Glycogen phosphorylase: a possible mechanism for the pH increase in High Pressure processed bull meat

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Abstract – This research shows that the denaturation of glycogen phosphorylase by high pressure processing (HPP) provides a mechanism for the higher pH of the resulting meat. Four bulls were slaughtered, the strip loins hot-boned and subjected to pre-*rigour* HPP at 175MPa within one hour. This resulted in a three-fold decrease in shear force and an increase in pH at 24 hours post-*mortem* when compared to control steaks. Comparison of protein profiles showed that HPP caused the movement of glycogen phosphorylase from the sarcoplasm to the myofibrillar fraction. This suggests that HPP has increased glycolysis causing a rapid decline in pH, conditions which have led to denaturation of glycogen phosphorylase and the subsequent higher pH at 24 hours.

Key Words – denaturation, sarcoplasm, tenderness,

I. INTRODUCTION

High pressure processing (HPP) of pre-*rigour* meat from bulls at pressures below 200MPa resulted in steaks of low shear force and acceptable colour without the need for ageing (Bickerstaffe et al, 2015; Morton et al, 2017). These were also judged as of significantly higher eating quality by consumers (Morton et al, 2017). This process was associated with a significant increase in the ultimate pH of the meat. The typical decline in pH as muscle turns into meat is largely the result of the continuation of anaerobic glycolysis post-mortem leading to an accumulation of lactic acid. The purpose of this research was to examine the proteins from HPP-treated meat and determine whether there were changes associated with this pH increase.

II. MATERIALS AND METHODS

Four bulls were slaughtered normally and the hot-boned at meat processing plant. The strip loins (*longissimus thoracis*) were removed within 50 minutes of slaughter and two portions, treated (T) and control (C), were vacuum packed. T was subjected to HPP at 175MPa for 3 minutes and then both T and C were sliced into 25mm steaks, held chilled at –1°C for 1 day and then frozen at -40°C. Subsequently 15mm cores were removed, diced and homogenised for protein analysis. The samples were separated into myofibrillar (MF) and sarcoplasmic fractions (SF) (Culler et al) These were electrophoresed on 3-8% Tris-Acetate gels (NuPAGE®) and stained with Coomassie blue. Bands where there were differences between treatments were trysin digested, analysed by MS/MS and the peptides identified using the NCBInr database. Subsequently the glycogen phosphorylase was immuno-detected by Western blotting using a monoclonal mouse Anti-PYGM (ab88078).

III. RESULTS AND DISCUSSION

The HPP treatment lead to a large decrease in shear force from 18.18 to 5.34kgF and an increase in pH from 5.62 to 5.85. Comparison of the sarcoplasmic protein profiles between control and treated meat revealed a significant decrease in a band at around 90kDa (Figure 1). This corresponded to an increase in the intensity of a band with the same molecular weight in the myofibrillar fraction. Glycogen phosphorylase was identified in this band by mass spectrometry. Western blotting was used to confirm that the changes in intensity of this band were a result of movement of glycogen phosphorylase from the sarcoplasmic proteins to the myofibrillar fraction (Figure 1).

The movement of glycogen phosphorylase from the sarcoplasm into the myofibrillar fraction has been reported to be an indicator of denaturation (Warner et al, 1997). The loss of active glycogen phosphorylase would slow or prevent the breakdown of glycogen and subsequent glycolysis providing a mechanism for the high ultimate pH. A similar denaturing effect on glycogen phosphorylase has been reported in pork *longissimus* held at 40°C for 6 hours (Liu et al, 2014). This was associated with a rapid decline in pH.

Pre-*rigour* HPP of beef *longissimus* caused a dramatic increase in the rate glycolysis resulting in a decline to pH 5.81 by one hour post-*mortem* (Kennick et al., 1980). At this time the muscles are still at temperatures between 35 and 40°C, conditions that should lead to the denaturation of glycogen phosphorylase. Thus the effect of pre-rigour HPP is a sharp drop in pH within the first hour followed by very limited further glycolysis and meat with a higher ultimate pH.

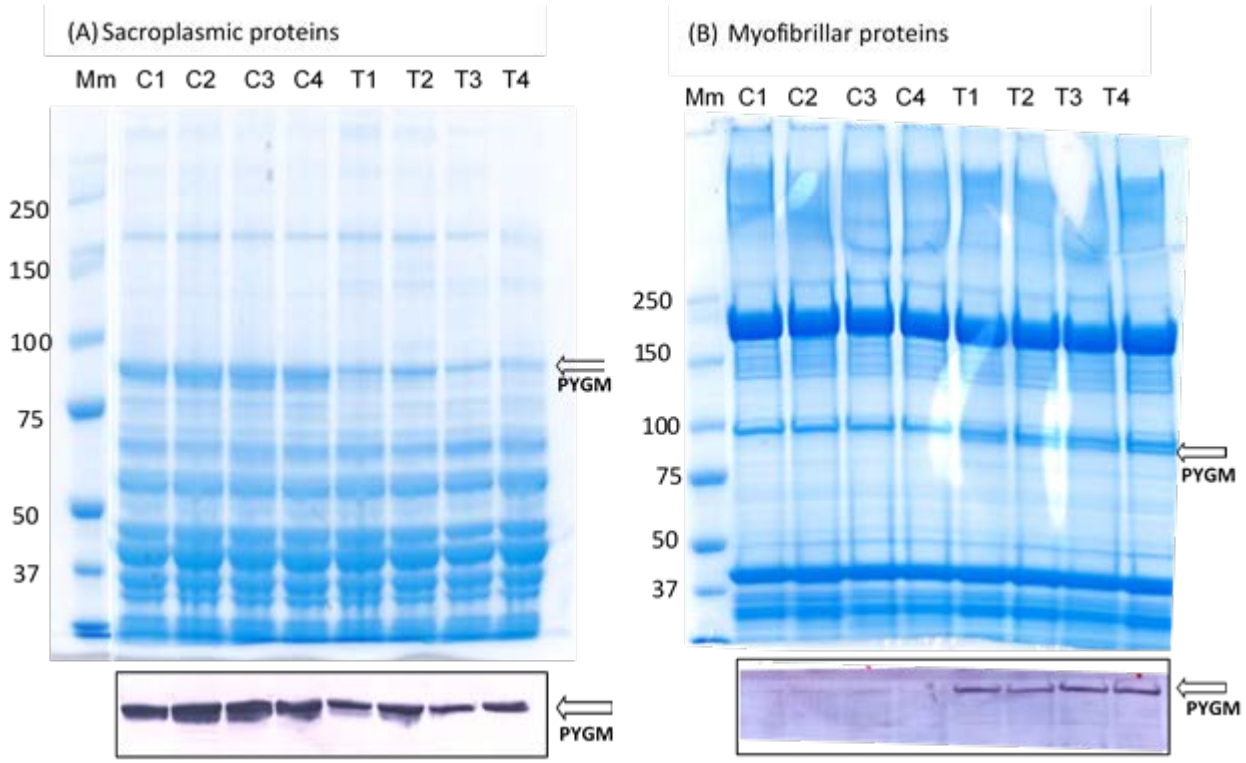

Figure 1. Protein profiles and immune-detection of sarcoplasmic (A) and myofibrillar (B) fractions from bull *longissimus*: four controls (non-treated, C1-C4)) with its respective four HPP treated (T1-T4) samples. (A) 19.5 µg sacroplasmic protein profiles and immuno-detection of glycogen phosphorylase (PYGM). (B) 25 µg myofibrillar proteins profiles and immuno-detection of PYGM. Protein profiles were carried out using NuPAGE® Novex® 3-8% Tris Acetate gel with molecular marker (Mm) (kDa, Precision Plus Protein™ Standards, Bio-Rad). Immuno-detection was carried out using anti-phosphorylase (1:2000, glycogen, muscle, mouse monoclonal ab88078, abcam®). Arrows indicate glycogen phosphatase which was significantly reduced after HPP treatment.

IV. CONCLUSION

Pre-*rigour* HPP of meat improves the meat quality and eating experience of bull *longissimus*. These changes are associated with the denaturation of glycogen phosphorylase and a rise in the ultimate pH of the meat.

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