Original Research Article

Kinetic study on the thermal denaturation of protein in yellowfin tuna meat

Pimravee Ngamkala[1], Thayawat Uthaijunyawong[1], Ratchaneeporn Ai-Tang[1], Chotika Viriyarattanasak[2] and Suwit Siriwatanayotin[1]*

[1]Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi 126 Pracha-u-Tid Road, Bangmod, Tungkru, Bangkok 10140, Thailand

[2]Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi 83 Moo 8 Thakham, Bangkhuntien, Bangkok 10150, Thailand

ARTICLE INFO

ABSTRACT

The purpose of this work was to study the kinetics of thermal denaturation of protein in tuna meat. Fresh yellowfin tuna meat was sealed into a stainless steel differential scanning calorimetry (DSC) pan, and subsequently heated at 45, 50, 60 and 65 °C for various interval time. The residual enthalpy of fresh and the heated tuna meat was determined by DSC at scanning rate 5 °C/min. The residual enthalpy of fresh tuna meat was used as a reference in calculation of denaturation degree. DSC thermogram of fresh tuna meat showed four denaturation peaks at 41, 49, 58 and 67 °C. It was suggested that first two peaks represented for myosin fractions, and the others are sarcoplasmic proteins and actin, respectively. The plot between logarithm of degree of denaturation and heating time revealed that there were two first-order rate constants for each heating temperature. This implied that there might be two mechanisms of protein denaturation in tuna meat. The mechanisms of these thermal denaturations probably come from the non-aggregated or reversible state and the stage of protein aggregation.

* *Corresponding author*: Tel.: 088 022 4385
Email: chotikav.kmutt@gmail.com

INTRODUCTION

Tuna fish is important in the food industry of Thailand. Tuna meats have high nutrition values. There are approximately 71.7% water, 25.9% protein, and 0.6% fat (Webb, 2003). Thailand's export of canned tuna has probably increased due to the demand for tuna, and this trend will keep increase constantly (Srijuntuk, 2013). The whole process of canned tuna production consists of thawing, pre-cooking, unwanted part removing, caning, retorting, cooling and labeling, respectively (Webb, 2003). Pre-cooking is the first thermal step after thawing processes. The pre-cooking process is the critical thermal processes before sterilization with retort. In this unit operation, tuna is cooked in a condition of atmospheric steam at 100 to 102°C (Zhang *et al.*, 2002). Pre-cooking causes the partial denaturation of protein in tuna meat. As a result, the muscle tissue is more compact, and easily removed from head, fins, skin and bones. However, over precooking reduces both yield and flavor of the tuna meat. Moreover, over temperature contributes to weight loss, texture change, and plant yield. In contrast, under precooking conditions make meat more difficult to pull from the bone and also reduces yield at final canning processes (Lassen, 1965; Bell *et al.*, 2001). Changes in texture of meat during precooking are consequences of both native protein thermal denaturation and loss of water holding capacity (Perez-Martin *et al.*, 1989). Furthermore, the thermal protein denaturation are abundantly depended on heating temperature and time; this is a reason why this work studied its kinetics. In general, kinetic study of thermal denaturation of muscle protein was performed via differential scanning caloriometry (DSC). For example, Kajitani *et al.* (2011) and Skipnes *et al.* (2008) studied kinetics of thermal denaturation of protein in cured pork meat and farmed atlantic cod, respectively, by using DSC techniques. During heating scan with DSC, the residual active protein was denatured, and therefore the enthalpy of this protein denaturation was detected as the first order phase transition observed from having step changes of enthalpy (Shakhnovich and Finkelstein, 1989). Therefore, the purpose of this study is to study kinetics of thermal denaturations of tuna meat via DSC.

MATERIALS AND METHODS

Raw Material

Frozen yellowfin tuna loins (*Thunnus albacares*) were obtained from local supermarket (Samut Phakarn, Thailand) and were used in this experiment. The meats were kept on ice during transportation. Subsequently, the meats were stored at -18 °C within one month until the experiment.

Sample Preparation

The frozen meat was thawed by using running water at ambient temperature ranging from 24 to 25 °C for 3 min. Then, it was randomly cut and ground. Subsequently, 50 ± 1.5 mg of sample was hermitically sealed in stainless steel DSC pan within 10 min after thawing.

In this study, there were five thermal treatments e.g. 45, 50, 60, 65 and 75 °C and various heating time levels or stopped when no more protein denatured. Firstly, the sample pan was placed in HPLC grade water beaker when the temperature reached the desired constant temperature controlled by water bath (GFL®, Burgwedel, Germany), and was hold for the desired time. After thermal treatment, the heated sample pan was immediately cooled in HPLC grade water at 0°C for 5 min.

Differential scanning calorimetry

The residual enthalpy of sample was determined by using Perkin-Elmer DSC 7 equipped with a cooler unit and dry box with nitrogen flush (Perkin-Elmer Corp., Norwalk, USA). A pan containing HPLC grade water equal to 70% of the ground meat weight in sample pan (the amount of water in the meat sample) was used as a reference (Bell *et al.*, 2001). Samples were scanned at 5°C/min over the range from 30°C to 95°C. Generally, the residual of denaturation enthalpy (ΔH) was defined as the area under the peak and over the base line (Skipnes *et al.*, 2008; Bell *et al.*, 2001). Temperature and enthalpy measurement of DSC were calibrated with indium. Each experiment was performed in two replications.

Kinetics data analysis

The residual enthalpy of denaturation of heat-treated samples was compared with its raw sample by calculating as the ratio of enthalpy change (ΔH_{ratio} , Equation (1)).

$$\Delta H_{ratio} = \frac{\Delta H}{\Delta H_0} \tag{1}$$

where ΔH is a residual denaturation enthalpy of the heated sample, and ΔH_0 is a protein denaturation enthalpy of untreated sample. Since, previous study revealed that thermal denaturation can be described by first-order reaction (Shakhnovich and Finkelstein, 1989), we hypothesized that kinetics of thermal denaturation of a tuna meat in this study could also be explained by as shown in Equation (2).

$$N \xrightarrow{k} D$$

$$\frac{d[N]}{dt} = -k[N] \tag{2}$$

where $[N]$ is the ratio of enthalpy change (ΔH_{ratio}), and k is the reaction rate constant. In case of isothermal experiments (i.e. rate constant not varying with time), Equation (2) can be integrated into Equation (3) (Marangoni, 2003).

$$\ln \frac{[N_t]}{[N_o]} = -kt \tag{3}$$

Arrhenius relationship was used to describe the relationship between temperature dependent rate constant and temperature. (Eq. (4))

$$\ln k = -\frac{E_a}{R_g}[\frac{1}{T} - \frac{1}{T_{ref}}] + \ln k_{ref} \tag{4}$$

where k is the inactivation rate constant at temperature T, E_a is the activation energy and R_g the universal gas constant (R_g = 8.314 J/kmol). k_{ref} is the inactivation rate constant at T_{ref}.

Results and Discussion

DSC thermograms of tuna samples

A DSC thermogram for a fresh tuna meat sample was shown in Figure 1. There were four independent denaturation peaks 41, 49, 58 and 67°C. Shakhnovich and Finkelstein (1989) reported that denanaturation peak in DSC thermogram was first order phase transition observed from having step changes of enthalpy. Moreover, appearance of four independent denaturation peaks implied that each protein fraction in tuna meat was denatured slowly and independently (Weijers, 2005).
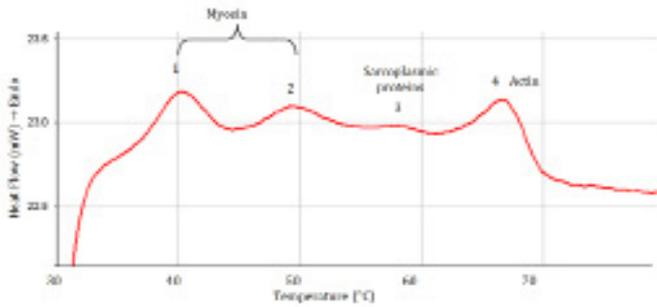

Figure 1 DSC thermogram of a raw yellowfin tuna sample (heating rate of 5 °C/min).

In literature review, denaturation of various proteins in several kinds of fish meats was reported (Table 1). Skipnes *et al.* (2008) demonstrated that in cod meat myosin was early denatured at 38-44.1°C. Moreover, sarcoplasmic protein and actin were subsequently denatured when temperature in DSC scan was increased to around 57.3°C and 76.1°C, respectively. The difference in denaturation temperature for the similar kind of protein might be caused by the difference in fish types and scanning rates (Lasocka, 1976; Savitri, 2011). According to these previous studies, it was suggested that four endothermic peaks observed in fresh tuna meat in our study should be responsible for myosin, sarcoplasmic protein and actin respectively. The endothermic peak at approximately 60°C was considered to represent denaturation peak of sarcoplasmic proteins instead of that of collagen. This was because the samples used in our study were taken from ordinary dorsal muscle which was abundant with myoglobin.

DSC thermograms for the heated samples were shown in Figure 2. It was found that heating at 45°C for 30 min had only a result in disappearance of myosin peak (Figure 2A). On the other hand, heating at 50°C for 10 min denatured the first two peaks (Figure 2B). This implied that different kinds of protein; e.g., sarcoplasmic protein, in tuna meat were denatured simultaneously during thermal treatment. Heat treatment at 60°C and 65°C showed in the similar trend. That is heating at 60°C or 65°C for 10 min denatured the first three peaks simultaneously. Moreover, the prolonged heating time at 60°C or 65°C caused decreasing in an area of actin denaturation peak. On the other hand, heating at 75°C for 30 sec could destroy all kinds of protein muscle, and there was no peaks left in the DSC thermogram (data was not shown). These results were in agreement with the report of Webb (2003) and Skipnes *et al.* (2008).
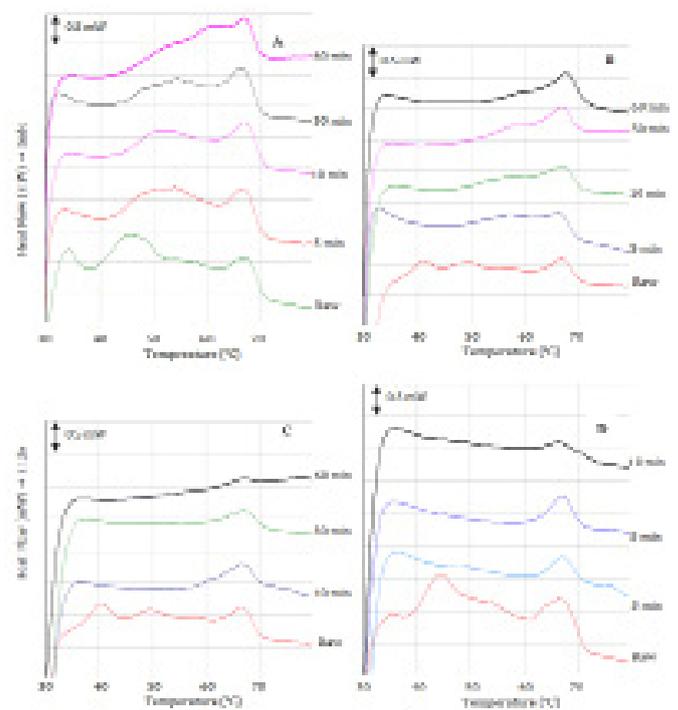

Figure 2 DSC thermograms of yellowfin tuna sample heated at 45 °C (A), 50 °C (B), 60 °C (C), 65 °C (D).

Table 1 Denaturation temperature for various fish meats.

Materials	Heating rate (ºC/min)	Peak Temperature (°C)				References
		Myosin	Sarcoplasmic protein	Collagen	Actin	
Skipjack tuna dorsal loins	5	52		59	68	Bell et al. (2001)
Skipjack tuna loins	10	49		59	68	Zhang et al. (2001)
Skipjack tuna loins	10	50.1	--------64.7--------		73.5	Webb (2003)
Farmed frozen cod	10	38.4, 44.1	57.3, 69.5		76.1	Skipnes et al. (2008)
Farmed Atlantic salmon loin muscle (ground fresh)	5	46.8, 59.3	68.6		77.3	Savitri (2011)
Yellowfin tuna dorsal loins	5	41, 49	58		67	Present work

Kinetics of protein denaturation in yellowfin tuna meat

The residual denaturation enthalpy (ΔH) was estimated from the total area of all peaks. The denaturation enthalpy of raw yellowfin tuna (ΔH_o) was 2.31±0.13 J/g (n=7). In addition, the relationship between natural logarithm of $\Delta H/\Delta H_o$ and heating time was plotted. Figure 3 revealed that there were two first-order rate constants for each heating temperature. This result was in contrast with the results of Skipnes *et al.* (2007), which generally showed the first-order reaction of actin denaturation in cod meat. These two step first-order reaction might be explained by Lumry and Eyring model. Lumry and Eyring model explained that native or folded protein (N) are reversibly denatured to unfolded proteins (U) with rate k_1, and most of this unfolded proteins are able to changed to irreversible proteins (I) leading to flocculation and precipitation ($k_2 \gg k_{-1}$ in Eq. (6)) (Brown *et al.*, 2013)

$$N \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} U \overset{k_2}{\to} I \tag{6}$$
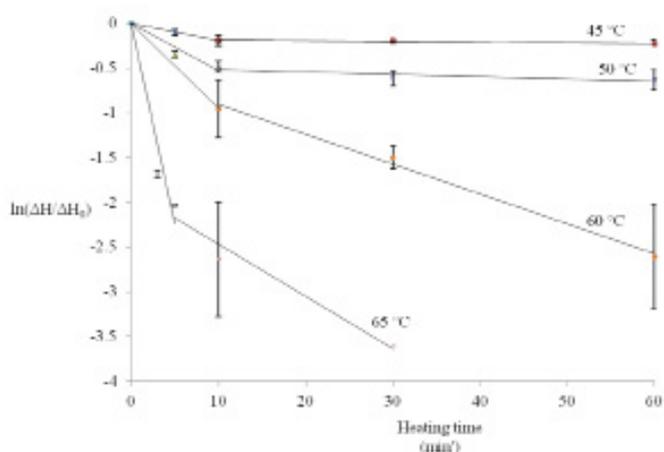

Figure 3 Natural logarithmic plots of the residual denaturation enthalpy of yellow fin tuna meat after heating temperatures at 45, 50, 60, and 65°C.

Therefore, we proposed that these two first-order reactions observed in our study might be responsible for unfolding process and aggregation or irreversible process of protein. The denaturation rate constant at unfolding process and irreversible process was -32.17×10⁻⁵ s⁻¹ and -1.50×10⁻⁵ s⁻¹ respectively for 45 °C; -88.33×10⁻⁵ s⁻¹ and -4.50×10⁻⁵ s⁻¹ respectively for 50 °C; -158.83×10⁻⁵ s⁻¹ and -55.97×10⁻⁵ s⁻¹ respectively for 60 °C; -747.33×10⁻⁵ s⁻¹ and -97.67×10⁻⁵ s⁻¹ respectively for 65 °C. Arrhenius's constant of both unfolding process and irreversible process of protein were 122.7 and 194.4 kJ/mol, accordingly. Thus, unfolded protein required more energy to form irreversible stage than native proteins to reversible stage. The Arrhenius equations that represent temperature dependence were able to predict rate constant at desired temperature (Figure 4).

The information from our study might be useful for the development of cooking process in tuna industry to control tuna meat quality.
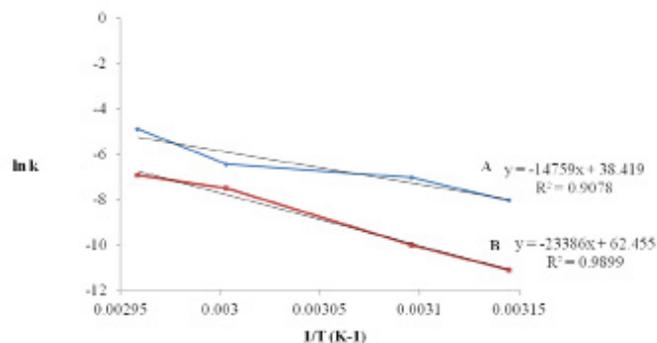

Figure 4 Arrhenius plot of thermal protein denaturation of tuna meat; unfolding process (A); irreversible process (B).

CONCLUSION

The kinetics of thermal denaturation of protein in tuna meat was studied at 45, 50, 60 and 65°C via DSC. DSC thermogram revealed that there were four denaturation peaks at 41, 49, 58, and 67°C in raw tuna meat. These peaks might be responsible for myosin, sarcoplasmic proteins and actin, respectively. When tuna meat was heated at different heating temperature and time, the degree of denaturation increased with increasing heating temperature for each heating time. Moreover, the plot between logarithm of degree of denaturation and heating time revealed that there were two first-order rate constants for each heating temperature. We proposed that these two first-order reactions observed in our study might be responsible for unfolding process and aggregation or irreversible process of protein.

REFERENCES

Ang, J.F. and Hultin, H.O. 1989. Denaturation of Cod Myosin during Freezing after Modification with Formaldehyde. Journal of Food Science 54 (4): 814-818.

Bell, J.W., Farkas, B.E., Hale, S.A. and Lanier, T.C. 2001. Effect of Thermal Treatment on Moisture Transport During Steam Cooking of Skipjack Tuna (*Katsuwonas Pelamis*). Journal of Food Science 66 (2): 307-313.

Brown, I., Dafforn, T.R., Fryer, P.J. and Cox, P.W. 2013. Kinetic Study of the Thermal Denaturation of a Hyperthermostable Extracellular A-Amylase from *Pyrococcus Furiosus*. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1834 (12): 2600-2605.

Kajitani, S., Fukuoka, M. and Sakai, N. 2011. Kinetics of Thermal Denaturation of Protein in Cured Pork Meat. Japan Journal of Food Engineering 12 (1): 19 – 26.

Lasocka, M. 1976. The Effect of Scanning Rate on Glass Transition Temperature of Splat-Cooled Te85ge15. Materials Science and Engineering 23 (2–3): 173-177.

Lassen, S. 1965. Tuna Canning and the Preservation of the Raw Material through Brine Refrigeration. In, Borgstrom, G. (Ed). Fish as Food, pp. 207-245. London: Academic Press.

Marangoni, A.G. 2003. Tools and Techniques of Kinetic Analysis. In Enzyme Kinetics: A Modern Approach, pp. 1-40. New York: Wiley.

Perez-Martin, R.I, Banga, M.G., Sotelo, Aubourg, S.P. and Gallardo, J.M. 1989. Prediction of Precooking Times for Albacore (*Thunnus alalunga*) by Computer Simulation. Journal of Food Engineering 10: 83–95.

Savitri, S. 2011. Heat Denaturation of Proteins from Atlantic Salmon (*Salmo salar*). Stavanger, Norway: University of Stavanger, Master's thesis.

Shakhnovich, E.I. and Finkelstein, A.V. 1989. Theory of Cooperative Transitions in Protein Molecules. I. Why Denaturation of Globular Protein Is a First-Order Phase Transition. Biopolymers 28 (10): 1667-1680.

Skipnes, D., Plancken, I.Vd., Loey, A.V. and Hendrickx, M.E. 2008. Kinetics of Heat Denaturation of Proteins from Farmed Atlantic Cod (*Gadus morhua*). Journal of Food Engineering 85(1): 51-58.

Srijuntuk, N. 2013. Situation for Tuna and Products First 6 Months of 2556. Downloaded from http://www.thai-frozen.or.th/news_47.php on 13/5/2014.

Webb, E.L. 2003. Process Control Parameters for Skipjack Tuna (*Katsuwonas pelamis*) Precooking, North Carolina, USA: North Carolina State University, PhD thesis.

Weijers, M. 2005. Aggregate Morphology and Network Properties of Ovalbumin, Wageningen, Netherlands: Wageningen University, PhD thesis.

Zhang, J., Farkas, B.E. and Hale, S.A. 2001. Thermal Properties of Skipjack Tuna (*Katsuwonis pelamis*). International Journal of Food Properties 4(1): 81-90.

Zhang,J., Farkas, B.E. and Hale, S.A. 2002. Precooking and Cooling of Skipjack Tuna (*Katsuwonas pelamis*): A Numerical Simulation. Lebensmittel-Wissenschaft und-Technologie 35 (7): 607-611.