COMBINED HIGH PRESSURE AND TEMPERATURE EFFECTS ON THE TEXTURAL QUALITY OF POST-RIGOR BEEF

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***Abstract*—The effects of high pressure on the texture of post-rigor beef muscle has been shown to differ depending on the temperature at which the pressure is applied; low temperature having no effect on tenderization, whereas high temperature has a marked effect on reducing the toughness of beef muscle cuts. We subjected post-rigor beef neck muscle (*M. sternomandibularis*) to different pressures and times (100 MPa for 2min, 200 MPa for 20 min) at a range of temperatures (5-80°C) and measured the texture of treated samples and determined the degradation of muscle fibres using light microscopy. The texture of beef neck muscle, as measured by Warner-Bratzler peak force, was tough (~80 N) when cooked from raw. Pressure treatment (100, 200 MPa) at low temperatures (<40°C) did not improve the tenderness of beef neck muscle, whereas a significant reduction in toughness was achieved (20-30 N) when pressure (200 MPa, 20 min) was combined with heat (40-80°C). Microscopy of whole muscle homogenates showed that there were differences in the appearance and the length of the myofibres. A strong correlation between peak force and fibre length was shown. This finding supports the suggestions that muscle proteins are affected differently when subjected to pressure or heat, and provides some understanding to the mechanisms of solubilization and aggregation of muscle structures after pressure treatment at low or high temperatures, respectively.**

***Index Terms*—beef, high pressure, light microscopy, temperature, texture.**

I. INTRODUCTION

The application of high hydrostatic pressure provides important opportunities in the processing of muscle-based food products. High pressure processing (HPP) is currently being used commercially for extension of shelf-life of packaged, sliced, cooked meats through its ability to reduce microbial populations. Research has also shown the effectiveness of high pressure treatments for the modification of textural and functional properties of meat, poultry and fish (Macfarlane, 1985; Cheftel and Culioli, 1997).

Post-mortem meat tenderization is generally assumed to result from softening of the myofibrillar and connective tissue proteins by endopeptidases, such as cathepsins and calpains and high pressure may also have a favourable effect on meat tenderness (Macfarlane, 1973; Jung, de Lamballerie-Anton and Ghoul, 2000a; Ichinoseki, Nishiumi and Suzuki, 2006; Sikes, Tornberg and Tume, 2010), but often conflicting results are obtained (Cheftel and Culioli, 1997; Jung, de Lamballerie-Anton, Taylor and Ghoul, 2000d; Cofrades, Banon, Carballo and Jimenez-Colmenero, 2003). Many studies indicate that high pressure can tenderize meat when applied pre-rigor (Macfarlane, 1973; Kennick, Elgasim, Holmes and Meyer, 1980) but has no marked effect on post-rigor meat at low temperature (Bouton, Ford, Harris, Macfarlane and O'Shea, 1977; Jung, de Lamballerie-Anton and Ghoul, 2000b; Jung, Ghoul and de Lamballerie-Anton, 2000c; Ma and Ledward, 2004).

High pressure treatment at different temperatures will induce different effects on meat texture since the weak linkages stabilizing the secondary, tertiary and quaternary structures of a protein respond differently to heat and pressure (Galazka and Ledward, 1998). Bouton et al. (1977) found that a combination of pressure and heat treatment of post-rigor muscle had a beneficial effect on the shear-force resistance of cooked meat but treatment at low temperatures was ineffective. Jung et al. (2000a & 2000b) also found this effect at low temperature but they demonstrated myofibrillar disorganization with pressures between 100 and 600 MPa.

Ma and Ledward (2004) studied the effects of high pressure (200-800 MPa) at a range of temperatures (20-70°C) for 20 min on the texture of post-rigor beef LD muscle. At temperatures below 60°C, there was no tenderizing effect but a hardening effect occurred. At temperatures of 60°C and 70°C, pressures of 200 MPa caused significant decreases in hardness.

It was the aim of this study to investigate the effects of high pressure at a range of temperatures on the textural quality and degradation of muscle fibres of beef muscle. These findings will assist in building an understanding of the mechanism of muscle protein modifications of beef muscle by high pressure and temperature combinations.

II. MATERIALS AND METHODS

A. Sampling and preparation

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 18 h 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. Preheating protocols for samples were determined prior to treatments in order to ensure that the samples reached the designated temperatures during the pressure treatment. Appropriate heat control samples (0.1 MPa, no pressure treatment) were heated in a water bath for corresponding times and temperatures. Samples were pressure treated at 100 MPa for 2 min at 5, 10, 20 or 40°C, and at 200 MPa for 20 min at 5, 20, 40, 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/s so that the time to reach 200 MPa was approximately 10 s. A decompression procedure of 'open' 5 s, 'closed' 2 s over a period of 45 s was used.

C. Warner-Bratzler (WB) shear force

Immediately following treatments, samples were cooked at 80°C for 60 min (in a waterbath), chilled, and stored overnight at 4°C. Each cooked sample was then cut into six sub-samples for shear force measurement (Bouton, Harris and Shorthose, 1971; Bouton and Harris, 1972). All measurements were made on a Lloyd Instruments LRX Materials Testing Machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire UK) as described by Sikes et al. (2010). The mean peak shear values for the six sub-samples were recorded.

D. Muscle homogenate preparation

Whole muscle homogenates were prepared using the method of Busch, Stromer, Goll and Suzuki (1972). A 10 g sub-sample of minced muscle was homogenized under standardized conditions for 30 s in a Waring blender in 100 mL of extraction buffer (50 mM Tris-HCl, 0.1 M KCl, 5 mM EDTA, pH 7).

E. Light microscopy for fibre length determination

Samples of muscle homogenates were viewed with a light microscope (Nikon Eclipse E200) using phase contrast with standardized magnification (40x). 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 of the muscle fibre fragments using a method developed for myofibrils (Olsson and Tornberg, 1992; Devine, Wahlgren and Tornberg, 1999).

III. RESULTS AND DISCUSSION

As shown previously (Bouton et al., 1977; Ma and Ledward 2004; Sikes et al., 2010), pressure-heat treatment (200 MPa, >40°C, 20 min) had a marked tenderizing effect on beef muscle when subsequently cooked at 80°C for 1 h, with WB peak force being reduced (P<0.001) from 80 N for the untreated sample to 31 N for P-H treatment (200 MPa, 60°C, 20 min) (Figure 1). When using 100 MPa for 2 min at low temperatures (5-40°C), the WB peak shear force values were lower (P>0.05) than those of the untreated control samples but there was a trend towards increasing toughness with increasing temperatures (Figure 1). Similarly, using a higher pressure (200 MPa) for a longer time (20 min) at low temperature (5°C) had no effect on tenderization of beef neck muscle. These results parallel those of Jung et al. (2000b) in that there was no significant tenderization effect on beef muscle with pressure treatment at low temperature, although they reported an increase in toughness with pressure treatment at low temperature (10°C). This difference may result from the higher pressure conditions used in their experiments (130, 520 MPa). However, with increasing temperatures (20-80°C) combined with 200 MPa and 20 min, the WB shear force values decreased significantly (P<0.005) compared to the untreated samples, with the greatest effect being seen at 60 and 80°C (Figure 1).

In the current study, the degradation of the myofibrillar structures was followed by measuring the fibre length of muscle

homogenates using light microscopy (Figure 2). Homogenates of raw muscle showed that many of the myofibres had been separated into individual fibres which had a mean fragmented fibre length of 305 μm. However, homogenates of heated muscle (60°C) had longer fibre fragments (437 μm) and there was evidence that the myofibres were more aggregated. Homogenates prepared from pressure-treated muscle (200 MPa) tended to have shorter fibre lengths compared to the raw control and the heated samples, but this was most evident at 60 and 80°C where the lengths were significantly smaller ($P<0.01$). Unlike the samples treated at 200 MPa, there was little difference in fibre lengths of the samples treated at 100 MPa at the lower end of the temperature range (Figure 2).

As can be seen in Figures 1 and 2, the application of pressure at higher temperatures (>40°C) has a dramatic effect on both the texture of the beef muscle (WB shear force) and the length of the muscle fibre fragments, resulting in a strong linear relationship ($R^2 = 0.73$) (Figure 3). We believe that the pressure-heat process leads to a complex series of events, including membrane disruption and the release of proteolytic enzymes which for maximal activity require high temperatures (Kurth, 1986) and altered protein structures. At lower temperatures, not only is there less proteolysis but the structural proteins are affected differently by pressure where there is greater solubilization and less denaturation, aggregation and strengthening. Thus according to our theory (Sikes et al., 2010), the potential for crack propagation leading to fibre fragmentation and therefore more tender meat will not be as great compared with pressure treatment at higher temperatures, where proteolysis, protein denaturation, aggregation and strengthening are higher, thus enabling shear fracturing of a more brittle structure.

IV. CONCLUSION

High pressure processing (up to 200 MPa), as an innovative technology, has been used in combination with low and high temperatures (5-80°C) to understand and make use of changes in protein structures of red meat. The results suggest that different mechanisms of protein modification occur under high pressure depending on the temperature applied. At low temperatures, solubilization of myofibrillar proteins occurs, whereas at higher temperatures, aggregation of muscle proteins causes a strengthening of structures. It has also been found that there is an impact on endogenous enzymes under pressure at high temperatures.

The application of high pressure processing combined with heat has shown a tenderizing effect on beef neck muscle. This large improvement in tenderness for commercially available low-valued meat cuts provide opportunities for the meat industry to provide healthy, convenient and economical alternative meat products with excellent functional properties and value for the consumer.

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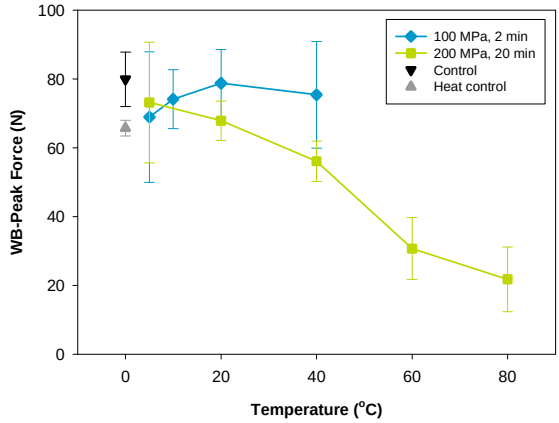

Figure 1: Effect of temperature at different pressures on WB peak force values of beef neck muscles (mean±SD, n=4).

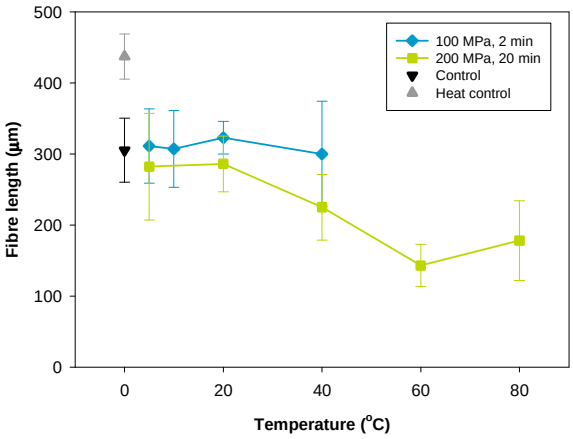

Figure 2: Effect of temperature at different pressures on the fibre length of muscle fibre homogenates of beef neck muscles (mean±SD, n=4).

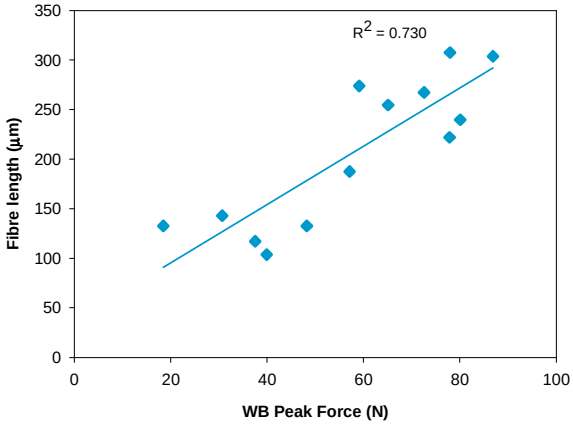

Figure 3: The relationship between WB peak force and fibre length of pressure-temperature-treated beef neck muscle.