CHARACTERIZATION OF THAI BEEF MACROMOLECULAR CHANGES UNDER VARIOUS SOUS-VIDE COOKING CONDITIONS: AN INFRARED MICROSPECTROSCOPY STUDY

P. Supaphon[1,2*], S. Kerdpiboon[1], A. Vénien[2], O. Loison[2] and T. Astruc[2]

[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand;

[2]INRA de Clermont-Ferrand-Theix, UR370 QuaPA, F-63122 St Genès Champanelle, France

*Corresponding author email: s7608037@kmitl.ac.th

I. INTRODUCTION

Conventional cooking of meat induced generally a shrinkage of myofibrillar mass of muscle fibers and of collagen from the intramuscular connective tissue [1,2]. Conversely, sous-vide cooking can lead to a swelling of the muscle fibers [3,4,5] and the mechanism is still unclear. To better understand this phenomenon, we investigate the effect of various sous-vide cooking parameters on the macromolecular changes of myofibrillar proteins and connective tissue. The macromolecular structure of proteins was assessed in situ, using mid infrared microspectroscopy.

II. MATERIAL AND METHODS

Sample preparation: Fifteen round muscle from local Thai beef (*Bos indicus*) were purchased from Huatakhe market, Bangkok province, Thailand. Sample were sliced into 7×7×7 cm, vacuum packed and sous-vide cooked at 60, 70 and 80°C for 6, 18 and 36 hours. Then sample were cut into 0.5×0.5×1 cm with fiber direction in the length of the sample. Samples were immersed in a solution of 4% formaldehyde in phosphate buffer (pH 7.2) until use. Sample were cryofixed in isopentane cooled with liquid Nitrogen. Cross cryosection (6 µm thick; cryostat CM1950, Leica Microsystems, Germany), were mounted on BaF2 windows and air-dried at room temperature. ***Infrared spectroscopy:*** Spectra acquisition was performed using an infrared microscope (Nicolet iN10, Thermo Fisher, USA). For each time/temperature condition, IR spectra were collected point-by-point in a 4000-650 cm^{-1} range and recorded at a spectral resolution of 4 cm^{-1}. 20 spectra were acquired in twenty muscle fibers (one acquisition by muscle fiber) with a scanning area of 30×30 µm (accumulation of 64 scans) while for perimysium 10 spectra were collected along a line in a selected area (spatial resolution of 10×10 µm, 64 scans). Spectra undergone an extended multiplicative signal correction and a second derivative. The 1500-1700 cm^{-1} part of the spectra most assigned to protein signal was selected for principal component analysis. Principal component analysis (PCA) were performed using The Unscrambler software version 9.8 (CAMO Software AS).

III. RESULTS AND DISCUSSION

The PCA score plot and loading of muscle fibers are shown in Fig. 1A and 1B, respectively. Whatever the cooking time, the samples from the 3 cooking temperatures and the raw samples were clearly separated on the PC1, the segregation between the 3 cooking temperature is seen on the PC2. Samples exhibited more variability when they were cooked at 60°C for 6 hours, but became more homogeneous for longer cooking times. The typical resulting second derivatives from infrared spectra between the range 1700-1500 cm^{-1} are shown in Fig. 1B. The 1655 cm^{-1} peak, assigned to alpha helix of the amide I band of proteins, decreased with increasing temperature while the 1624 cm^{-1} peak assigned to aggregated beta sheets structure increase, reflecting protein denaturation [6,7]. Connective tissue also denatured with cooking temperature and time (Fig. 2). After 6 hours cooking, there is a clear separation of the samples heated at 60°C from the others one, while after 18 hours cooking, there is a shift of samples heated at 60°C in the direction of those heated at 70 and 80°C. After 36 hours heating, the samples heated at 70°C are mixed with those heated at 80°C reflecting the same rate of collagen denaturation.

IV. CONCLUSION

Whatever the targeted proteins (intrafibrillar or connective tissue), cooking lead to their denaturation. Proteins from 80°C cooked samples seems to be highly denatured after 6 hours of heating and perhaps do not evolve when increasing the cooking time. However, denaturation increased with time for samples

cooked at 60 and 70°C until 36 hours of heating. Further statistical analysis of the data, such as anova on the absorbance rate at 1655 and 1624 cm^{-1} will allow us to refine the results.

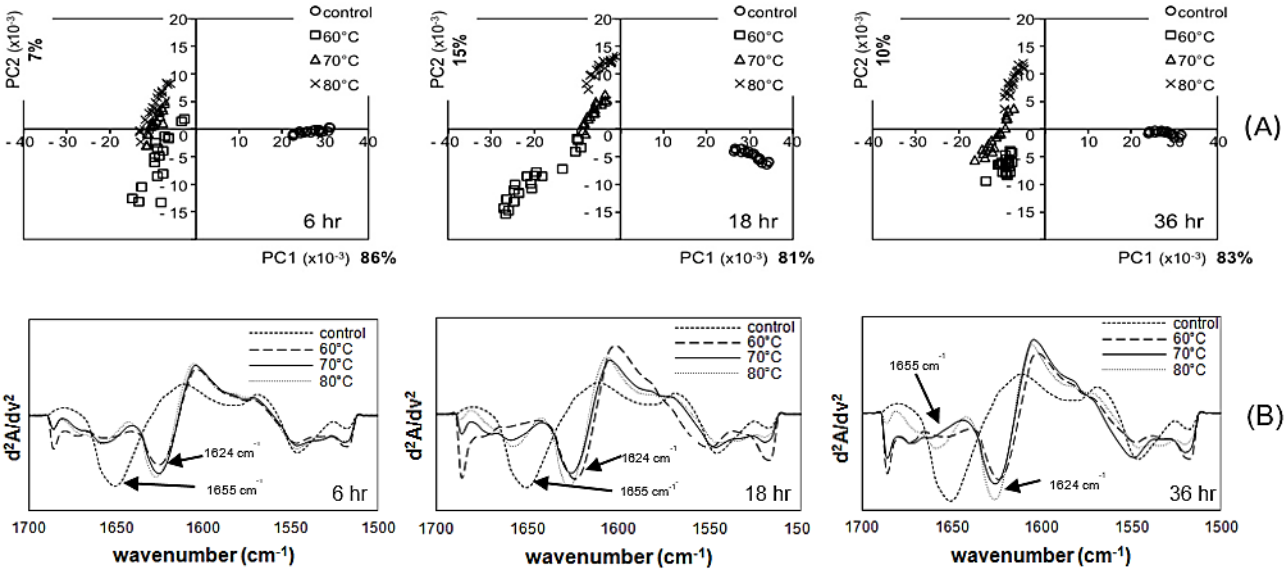

Figure 1. PCA score plot of the IR spectra obtained from myofibers (1700-1500 cm^{-1}) of control and cooked samples at 6, 18 and 36 hours for 60, 70 and 80°C. The second derivative of the IR spectra (range 1700-1500 cm^{-1}) obtained from myofibers of control and cooked samples (B).

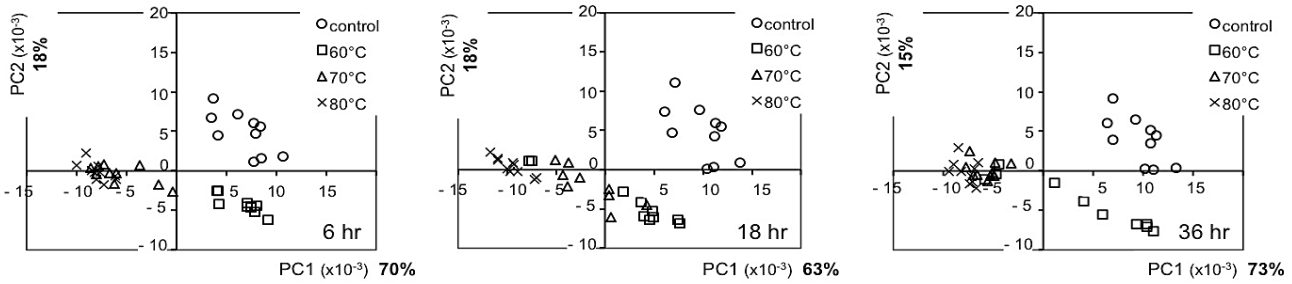

Figure 2. PCA score plot of the IR spectra obtained from connective tissue (1700-1500 cm^{-1}) of control and cooked samples at 6, 18 and 36 hours for 60, 70 and 80°C.

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REFERENCES

1. Davey, C. L. & Gilbert Kevin, V. (2006). Temperature-dependent cooking toughness in beef. Journal of the Science of Food and Agriculture 25: 931-938.
2. Tornberg, E. (2005). Effects of heat on meat proteins-Implications on structure and quality of meat products. Meat Science 70: 493-508.
3. Wattanachant, S., Benjakul, S. & Ledward, D. A. (2005). Effect of heat treatment on changes in texture, structure and properties of Thai indigenous chicken muscle. Food Chemistry 93: 337-348.
4. Roldán, M., Antequera, T., Martín, A., Mayoral, A. I. & Ruiz, J. (2013). Effect of different temperature-time combinations on physicochemical, microbiological, textural and structural features of sous-vide cooked lamb loins. Meat Science 93: 572-578.
5. Supaphon, P., Kerdpiboon, S., Vénien, A., Loison, O. & Astruc T. (2018). Structural changes of local Thai beef during sous-vide cooking. In Proceedings 64th International Congress of Meat Science and Technology, 12-17 August 2018, Melbourne, Australia.
6. Motoyama, M., Vénien, A., Loison, O., Sandt, C., Watanabe, G., Sicard, J., Sasaki, K. & Astruc, T. (2018). *In situ* characterization of acidic and thermal protein denaturation by infrared microspectroscopy. Food Chemistry 248: 322-329.
7. Astruc, T., Peyrin, F., Vénien, A., Labas, R., Abrantes, M., Dumas, P. & Jamme, F. (2012). *In situ* thermal denaturation of myofibre sub-type proteins studied by immunohistofluorescence and synchrotron radiation FT-IR microspectroscopy. Food Chemistry 134: 1044-1051.