KINETICS FOR DENATURATION AND REDUCTION OF PORCINE METMYOGLOBIN UNDER HIGH HYDROSRATIC PRESSURE

G.P. Hong*, K.B. Shim, M.J. Choi, S.H. Ko, E.J. Seo, and S.G. Min

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143701, Korea, E-mail : genpro@konkuk.ac.kr

Key Words: Metmyoglobin, reduction, denaturation, pressure, kinetics

Introduction

High pressure processing is being applied in food industry since a decade. In meat application, high pressure can improve water-holding capacity and texture, whilst pressure-induced meat discoloration is the most concern in processor. In general, meat discoloration through pressure processing may result from a whitening effect in the range 200-350 MPa, due to globin denaturation and/or to heme displacement or release, and oxidation of ferrous myoglobin (Mb) to ferric metmyoglobin (metMb), at or above 400 MPa (Carlez et al., 1995). On the contrary, Cheah and Ledward (1997) observed that, at moderately low pressure, approximately 100 MPa, meat appeared to bloom more readily after pressurisation and retain its red colour longer. Jung et al. (2003) also reported an increase in redness with increasing pressure until around 350 MPa. They concluded that enzymatic system implicated in reduction of metMb could be activated and lead to the decrease of metMb for moderate pressure, whilst the enzymatic system and the reactions involved in the formation of metMb could be disturbed by changes in the enzymatic system itself or the environment of the enzymes at higher pressure.

In our laboratory, changes in meat colour after pressure treatment were determined, however, the results were some contradictory depending upon applied pressure conditions such as holding time and temperature (Hong et al., 2005; Hong et al., 2006). In consequence, we made a hypothesis that reaction rate of Mb denaturation is more rapid than that of metMb oxidation at below a specific pressure level and this phenomena is reversed above this pressure range. Therefore this study was aimed to compare the reaction rates for Mb denaturation and for metMb oxidation (or reduction) after pressurisation.

Materials and Methods

Porcine *m. longissimus dorsi* was obtained at 24 h post mortem from local abattoir. Myoglobin extraction was conducted according to Osborn et al. (2003) with some modifications. Meat was trimmed all visible fat and connective tissue and ground three times using 8 mm plate. Meat was added into 40 mM Na/K phosphate buffer (pH 6.8) as a weigh ratio of 2:1 and stored overnight at 2ºC for myoglobin extraction. Meat extracts was centrifuged at 5,000 rpm for 30 min at 2ºC and the supernatant was filtered through Whatman No. 1 filter paper. Approximately 5 mL of sample was filled into polyethylene pouch and stored at 2ºC prior to pressurisation. After pressurisation, sample was centrifuged at 5,000 rpm for 10 min at 2ºC and diluted the same volume of 40 mM Na/K phosphate buffer (pH 6.8). Sample was filtered through Whatman No. 1 filter paper and then 0.45 μm syringe filter. Myoglobin and metmyoglobin concentration was determined by the method of Tang et al. (2004).

Experiment was conducted by 3 (30, 40 and 50ºC of temperature) × 3 (10, 20 and 30 min of holding time) × 3 (100, 200 and 300 MPa of pressure) factorial design. Data obtained from three replications were analysed, using SAS program as a non-linear regression model. Activation energies for myoglobin denaturation and reduction were calculated from the regression model.

Results and Discussion

Denaturation of Mb was significant ($p<0.001$) with increasing either temperature or pressure, whilst metMb reduction was significant ($p<0.001$) with increasing pressure alone (Figure 1). For metMb reduction, reduction was initialised at 50 MPa and optimal temperature for metMb reduction was approximately 30 and 35ºC. The result was also confirmed by rate constant determination. Maximum reduction rate was obtained at 300 MPa and 30ºC, at which Mb showed moderate denaturation.

At mild pressure, activation energy for reduction of metMb was lower than that for Mb denaturation, whilst the value was reversed with increasing pressure level (Figure 2). In addition, activation energies for both Mb denaturation and metMb reduction showed same value at 350 MPa. The result indicated that pressure-induced metMb reduction was occurred at less than 350 MPa as reported by Jung et al. (2003). On the contrary, increasing pressure level favoured both Mb denaturation and metMb oxidation, resulted in low redness as observed other literature (Carlez et al., 1995; Jung et al., 2003; Hong et al., 2006).

According to this study, increased lightness with increasing pressure level resulted from myoglobin denaturation, particularly from globin denaturation, whilst increased redness was due to high metMb reduction kinetics at moderate pressure level. In consequence, pressurisation could be taken as a colour improvement or an

elongation of shelf-life depending upon applied pressure level or upon the final meat form commercialisation as concluded by Jung et al. (2003).

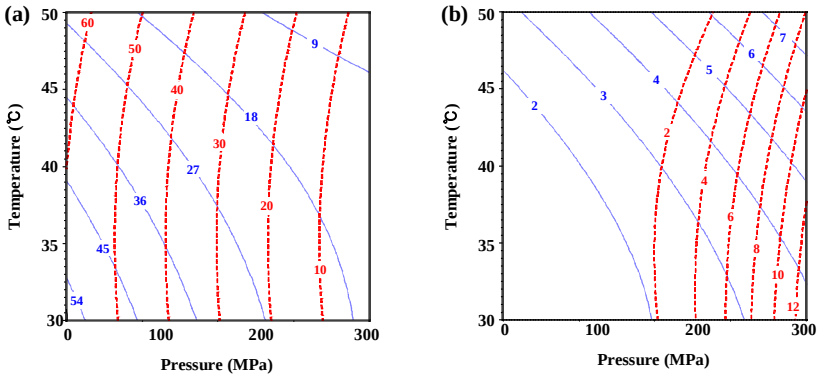

Figure 1. Contour plots for (a) myoglobin (μM/L, r^2 = 0.8472, solid line) and metmyoglobin contents (%, r^2 = 0.8802, dotted line), and (b) rate constant (/100 min) of myoglobin denaturation (r^2 = 0.9934) and metmyoglobin reduction (r^2 = 0.9904) as a function of pressure and temperature. Sample was pressurised for 30 min.

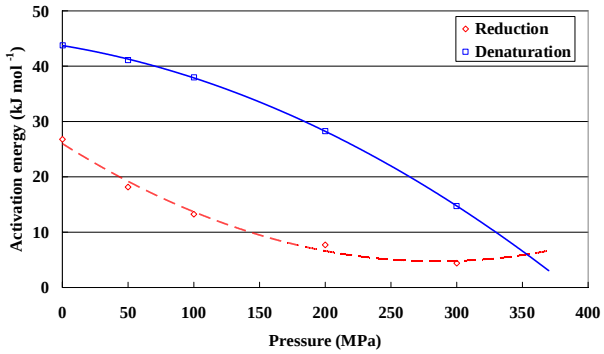

Figure 2. Change in activation energy as a function of pressure. Solid and dotted line presented denaturation and reduction of pork metmyoglobin, respectively.

Conclusion

In the current study, 250-300 MPa was a critical pressure level at which denaturation and reduction rate of Mb were reversed. Therefore, the result indicated that moderate pressure was potential benefit in fresh meat retails and that a mechanism by which metMb could be reduced was possibly presented. Furthermore, pressure more than 300 MPa for calculating accurate phase boundaries of Mb denaturation and metMb reduction was required.

References

1. Carlez, A., Veciana-Nogues, T., and Cheftel, J.C. (1995). Changes in colour and myoglobin of minced beef meat due to high pressure processing. *Lebensmittel Wissenschaft und Technologie, 28,* 528-538.
2. Cheah, P.B. and Ledward, D.A. (1997). Inhibition of metmyoglobin formation in fresh beef by pressure treatment. *Meat Science, 45,* 411-418.
3. Hong, G.P., Park, S.H., Kim, J.Y., Lee, S.K., and Min, S.G. (2005). Effects of time-dependent high pressure treatment on physico-chemical properties of pork. *Food Science and Biotechnology, 14,* 808-812.
4. Hong, G.P., Park, S.H., Kim, J.Y., and Min, S.G. (2006). The effects of high pressure and various binders on the physico-chemical properties of restructured pork meat. *Asian-Australasian Journal of Animal Science, 19,* 1484-1489.
5. Jung, S., Ghoul, M., and De Lamballerie-Anton, M. (2003). Influence of high pressure on the color and microbial quality of beef meat. *Lebensmittel Wissenschaft und Technologie, 36,* 625-631.
6. Osborn, H.M., Brown, H., Adams, J.B., and Ledward, D.A. (2003). High temperature reduction of metmyoglobin in aqueous muscle extracts. *Meat Science, 65,* 631-637.
7. Tang, J., Faustman, C., and Hoagland, T.A. (2004). Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *Journal of Food Science, 69,* C717-720.