PE7.13 Activation of Proteolytic Enzymes with Pressure-Heat Treatment to Tenderize Beef 160.00

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***Abstract*—High pressure-heat (P-H) treatment of post-rigor beef muscle has previously been shown to improve tenderness. The approach we have taken, using beef neck muscle (*M. sternomandibularis*), was to investigate the effect of pressure-heat (200 MPa, 60°C, 20 min) compared with controls of heat treatment alone (60°C, 20 min) and raw muscle. Also, in order to investigate whether this improvement in tenderness with P-H was due to the stimulation of proteolytic activity, beef neck muscles were subjected to 200 MPa pressure for 20 min at temperatures of 5, 60 or 80°C. The tenderness was determined following cooking and the degradation of muscle fibre structures was examined using light microscopy.**

The texture of beef neck muscle, as measured by Warner-Bratzler Peak Force, was very tough (~ 10 kg) when cooked from both raw or previously heated (60°C, 20 min), whereas a significant improvement in tenderness was achieved (4-5 kg) when pressure-heat (P-H) treated muscle (200 MPa, 60°C, 20 min) was cooked. Microscopy of whole muscle homogenates following various treatments, showed that there were differences in the appearance of the myofibres, suggesting there had been greater proteolysis in the P-H treated samples.

This finding provides some understanding to the underlying mechanism of the effect of high pressure-heat treatment on the tenderization of beef muscle.

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***Index Terms*—beef, heat, high pressure, light microscopy, tenderisation.**

I. Introduction

THE potential benefits of high pressure processing (HPP) as a tool for increasing the value of meat products was discovered in the 1970's with regard to both improved tenderness and longer shelf life. Commercially however, this technology is used essentially for the extension of shelf-life of processed meat products, as high pressure at low or moderate temperatures reduces the microbial population without the need for further heat or preservatives. Therefore, an opportunity exists to improve the tenderness of lower-valued meat cuts that have higher connective tissue contents and are usually tough.

High pressure (103 MPa, 30-35°C, 1-4 min) was first used to tenderize pre-rigor meat [1] but it was subsequently found that pressure at temperatures below 30°C had no beneficial effect on post-rigor meat [2]. These same authors however, found that by using a combination of heat with high pressure, post-rigor meat could be tenderized at an optimum temperature of 55-60°C.

Ma and Ledward [3] used high pressures of up to 800 MPa at different temperatures (20-70°C) for 20 min with post-rigor beef *M. longissimus dorsi* and found that there was no tenderizing effect but a hardening effect when high pressure was applied at temperatures below 60°C. A significant decrease in hardness was only observed at 60-70°C and 200 MPa.

Muscle tissue contains various proteolytic enzymes that are believed to play an important role in the tenderization of meat post-mortem [4]. Lysosomes store a variety of enzymes including lipases and proteases, especially cathepsins B and L that can be released into the cytosol leading to tissue disruption. It has been shown that HPP can lead to the disruption of lysosomes [5, 6], therefore leading to the release of catheptic and other activities. Whilst this may lead to greater interaction between the enzyme and its substrate, higher pressures may result in partial denaturation of cathepsin and therefore lessen its effect.

The purpose of this work was to investigate whether proteolytic activity is stimulated during high pressure and heat treatment of beef muscle that results in improved tenderness. By understanding the mechanism of action, it should be possible to control and optimize the treatment conditions for production of tender meat.

II. Materials and Methods

A. Sample collection

Pre-rigor beef neck muscle (*M. sternomandibularis*) was obtained at slaughter at a local abattoir. Within an hour,

each muscle was tightly wrapped with GladWrap® followed by packing tape and kept for approximately 18h at 15°C and then chilled to 5°C. This procedure minimized the possibility of cold-shortening and also ensured that the muscles were in the normal pH range for post-mortem beef (approximately pH 5.50 to 5.80).

B. High pressure processing

Pressure treatments were performed using an 850 Mini FoodLab 0.3L high pressure vessel (Stansted Fluid Power Ltd, Stansted, UK) with temperature control. The compression fluid used in the sample chamber was 30% propylene glycol in water (v/v).

On the day of pressure treatment, muscles were unwrapped and cut into uniform lengths and cross-sections, approximately 150 x 35 x 35 mm with fibres parallel to the long axis. All samples were individually sealed in vacuum bags and maintained at 5°C until pressure treatment. Samples were pressure treated at 200 MPa for 20 minutes at 5, 60 or 80°C. Following release of pressure, all samples were cooled in an ice slurry for 20 min and stored at 5°C until required for analysis. The inherent ramp rate was 20 MPa/sec so that the time to reach 200 MPa was approximately 10 seconds. A decompression procedure of 'open' 5 sec, 'closed' 2 sec over a period of 45 sec was used.

Heat control samples (0.1 MPa, no pressure treatment) were heated in a water bath at 60°C for 20 minutes.

C. Warner-Bratzler (WB) shear force

Immediately following treatments, samples were cooked at 80°C for 60 minutes (in a waterbath), chilled, and stored overnight at 4°C. On the following day, the cooked samples were cut into six sub-samples for shear force measurement; the details of sample thickness, shape and fibre orientation are described by Bouton et al [7, 8]. Warner-Bratzler shear force measurements were made on a Lloyd Instruments LRX Materials Testing Machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire UK). The force required to shear through the clamped sub-sample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured as shear force. The mean for the sub-samples was recorded.

D. Muscle homogenate preparation

Whole muscle homogenates were prepared using the method of Busch et al [9]. A 20 g sub-sample of minced muscle was homogenized under standardized conditions for 30 seconds in a Waring blender in 200 mL of extraction buffer (50 mM Tris-HCl, pH 7.0, 100 mM KCl, 5 mM EDTA).

E. Light microscopy

To determine if there were any visual differences resulting from P-H treatment, the whole muscle homogenates of each of the muscle tissue preparations were viewed by light microscopy. Samples were viewed with a Nikon Eclipse E200 using phase contrast with 400x magnification. Images were obtained with a Tucsen 5.0 MP camera (Tucsen Digital Imaging Technology, China) and analyzed using the accompanying image analysis software (TS mini) to determine the length and width of the muscle fibre fragments using a method developed for myofibrils [10, 11].

III. RESULTS AND DISCUSSION

Pressure-heat treatment had a marked tenderizing effect on beef neck muscle when subsequently cooked at 80°C for 1 hour with Warner-Bratzler peak force being reduced (Fig. 1). The cooked but otherwise untreated beef neck muscles were very tough (mean force of about 10 kg), as were the 60°C heated controls. This P-H treatment not only reduced peak force values of beef neck muscle to less than 5 kg but resulted in the production of a very consistent product; the standard deviation of the mean value was very small.

It can also be seen from Figure 1 that P-H treatment had a very large effect on initial yield (IY) thereby accounting for most of the reduction in peak force. This suggests that myofibrillar structures were mainly affected and contributed largely to the improvement in tenderness. It can also be seen that PF-IY increased with P-H treatment suggesting that connective tissue structures may have strengthened.

Light microscopy of whole muscle homogenates (Fig. 2) showed that there were differences in the appearance of the myofibres dependent upon treatment. For raw muscle homogenates (Fig. 2a), myofibres were generally short in length, having a mean fibre length of 296±35.3 μm and a diameter of 48±2.7 μm. Further, myofibrils of varying length can be seen protruding from the broken ends of the myofibres indicating that the break had not been clean cut.

However, compared with that from raw muscle, homogenates of muscle heated at (60°C) had significantly longer fibres (624±147.4 μm) and were thinner (38±5.0 μm), and it was also evident that the myofibres were more aggregated and clumped (Fig. 2b). The reduction in fibre widths may have resulted from lateral contraction caused by the heat denaturation of the myofibrillar proteins that has been reported when muscle is heated above 40°C [12].

The appearance of homogenates prepared from P-H

treated muscle was very different from the raw and heat-treated samples. It was evident that fibres had been degraded into smaller lengths (104±12.4 μm) with pressure-heat treatment (Fig. 2c). Also, fibre widths (46±2.8 μm) were not markedly changed (compared with the raw muscle). However, the broken ends of the myofibres were sharp and at 90° to the fibre length, giving the myofibres a block-like appearance. As the myofibres had been degraded into smaller lengths by the P-H treatment, this is further support for the argument that the tenderizing effect of the process had been aided by the action of proteases, possibly cathepsins.

Further evidence to support the case for stimulation of proteolytic activity with pressure comes from the effect of temperature at which the pressure is applied. There is evidence that whilst cathepsins are very active at temperatures of 50-60°C, they are denatured once temperatures reach 70-80°C [13]. We hypothesized that the tenderizing effect at 80°C would be less than that at 60°C because of the partial inactivation of the proteases by heat. At 5°C, we would expect little or no change in tenderness because, although the enzymes may be released from the pressure-damaged lysosomes into the cytosol, their activities against the substrates would be very low at that temperature.

As in the previous experiments, we found in this investigation that cooked raw muscles had a mean peak force of 9.69 kg and this was significantly reduced to 4.92 kg by pressure treatment at 60°C (N=3). Pressure treatment at 5°C did not result in a different tenderness from the Control (8.76 kg). However, pressure treatment at 80°C still resulted in tenderization but the effect was not as great as that observed at 60°C (5.23 kg). Although this work suggests that the high temperature has had a slight effect on reducing tenderness through inhibition of proteolytic activity, it would seem that the high temperature was not achieved rapidly enough, still allowing significant activity to occur.

Despite this uncertainty, observations of the length and width of myofibres in the homogenized samples suggest that proteolytic activity was lower at the higher temperature (Table 1). The micrographs of raw and high pressure treated muscles at 5, 60 and 80°C shown in Fig. 3, used a lower magnification (100x) compared with the previous images, thus allowing an easier overview of the meat fibres. In Fig. 3a, the fibres of the raw meat control can be seen. There is a large variation in fibre size as well as evidence of a large amount of myofibres being extracted into the medium (small particles).

For the muscle treated with high pressure at the low temperature of 5°C (Fig. 3b), the fibres seem to be in general longer and they are also more swollen than those present in the raw preparation. It is assumed that the cathepsins would have not been very active at this low temperature. However, some swelling is evident, probably as a result of solubilization of the actomyosin, which is favored at these low temperatures with pressure treatment. Solubilization usually begins with the swelling of the fibres [14].

The high pressure treated muscle at 60°C can be seen in Fig. 3c and in this micrograph it is clearly evident that fibres have been degraded to a smaller length. This is indicative of a higher activity of the cathepsins at this temperature as has been shown by Kurth [13] and others. Kurth also showed that cathepsin activity was reduced when high pressure was performed at 80°C and in this work (Fig. 3d), it can be seen that the myofibres are longer than those that were pressure treated at 60°C. It can also be seen that for the high pressure treated muscle at 80°C, the fibres are again thinner, especially in comparison with those high pressure treated at 5°C (Fig. 3b). That may be due to the lateral contraction of the fibre on heat denaturation up to such a high temperature of 80°C.

IV. Conclusion

It was demonstrated that high pressure, in combination with heat, resulted in a large improvement in the tenderness of beef neck muscle, which is generally regarded as a tough muscle. Light microscopy and resultant fibre length and width measurements suggests a role for proteolysis in the improved tenderization of muscle when subjected to high pressure-heat treatment (200 MPa, 60°C, 20 min).

Acknowledgement

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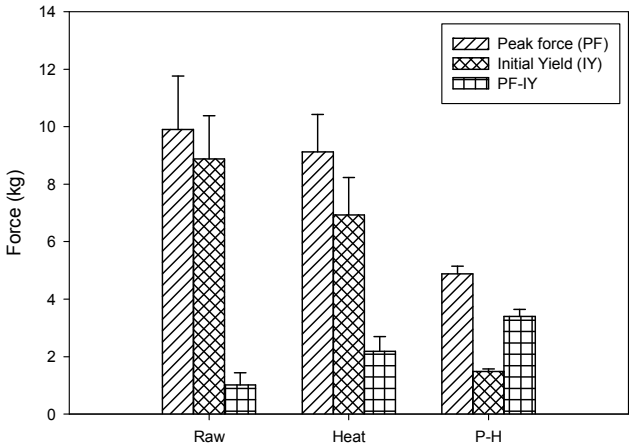
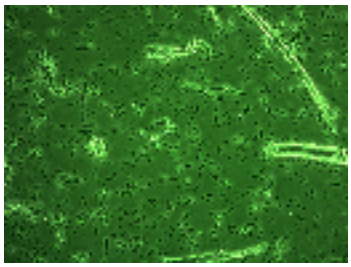


Fig. 1: Effect of heat and pressure-heat treatment on Warner-Bratzler shear forces of beef neck muscles (mean ± SD, N=4).

(a)



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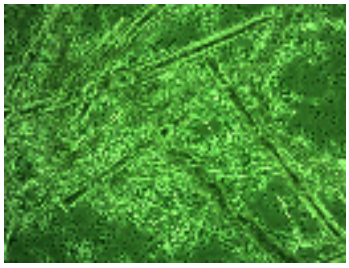

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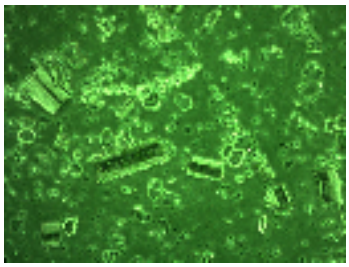
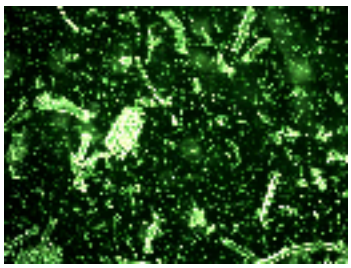


Fig. 2: Muscle fibre homogenates of (a) raw control, (b) 60°C heat treated, and (c) pressure-heat treated (200 MPa, 60°C, 20 min).

Table 1: Myofibre lengths and widths (mean ± SD) obtained from light micrographs of whole muscle homogenates from high pressure (200 MPa, 20 min) treated muscles at different temperatures.

Treatment	*Fibre Length (µm)*	*Fibre Width (µm)*
Raw	159 ± 44.3	40 ± 4.8
5°C	297 ± 105.8	60 ± 9.3
60°C	133 ± 41.0	37 ± 4.6
80°C	310 ± 99.7	34 ± 1.8

a)



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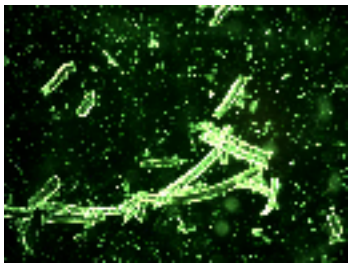

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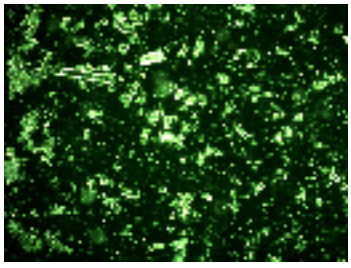

(d)

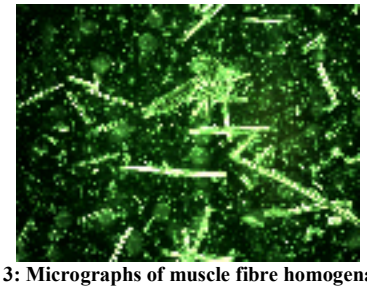

Fig. 3: Micrographs of muscle fibre homogenates of (a) raw muscle, (b) 200 MPa, 5°C, 20 min, (c) 200 MPa, 60°C, 20 min, and (d) 200 MPa, 80°C, 20 min.