observed to be in the 173,000 and 153,000-dalton regions and it is suggested these two bands were

Table 1 - Changes in specific activities of β-Glucuronidase in the sedimentable and unsedimentable fraction from control and pressure treated bovine logissimus muscles with postmortem times.

Post-mortem Time(hr)	Treatment[b]	Specific Activities of Fraction[a] U	S	% Bound Activity[c]
1½	CON	0.164[d]	0.202[h]	55.50
	PRP	0.231[d]	0.113[h]	32.90
24	CON	0.268[e]	0.171[i]	39.00
	PRP	0.356[e]	0.084[i]	19.10
168	CON	0.341[f]	0.141[k]	29.20
	PRP	0.392[g]	0.072[k]	15.50

[a] Specific activities were expressed as nM substrate hydrolyzed/mg protein/min. Each value is a mean of eight observations. U = unsedimentable fraction; S = sedimentable fraction.

[b] CON = control; PRP = prerigor pressurization. Conditions of pressure treatment: 103.5 MNm^{-2}, 37°C and 2 min. duration.

[c] % bound activity = S-activity ÷ (U-activity + sedimentable activity).

[d, e, f, g, h, i, k] means in the same postmortem period and the same column, carrying the same superscript are significantly different ($P<0.05$)

mobilized by the PRP treatment. The nature of these two bands is not known, but we believe them to be natural proteinase inhibitors. A third band at 130,000-dalton region almost vanished from the PRP sample (gel 1). When these gels were scanned, the intensity of some other minor bands were observed to be affected by the pressure treatment. There were no differences in the banding pattern of the electrophoretograms obtained from the total extract of the PRP (gel 3) and in the CON (gel 4). Only minor differences in the intensity of these bands can be observed.

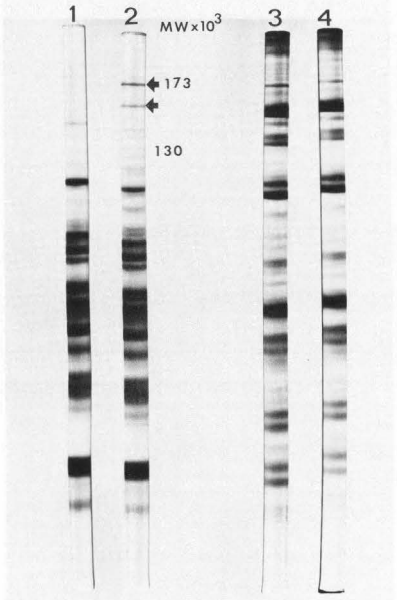

Fig. 3: SDS-gel electrophoresis of total extract and U-fractions prepared from CON and PRP samples 1½ hr postmortem. Gels 1 and 2 are U-fractions from PRP and CON samples respectively. Gels 3 and 4 are total extracts from PRP and CON respectively. Conditions of the PRP: 103.5 MNm^{-2}, 37°C and 2 min. duration. Note that CON has two bands (arrows) more than the PRP samples.

Figure 4 shows the electron micrographs of pressure treated and CON samples. It is obvious that the Z-lines of the pressure treated samples were extensively disintegrated. Calcium-activated factor (CAF) (Penny, 1974; Dayton et al., 1976) and cathepsin D (Eino and Stanley, 1973) were implicated in postmortem Z-line degradation. At a pH <6.0 Suzuki et al. (1982), observed that CAF was less effective in degrading the Z-line of the myofibrils, and in our study, the pH of the muscle is below 6.0 immediately after pressurization. Several studies (Eino and Stanley, 1973; Robbins and Cohen, 1976) have indicated that cathepsin D is effective under these conditions. Since cathepsin D is lysosomal in origin (Canonico and Bird, 1970) the Z-line degradation observed here (Fig. 4) can be taken as evidence of an early release of lysosomal enzymes induced by PRP treatment.

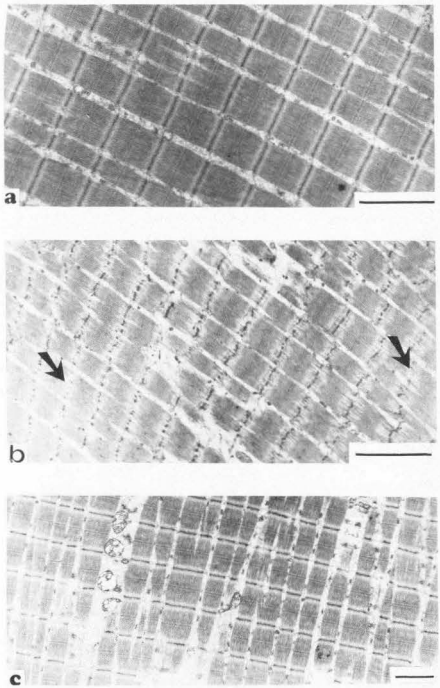

Fig. 4: Transmission electron micrograph of longissimus muscle sampled and fixed 1½ hr postmortem: a) longitudinal section from CON sample; b) longitudinal section from PRP sample; c) longitudinal section from CON sample, sampled and fixed at 24 hr postmortem. Note Z-line disintegration of PRP (arrows). There was no Z-line disintegration in the CON samples even with aging up to 24 hr postmortem. Bars in a and b equal 3.1 μm, bar in c = 2.0 μm.

In view of the results presented here, there is no doubt that PRP caused an early release of lysosomal enzymes at a time when the temperature and pH conditions of the muscle were conducive to a greater and faster enzymatic action. Therefore, at least part of the improvement in tenderness induced by PRP treatment could be attributed to the early action of the lysosomal enzymes.

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References

Canonico PG, Bird JWC. (1970). Lysosomes in skeletal muscle tissue. Zonal centrifugation evidence for multiple cellular sources. J. Cell. Biol. 45,321-333.

Dayton WR, Reville WJ, Goll DE, Stromer MH. (1976). A Ca^{+2} activated protease possibly involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. Biochem. 15,2159-2167.

Dutson TR, Smith GC, Carpenter ZL. (1980). Lysosomal enzyme distribution in electrically stimulated ovine muscle. J. Food Sci. 45,1097-1098.

Eino MF, Stanley DW. (1973). Catheptic activity, textural properties and surface ultrastructure of postmortem beef muscle. J. Food Sci. 38,45-50.

Elgasim EA. (1977). The effect of ultrahydrostatic pressure of prerigor muscle on characteristics of economic importance. M.S. thesis, Oregon State University, Corvallis, Oregon.

Elgasim EA, Kennick WH. (1982). Effect of high hydrostatic pressure on meat microstructure. Food Microstructure. 1(1),75-82.

Elgasim EA, Kennick WH, Anglemier AF, Elkhalifa EA, Koohmaraie M. (1982). Effect of prerigor pressurization on the emulsifying capacity of muscle protein. J. Food Sci. 47,861-863.

Gornall AG, Bardawill CJ, David MM. (1949). Determination of serum-proteins by means of the biuret reaction. J. Biol. Chem. 177,751-766.

Horgan, DJ. (1981). Effect of pressure treatment on sarcoplasmic reticulum of red and white muscles. Meat Sci. 5,297-305.

Horgan DJ. (1979). ATPase activities of sarcoplasmic reticulum isolated from rabbit and bovine muscle subjected to prerigor pressure treatment. J. Food Sci. 44,492-493.

Ikkai T, Ooi T. (1966). The effect of pressure on F-G transformation of actin. Biochem. 5,1551-1560.

Ivanov II, Berg IN, Labedeva NN. (1960). Changes in some properties of myosin, actomyosin and actin under the influence of high pressure. Biokhemeya. 25,505-510.

Joseph R, Harrington WF. (1968). On the stability of myosin filaments. Biochem. 7,2834-2847.

Kennick WH, Elgasim EA, Holmes ZA, Meyer PF. (1980). The effects of ultrahydrostatic pressurization of prerigor muscle on postrigor meat characteristics. Meat Sci. 4,33-40.

Macfarlane JJ. (1973). Prerigor pressurization of muscle: Effect on pH, shear value and taste panel assessment. J. Food Sci. 38,294-298.

Miyagawa K, Suzuki K. (1963a). Pressure inactivation of enzyme: Some kinetic aspects of pressure inactivation of Trypsin. Rev. Phys. Chem. Japan. 32,43-50.

Miyagawa K, Suzuki K. (1963b) Pressure inactivation of enzyme: Some kinetic aspects of pressure inactivation of chymotrypsin. Rev. Phys. Chem. Japan. 32,51-56.

Moeller PW, Fields PA, Dutson TR, Landman WA, Carpenter ZL. (1976). Effect of high temperature conditioning on subcellular distribution and levels of lysosomal enzymes. J. Food Sci. 41,216-217.

Ono K. (1970). Lyososomal-type enzymes in beef longissimus dorsi muscle. J. Food Sci. 35,256-257.

Penny IF. (1974). The action of a muscle protease on the myofibrillar proteins of bovine muscle. J. Sci. Fd. Agric. 25,1273-1284.

Porzio MA, Pearson AM. (1977). Improved resolution of myofibrillar proteins with sodium dodecyl sulfate - polyacrylamide gel electrophoresis. Biochem. Biophys. Acta. 490,27-34.

Robbins FM, Cohen SH. (1976). Effects of cathepsin enzymes from spleen on the microstructure of bovine semimembranous muscle. J. Texture Stud. 7,137-142.

Sawant PL, Desai ID, Tappel AL. (1964). Factors affecting the lysosomal membrane and availability of enzymes. Arch. Biochem. Biophys. 105,246-253.

Stagni N, Bernard BDE. (1968). Lysosomal enzyme activity in rat and beef skeletal muscle. Biochem. Biophys. Acta. 170,129-139.

Steel FGD, Torrie JG. (1960). Principles and procedures of statistics. McGraw-Hill, New York, 78-81.

Suzuki A, Matsumoto Y, Sato T, Nonami Y, Saito M. (1982). Ca^{2+}-activated protease in stored muscle. Meat Sci. 7,269-278.

Discussion with Reviewers

P.B. Addis: The authors report that pressure causes a more rapid pH drop in muscle. However, this experiment is confounded by the fact that the control side was intact whereas the treated side was excised, and excision by itself is able to stimulate contraction and pH decline. Furthermore, pH decline is a factor which can stimulate release of lysosomal enzymes.

Authors: We measured pH before and after excision and we observed very little change as a result of excision. It is to be noted that our measurements were taken within ½ to 1 min. after excision. We think excision will become a factor in pH decline if there is a time gap between excision and pH measurement. We are quite confident that the substantial change in the pH of the pressure treated sample is due to the treatment itself and not the excision of the muscle. We do agree with you that pH decline is a factor that can stimulate release of lysosomal enzymes and this is the main reason for presenting the pH results in this study.

T.R. Dutson: Was care taken not to remove samples from any area of muscle that had previously been cut for pH measurement?
Authors: Generally we avoided any muscle area that had been exposed for a longer period.

R.J. Carroll: Why were utility grade cows used? Would not the connective tissue content be higher, and, therefore, less tender meat?
Authors: It happened that they were the samples available to us. We agree with you that they are less tender, however, we think it is due mainly to the quality of connective tissue rather than the quantity. Connective tissue becomes more cross-linked with maturity, thus more stable to heating (cooking).

Reviewer VI: Can you please comment further on the gel electrophoresis?
Authors: Two techniques were used to perform gel electrophoresis. One was Laemmli (1970) with some modification (slab gel instead) and the other was the Porzio and Pearson (1977) technique. The latter has given a much better resolution and a clear electrophoretogram is presented (Fig. 3). With Laemmli's procedure the two missing bands were observed at 179,000 and 126,500-dalton. With Porzio and Pearson's procedure the two missing bands were observed at 173,000 and 157,200-dalton. A third band at the 130,000-dalton region almost vanished.

T.R. Dutson: Are these micrographs (Fig. 4) representative of the micrographs of all samples? How many animals and how many samples per animal were evaluated?
Authors: Samples were obtained from two animals with two observations per animal. Over the years we have taken many electron micrographs and feel confident that the effect of pressure on the ultrastructure of muscle is very consistent.

R.J. Carroll: In the 'Results' section in discussing Table 1, you mention "approximately 23% and 20% increases in free activities...". Can you please explain this further?
Authors: Free activity is the enzyme activity in the unsedimentable fraction (supernatant). 23% and 20% came from Table 1 by subtracting and rounding to whole numbers, the % bound activity in the PRP sample from that of the CON at 1½ and 24 hr postmortem, respectively.

Reviewer VI: Please give further details about the β-Glucuronidase standard.
Authors: Purified enzyme with known protein content and enzyme activity (3,500,000 units/gm solid) obtained from Sigma Chemical, St. Louis, MO.

R.J. Carroll: ...48% of carcass length contraction after PRP treatment (Kennick et al., 1980), but on page 77 (Elgasim and Kennick, 1982, Food Microstructure) the sarcomeres for longissimus muscle were only 8.2% shorter. Can you clarify this discrepancy?
Authors: There is no discrepancy. In the first study (Kennick et al., 1980) we are looking to the overall shortening of the whole muscle relative to its resting length. In the second study (Elgasim and Kennick, 1982) the measurement was on the sarcomere, which is the smallest unit of the muscle. We have encountered some difficulties measuring the sarcomere length of the pressure treated samples. It is observed that the contraction generated by the pressure treatment is not uniform. We observed areas where the sarcomeres are stretched and in other areas supercontracted.

T.R. Dutson: Were gels produced from the tissue before 105,000 XG centrifugation or were the two fractions combined after centrifugation?
Authors: After centrifugation.

Reviewer VI: In connection with Fig.3, can you please explain relative intensity further? What was the activity of S-fractions for these samples?
Authors: In our case β-Glucuronidase enzyme solution with the highest concentration was used to set the relative intensity scale at 80. Usually quinine sulfate solution is used to calibrate the machine, but we did not use it in our study because the excitation and emission wave lengths of our samples are different from those of quinine. Unfortunately with our limited resources we did not measure the activity of the S-fraction, but our assumption is that it will show a pattern similar to that in Table 1.

P.B. Addis: Does prerigor pressurization affect rancidity of tissue? The disruption of membranes could, if severe, lead to phospholipid oxidation.
Authors: This study is in progress. We observed that there is more free fatty acid (FFA) in the PRP sample than their corresponding control. However, the peroxide values of the PRP samples are less than those of the control. It is too early to speculate what happened exactly.

Reviewer V: Another way to look at the data in Table 1, which I think is easily defendable, is to sum the activities of the "U" and "S" fractions and look at the total activity. If this is done, one finds differences due to treatment that do not appear to be significant. Thus, it may be postulated that the treatment only affects the equilibrium between the two fractions. Please comment.
Authors: There is more than one way of looking at the data. Based on the objective that we have stated for this study we looked at the data from

the distribution point of view (distribution of β-Glucuronidase in the S and U-fractions). We don't see any way of reaching the conclusion that the treatment has affected the equilibrium (we believe you mean distribution) between the two fractions without presenting the data the way we did in Table 1. Dutson et al. (1980) found that electrical stimulation affects the distribution of lysosomal enzymes. Prerigor pressurization effect is similar to electrical stimulation, just more vigorous than it, therefore it is not surprising that it affects the distribution in the manner discussed in this paper.

R.J. Carroll: Since the pH is a major variable in this study, why did you not fix each sample at the pH of the muscle at the time of sampling to minimize possible artifacts?
R.J. Carroll: Have you carried a restrained muscle through the prerigor pressurization treatment? How would this effect sarcomere lengths? Meat tenderness?
Authors: We agree with you that the effect of pH is very important. This study as well as the study you mentioned in your second question are in progress at this time and we hope to report about them in the near future.

P.B. Addis: Are any commercial applications of this technology currently being utilized?
Authors: So far no, but we think we are getting there.

S.H. Cohen: The authors should make note of the range of activity of the CAF vs catheptic enzymes, since the cathepsins are active at postmortem pH, whereas CAF is not.
Authors: CAF was dealt with in a separate study and its manuscript is under preparation.

Reviewer V: There is no evidence to support the statement that, the Z-disc removal is evidence of lysosomal activity.
Authors: A study on CAF from two different muscles (Koohmaraie, Kennick and Elgasim, in progress) shows that the control had more CAF activity, at all postmortem periods investigated, than the PRP samples. Our preliminary results with cathepsin D indicate that the PRP samples have more activity than their corresponding controls, that is immediately after the pressure treatment (≈1½ hr postmortem). Since cathepsin D and CAF are the two enzymes implicated in the Z-disk removal and since the conditions of the pressure treatment are not in favor of the CAF activity, one would tend to lean towards cathepsin D (a lysosomal enzyme) as a possible causative of the Z-disk degradation observed in the study. Also please refer to S.H. Cohen's comment above.

P.B. Addis: Does hyperbaric treatment of the type reported here result in more dissolved gasses (nitrogen and oxygen) in the aqueous phase of the meat?
Authors: Both subjective (consumer panel) and objective (Hunter Color Difference Meter) evaluations indicate that PRP improves the color of the meat. The mechanism(s) by which it does so is not clear. Some possible mechanisms are: 1) pressure treatment induces the expansion of muscle pigments (Marshland, 1944); 2) accelerated lactic acid production or 3) the improvement observed in color is due simply to more oxygen dissolved in the aqueous phase of the meat. We do not know.

Discussion References

Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T_4. Nature. 227,680-685.

Marshland DA. (1944). Mechanism of pigment displacement in unicellular chromatophores. Biol. Bull. 87,252-261.

Additional discussion with reviewers of the paper "Ultrastructure Studies of Pasta. A Review" continued from page 12.

E.A. Davis: Figure 4 shows "water" around starch and membrane residues. In our studies we observe this "water" or "ridge area" forming just as the granule begins to swell. What model system evidence do you mean by "membrane residue"?
Authors: By FF a ridge area of the granule cannot be clearly shown, because etching is not performed. The material around the granule that we indicated as "membrane residue" ("m" in Fig. 4c) exhibits the characteristic features of the material shown at the surface of the granule after a surface-fracture (Fig. 4b).

J.E. Dexter: How did the authors determine cooking time? Why was group B cooked 5 minutes longer than group A? Assuming strand diameter is similar for all samples I would not expect cooking time to vary by more than one minute between samples.
Authors: Cooking time is determined as written in the Appendix. Group B always shows a much longer cooking time than Group A even with the same strand diameter. We think that this is probably due to the starch and protein modifications caused by the heat treatment producing perhaps a lower rate of water penetration into the strand of the spaghetti (Wyland and D'Appolonia, [81]). However, in our opinion, it is difficult to foresee the cooking time of unknown products on the basis of conventional pasta cooking time.

R. Moss: How are protein and starch components identified, particularly in cooked pasta – in many of the Figures the protein appears to have a finer granular structure than the starch? Is this the case?
Authors: In FF cooked samples the protein exhibits always a finer structure than the starch because, under hydrated state, the protein subunits have a diameter at least 3 times smaller than starch spherulites. This is one of the differential features of the two components. Furthermore, the protein matrix shows often inclusions of lipid and other material, never found into the starch, which help in the interpretation of the micrographs.