COMBINED HIGH PRESSURE AND THERMAL TREATMEAT ON PROTEASE ACTIVETIES IN BEEF MUSCLE

H. J. Ma[1], G. H. Zhou[2], X. L. Xu[2], Zh. J. Song[1], and D. A. Ledward[3]

[1]*School of Food, Henan Institute of Science and Technology, 453003 Xinxiang, Henan, China*
[2]*College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China*
[3]*School of Food Biosciences,University of Reading, P.O.Box 226,Whiteknights, Reading RG6 6AP, UK*

Key Words: high pressure; thermal treatment; protease activities

Introduction

High pressure treatment can tenderize meat when applied pre-rigor (Macfarlane, 1973), but has no marked effect on post-rigor meat at low temperature (Jung et al, 2000). However, it is difficult for pre-rigor meat to be treated by high pressure in practice but high pressure technology may be applied with heat treatment on post-rigor meat to induce tenderization (Ma and Ledward, 2004).

Several studies on the effect of high pressure combined with heat treatment on the texture of post-rigor meat have been done, showing that high pressure treatment combined with temperature below 30□ has no marked effect on tenderness of post-rigor muscle whereas above 30□ tenderization occurs (Beilken et al, 1990). Our previous work also showed that a pressure of 200 MPa at 60 and 70℃ can lead to significant increases in tenderness of post-rigor beef muscle, which was attributed to enzymic activity in beef muscle due to the combination of high pressure, which acts instantaneously with slowly rising temperature (Ma and Ledward, 2004).

Numerous authors have studied the effect of high pressure treatment on the proteases in rabbit or beef (Ohmori et al, 1991; Jung et al, 2000; Homma, 1994, 1995), showing that pressure treatment increased the release of lysosomal enzymes into cytosol and improved the enzymes' relative activities at lower pressure, the total activities of calpains can also be increased by pressure treatment at less 200 MPa.

Although the effects of high pressure on the proteolytic enzymes in muscle have been studied, previous studies are limited to ambient temperature. In this paper, the protease activities at pH 3.0, 5.0 and 7.5 in beef muscle after high pressure combined with temperature treatments have been applied, the objective was to further understand the relationship between protease activities and muscle texture.

Materials and methods

Preparation of samples: The beef Longissimus dorsi was obtained from a 2 year old crossbreed (Chinese yellow cattle ×Limusin) and its treatment was as described by Ma and Ledward (2004).

High pressure and heat treatment: Samples were pressurized at 100 to 600MPa at room temperature and 200MPa and 600MPa at 40 and 60℃ for 20 min as described by Ma and Ledward (2004). Some samples were heated in water baths at 40 or 60℃ for 30 min.

Preparation of the beef extracts: The pressurized beef blocks were chilled in an ice bath and then homogenized in 3 volumes of chilled distilled water with a Waring Blender (Stomacher 400, France). Homogenization was performed three times for 30 sec at 10,000 rpm, with a 30-sec cooling interval between each burst. After standing for 1 hr in an ice bath, the homogenates were centrifuged at 12,000×g for 20 min and the supernatants obtained were filtered.

Protease activities assay: The protease activities of the beef extracts were assayed as described by Ohmori et al (1991) with a slight modification. The activity was determined by incubating the reaction mixture at 37℃ for 4 hr in 50mM buffer (sodium acetate-HCl for pH 3.0, sodium citrate for pH 7.0 and Tris-HCl for pH7.5), 5mg/ml bovine erythrocyte, 10mM 2-mercaptoethanol, 1mM NaN3, 0.5ml beef extract (2mg protein/ml) in a final volume of 3ml. The reaction was stopped by adding an equal volume of 10% trichloroacetic acid (TCA). The mixtures were then centrifuged at 5,000 g for 15min, and the absorbance of the supernatant measured at 280 nm. The protease activities are expressed as the increase in A280 after bovine erythrocyte digestion at pH 3.0, 7.0 and 7.5, respectively. One unit of activity is defined as the amount of enzyme protein which increased the A280 by1.0

Results and discussion

Changes of protease activity of the beef extracts from the pressurized muscle are presented in Fig 1. There is no obvious change in protease activity in the samples treated at 100 MPa at ambient temperature ($P > 0.05$), but above 100 MPa, the activities of proteases decreased rapidly with increasing pressure up to 400 MPa, after which there were no significant changes on further increases in pressure. This result is similar to that of Ohmori et al (1991) who found that when beef round was subjected to pressure at 1000-5000 atm, the activities of acid

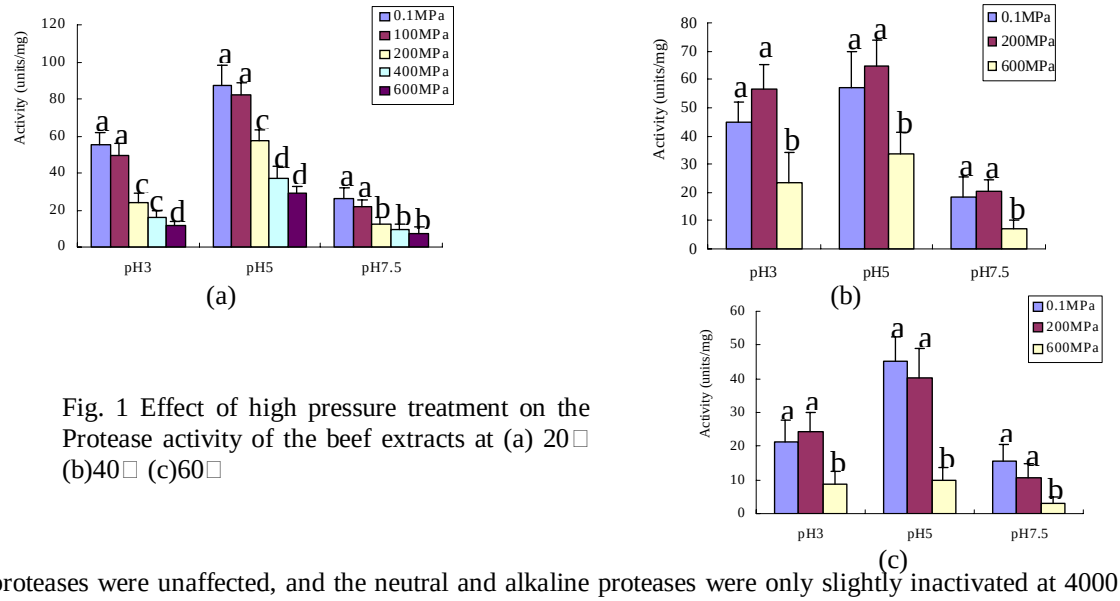

Fig. 1 Effect of high pressure treatment on the Protease activity of the beef extracts at (a) 20□ (b)40□ (c)60□

proteases were unaffected, and the neutral and alkaline proteases were only slightly inactivated at 4000 atm or higher.

Although the activities of protease were still measurable after pressure treatment at 400 MPa and higher, they were very low, especially at pH 3 and 7.5. Homma et al (1994, 1995) reported that the activity of calpain decreased rapidly when rabbit muscle was subjected to pressures above 100 MPa and was almost completely inactivated at 300 MPa. Acid phosphatase activity increased with increasing pressure when applied to beef muscle up to 500 MPa. Activity of cathepsin B, D, L increased up to 400 MPa, and then tended to decrease at 500 MPa. Cathepsin H and aminopeptidase B decreased with increasing pressure. Numerous authors have shown that moderate pressure (about 200 MPa) treatment denatured some sarcoplasmic proteins and inactivated proteases. At the same time, pressure treatment also leads to destruction of the lysosomal membrane and subsequent leakage of more protease(Elgasim et al, 1982) and thus enhanced the relatively activity (Ohmori et al 1991). But with further increasing pressure, more and more proteases are inactivated and until complete loss of activity occurs.

On heat treatment at 40℃ at ambient pressure it is seen from Fig 1 that there is a slight decrease in protease activities, although the release of enzymes from lysosomes will be increased (Shiono et al, 1983), enzymes inactivation by heating may dominant over the increasing release into the cytosol. 200 MPa treatment at 40℃ causing no significant change but 600 MPa induced marked decreases in protease activities which has been attributed to denaturation (Homma et al, 1994). On heating at 60℃, most enzymes were inactivated except some proteases, e.g. dipeptidase □、□ which have higher optimum temperatures (45～55℃ and 65℃, respectively; Sentandreu et al, 2002). Pressure treatment at 200 MPa and 60℃ caused no significant changes in protease activities but treatment at 600 MPa inactivated almost all proteases.

Conclusions

The protease activities decreased with increasing pressure at ambient temperature, but when pressure was applied at 40 or 60℃, the protease activities were not affected in beef muscle up to 200 MPa, which may result in tenderization of meat.

References

1. Beilken, S. L., Macfarlane, J. J. and Jones, P. N. (1990). Effect of high pressure during heat treatment on the warner-bratzler shear force values of selected beef muscles. Journal of Food Science,55, 15-18.
2. Homma, N., Ikeuchi, Y. and Suzuki, A. (1994). Effects of high pressure treatment on the proteolytic enzymes in meat. Meat Science, , 38, (2), 219-228
3. Homma, N., Ikeuchi, Y. and Suzuki, A. (1995). Levels of calpain and calpastatin in meat subjected to high pressure. Meat Science, 41, (3), 251-260
4. Jung, S., Ghoul, M. and De Lamballerie-Anton, M. (2000). Changes in lysosomal enzyme activities and shear values of high pressure treated meat during ageing. Meat science, 2000, 56, 239-246.
5. Ma, Hanjun & Ledward, D. A. (2004). High pressure/thermal treatment effects on the texture of beef muscle. Meat Science, 68, pp347-355
6. Macfarlane, J. J.. Pre-rigor pressurization of muscle(1973). Effects on pH, shear value and taste panel assessment. [J]. Journal of Food Science, 38, 294-298.
7. Ohmori, T., Shigehisa, T., Taji, S. and Hayashi, R.(1991). Effect of high pressure on the protease activities in meat. Agric. Biol. Chem., 55, 2, 357-361