CHAPTER IV

Development of Surimi Gel

Since surimi gel is a heat-induced gel, heating time-temperature is therefore essential to induce gelation of myofibrillar proteins to form a unique elastic textural property. The mechanisms of gelation comprise two steps: (1) the conversion of minced fish into sol after mixing with salt, leading to the dissociation of myofibril structures by protein solubilization; (2) the conversion of sol into gel by heat treatment resulting in the partial unfolding of protein structure and the aggregation of unfolded protein via both covalent and noncovalent bonds to form a three-dimensional network (Benjakul et al., 2001; Hall and Ahmad, 1997; Stone and Stanley, 1992).

Generally, surimi gel production involves two heating steps: (1) the setting step in which surimi sol is set at low temperature ($\leq$ 50 °C) to form a translucent gel and (2) the cooking step in which the gel is processed at a higher temperature ($\geq$ 80 °C) to produce an opaque, highly elastic and strengthened gel (Lanier and Lee, 1992). The setting period is an important step in surimi gel development. This is because the setting at low temperature prior to heating at a higher temperature allows slow ordering of the protein molecules, resulting in good gelation, fine structure and great elasticity (Lanier and Lee, 1992; Hermansson, 1978). However, temperature does not only play an important role in the rearrangement of protein restructure which leads to gelatinization of surimi, but it also activates endogenous enzymes, transglutaminase and proteinase, that naturally exist in fish muscle. During gelation, endogenous transglutaminase enhances the gel strength of surimi by crosslinking myosin. On the contrary the proteinase enzymes degrade the gel strength of surimi by hydrolyzing myosin. Low-temperature setting is associated with transglutaminase activity. Transglutaminase catalyzes acyl-transfer reactions in which the γ-carboxamide group of peptide-bound glutaminyl residue is the acyl donor. When

the ε-amino group of peptide-bound lysine acts as the accepter, the ε-(γ-glutamyl)lysine bond is formed between the proteins, resulting in their crosslinking and possible enhancement of surimi gelling properties (Lee et al., 1997; An et al., 1996; Seguro et al., 1995). On the other hand, gel deformation is induced by endogenous proteinase. The proteinase activity causes the rapid and severe degradation of myofibrillar proteins, particularly myosin, effecting surimi quality, and substantially decreasing gel strength (An et al., 1996; Morrissey et al., 1993).

The qualities of surimi gel, notably gel strength and structure, can be affected by the setting temperature and the rate of heating during the setting step. Hence, in this chapter, an attempt to describe the effect of heat during the setting period on the surimi gel formation and its final structure is presented. A kinetic model was developed to describe the change in gel texture represented by gel strength. Examination of the microstructure was performed to reveal the correlation between gel strength and gel texture using a solid fraction analysis and scanning electron microscopy (SEM).

4.1 Effect of Setting Temperature and Time on Gel Formation and Gel Texture

In order to determine the setting time-temperature combination, the preliminary experiments were carried out by using threadfin bream surimi, which was purchased from Sea Royal Marine, Co LTD, Thailand. As mentioned previously, temperature is the main factor for gel formation and endogenous enzymes activation. It has been reported that setting temperatures between 35 and 50 °C is optimum for transglutaminase enzyme to enhance gel formation (An et al., 1996). Hence, this temperature range was selected for the current experiment. The change of gel texture was observed between setting times of 0 and 60 minutes by measuring the gel strength. The results in Figure 4.1 show that the different combinations of setting time-temperature in the initial step of gel formation have effects on final gel strengths. The final gel strengths increased to a maximum value and then decreased to the plateau in all setting temperature. Moreover, different gel

strength provided different gel network as can be seen in the scanning electron microphotographs (Figure 4.2). The electron microphotographs also revealed that higher gel strengths obtained from denser gel networks. Therefore setting time-temperature combination is the main factor determining the gel strength.

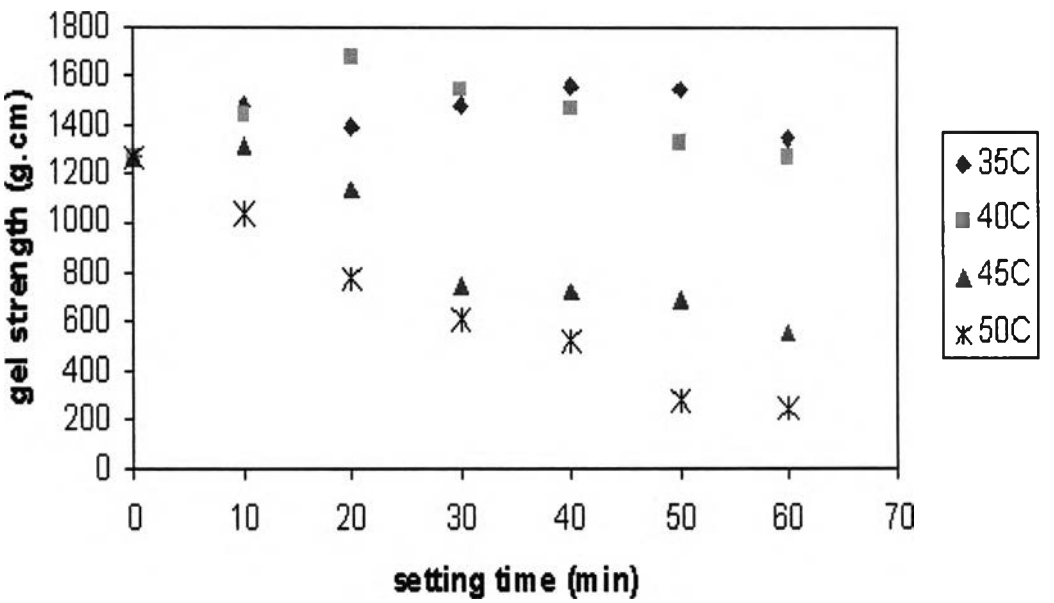

Figure 4.1 Gel strength of threadfm bream surimi at various setting time-temperature combinations.

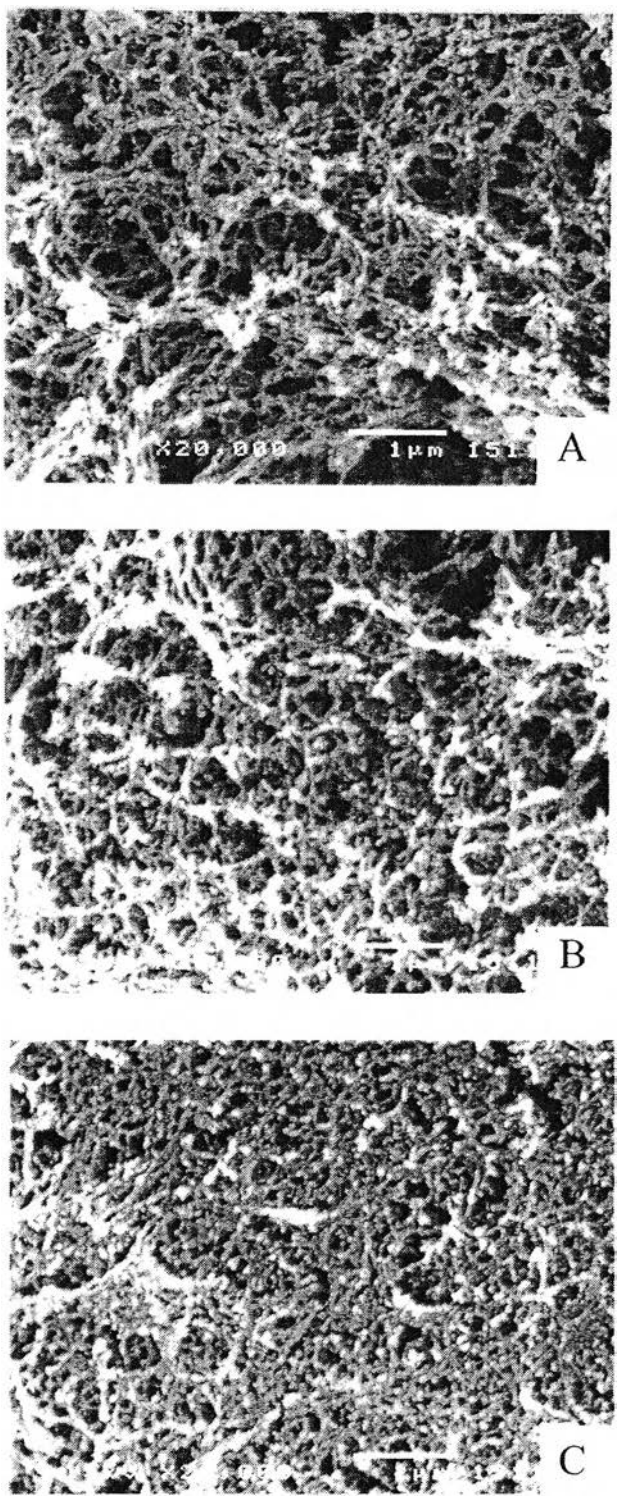

Figure 4.2 Scanning electron microphotographs of threadfin bream surimi gels setting at (A) 50 °C, 40 minutes (gel strength 520 g.cm) (B) 40 °C, 60 minutes (gel strength 1,269 g.cm) and (C) 40 °C, 20 minutes (gel strength 1,670 g.cm). Magnification = 10,000x.

In the second experiment, surimi samples were also prepared from cod. A temperature of 60 °C has been reported to be optimal for proteinase activity and temperatures greater than 70°C is shown to lower enzyme activity (Yongsawatdigul et al., 1997). Therefore, the effect of temperatures at 60 and 80 °C on gel structure was also investigated. Gel strengths of cod surimi gels, obtained under various setting conditions, are shown in Figures 4.3A and 4.3B. Different combinations of setting time-temperature provided the products with different gel strengths. In all cases, gel strength passes through its maximum value at a time depending on the setting temperature. The trend obtained is similar to the one obtained with threadfin bream surimi gel in the previous study (Figure 4.1).

Figures 4.3A and 4.3B show that at setting temperatures in the range 35 - 60 °C, the gel strengths rapidly increased with setting time, reached a peak, then gradually decreased to plateau values. However, the gel strength at 80 °C did not significantly change ($p>0.05$) with setting time after reaching its peak. Figures 4.3A and 4.3B also show that the maximum gel strength of each sample depended on the setting temperature. Within the setting temperature range between 35 to 50 °C, the highest maximum gel strength was obtained at 40 °C.

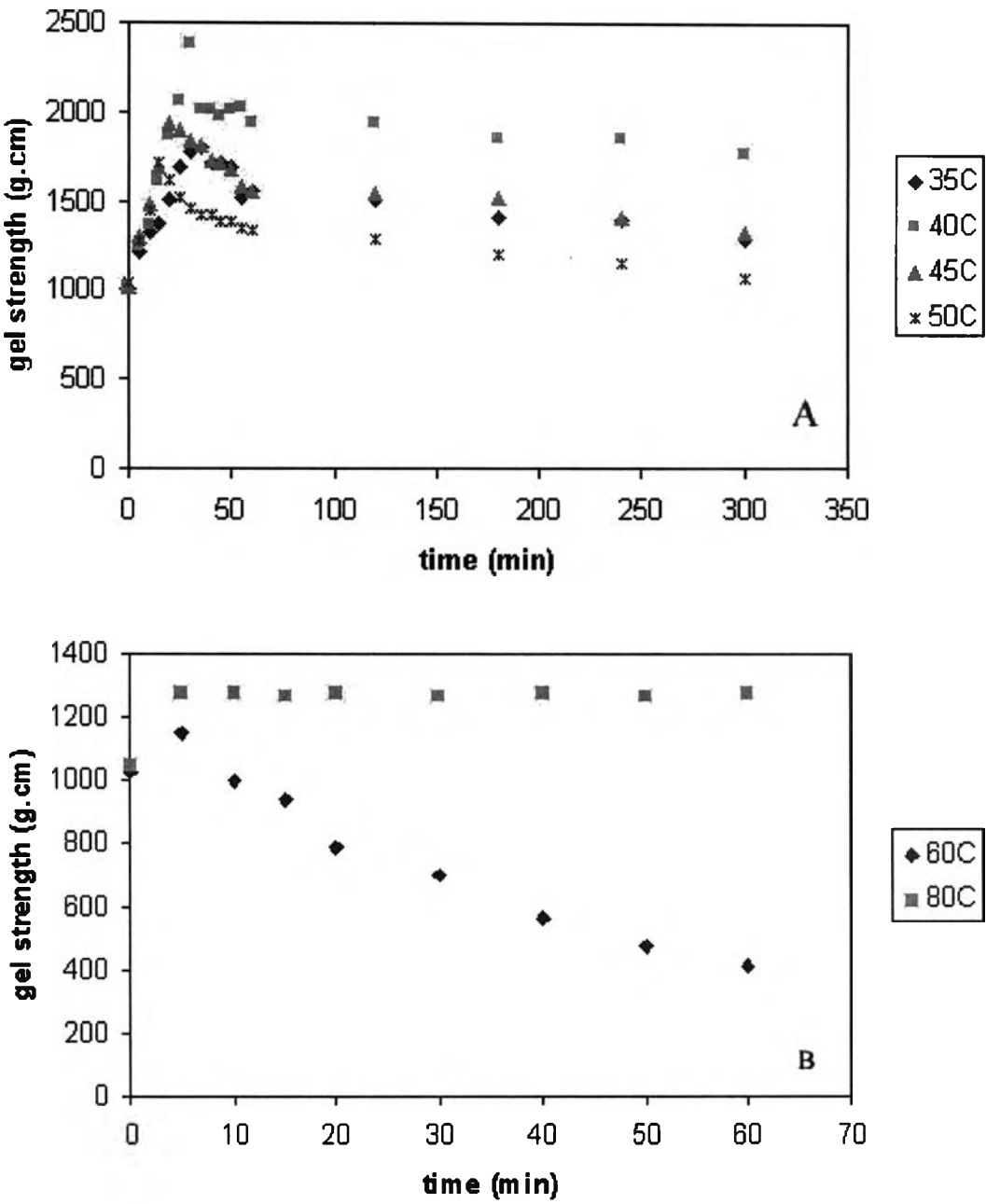

Figure 4.3 Gel strengths of cod surimi gels at setting time-temperature of 35-50 °C (A) and 60-80 °C (B).

The results at setting temperatures between 35 and 60 °C are qualitatively compatible with two crucial reactions involving the gel development: first, gel formation, and second, gel breakdown. Mechanistic arguments suggest that the gel formation process should be induced by endogenous transglutaminase, which can catalyze the formation of ε-(γ-glutamyl) lysyl cross-links, leading to improved functionality of surimi gels, especially the gel strength (An et al., 1996; Jiang et al., 2000). Also, Sankar and Ramachandran (2002), Jiang et al. (2000) and Lee et al. (1997) found that crosslinking of myosin by endogenous transglutaminase was related to gel texture in surimi made from Indian carps (rohu, catla and mrigal), Alaska pollock and golden threadfin bream surimi. Seguro et al. (1995) investigated the effect of the addition (at a level of 0.01-0.07% w/w) of microbial transglutaminase on surimi gel at low- and high-temperature preincubation and also reported similar findings. Their results showed an increase in breaking strength at setting temperatures of 10 °C and 45 °C with 0.03% microbial transglutaminase. In addition, the optimal temperature for enzyme activity is normally between 35 and 50 °C. Thus, samples which were set between 35 and 50 °C have more than 1.3 times the maximum gel strength of the samples set between 60 and 80 °C. Results from the current experiment also indicated that the highest maximum gel strength of cod surimi was obtained from the setting time-temperature of 20 minutes, 40 °C, prior to further cooking at a constant temperature of 90 °C for 20 minutes. This optimal setting temperature (40 °C) was also the temperature that the endogenous transglutaminase gave the maximum cross-linking reaction in croaker surimi as reported by Kamath et al. (1992).

In relation to network degradation, it was reported that the proteinase enzyme in the surimi gel was the main cause of change in gel structure. This enzyme can destroy the network of myofibrillar proteins, particularly myosin and result in decrease of gel strength. Proteinase activity in muscle is high at temperatures above 50 °C and the optimal temperature is around 60 °C; however, it is not detected above 70 °C (An et al., 1996; Boye and Lanier, 1988; Makinodan et al., 1985; and Cheng et al., 1979). This is also shown in the results of the current experiments that cod surimi

gel strengths which were set between 35 and 60 °C decreased after reaching its peak. In particular, at the setting temperature of 60 °C, rapid and severe degradation of gel strength can be observed. At the same time, the results of the current experiment revealed no sign of gel degradation at setting temperature of 80 °C for all setting times. This result could due to the lack of the proteinase activity above 70 °C. Similarly, Yongsawatdigul et al. (1997) examined the degradation kinetics of whiting surimi gel texture and reported that the proteolytic activity of endogenous proteinase was the main cause of changes in textural properties of whiting surimi gel. Textural breakdown of surimi gels occurred at 40 °C and the most severe deterioration was detected at 55-60 °C while the textural properties of the samples held at 75 and 80 °C did not significantly change with holding times. Also gel degradation in other fish species, such as bigeye snapper, lizardfish and paddlefish, has been ascribed to the degradation of myosin by endogenous proteinases (Benjakul et al., 2003a; Benjakul et al., 2003b; and Lou, et al., 2000). It can be concluded that the gel strength of cod surimi is controlled by the reactions from both transglutaminase and proteinase activities.

4.2 Kinetic Model for Texture Development

As already been mentioned, the gel formation reaction involved two major enzymes, transglutaminase and proteinase in fish. Transglutaminase induces the reaction of gel formation which enhance the crosslink between uncoiled myofibrillar protein during setting stage. On the other hand, proteinase decreases crosslink of myofibrillar networks. Thus, the gel formation reaction can be depicted as shown in Figure 4.4.

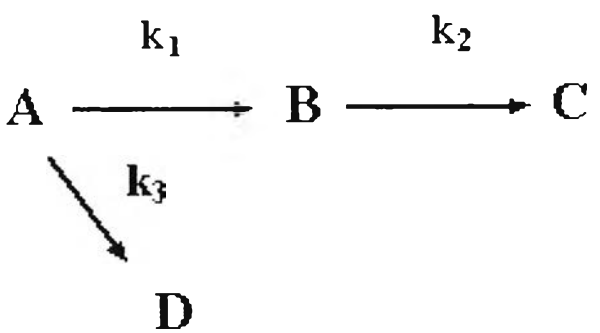

Figure 4.4 A reaction network for texture development of cod surimi gel

where A = starting paste

B = gel

C = disrupted gel (proteolytic breakdown)

D = disrupted fish protein (proteolytic breakdown)

k_1, k_2 and k_3 are the respective rate constants (min^{-1})

It is hypothesized here that texture development can be considered as the net result of two competitive-consecutive reaction processes (1) gel formation (A→B) and (2) gel and protein breakdown through proteolysis (B→C and A→D). Further it is assumed that these processes are first order in relation to the substrate concentration. So in a batch process, the reaction rate of decreasing of the quantify protein which affects gel texture can be expressed as Equation 4.1. And the rate at which protein responsible for gel texture formation can be written as Equation 4.2.

$$\frac{dA}{dt} = -(k_1 + k_3)A \tag{4.1}$$

$$\frac{dB}{dt} = k_1 A - k_2 B \tag{4.2}$$

With initial conditions B(0) = B_0 and A(0) = A_0, and assuming an isothermal process the change in gel texture (B(t)) can be represented as follows:

$$B(t) = \frac{k_1 A_0}{k_2 - k_1 - k_3}(e^{-(k_1+k_3)t} - e^{-k_2 t}) + B_0 e^{-k_2 t} \tag{4.3}$$

where A_0 = initial changeable protein

t = setting time (min)

Under isothermal setting conditions, the rate constants may be determined by fitting the change of gel strength with time to the model. Furthermore, the role of setting temperature on the reaction rate could be expressed by the Arrhenius law as follow:

$$k = \alpha \cdot e^{-\Delta E/RT} \tag{4.4}$$

where α = pre-exponential factor (min^{-1})

ΔE = activation energy ($kJ.kmol^{-1}$)

R = gas constant (8.314 $J.mol^{-1} K^{-1}$)

T = absolute temperature (K)

According to Equation 4.3, the competitive-consecutive first order kinetic model gave a good fit with the change of gel strength with time for all setting temperature as shown in Figure 4.5. In addition, all parameters, k_1, k_2 and k_3 deduced from the fit are shown in Table 4.1.

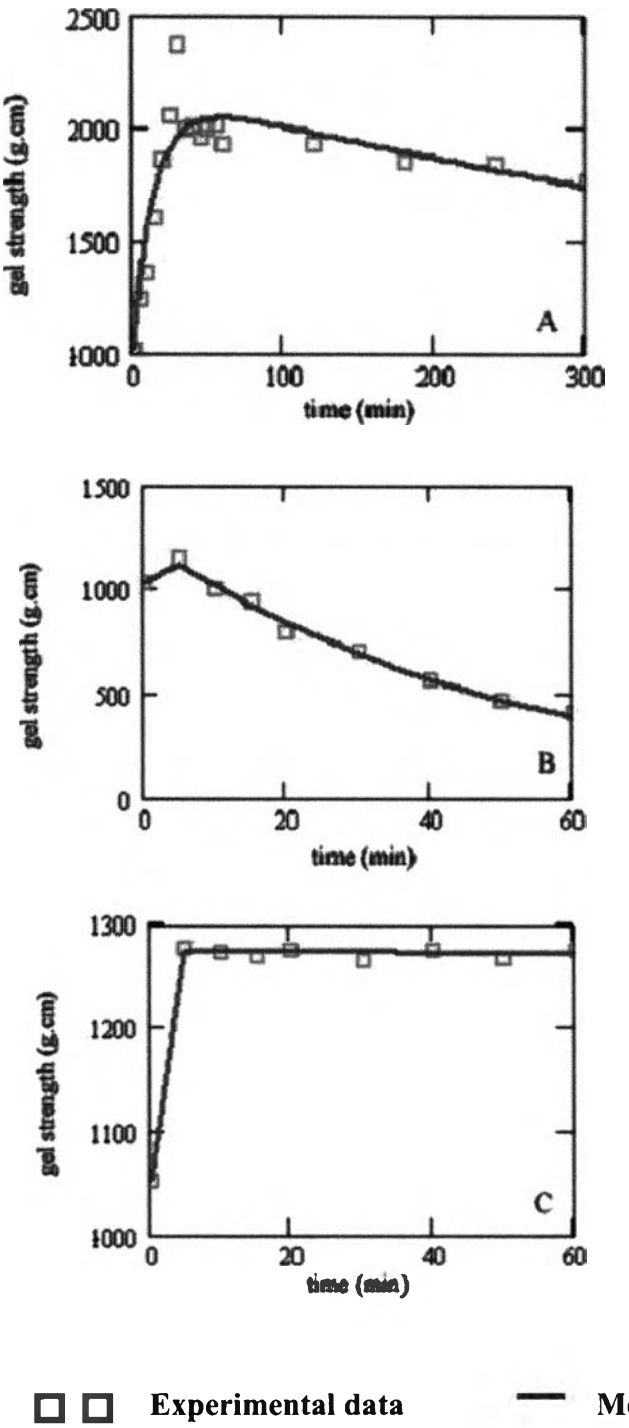

Figure 4.5 Comparison between experimental gel strength values for cod surimi during setting at (A) 40 °C; (B) 60 °C; (C) 80 °C, deduced from Equation 4.3 using the best fit values of k_1, k_2 and k_3.

Table 4.1 Values of k_1, k_2 and k_3 (Equation 4.3) at various setting temperature.

setting temperature (°C)	k_1 (min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})
35	0.067	1.19×10^{-3}	3.04×10^{-3}
40	0.060	7.25×10^{-4}	7.84×10^{-3}
45	0.123	1.18×10^{-3}	3.45×10^{-3}
50	0.213	1.32×10^{-3}	1.80×10^{-2}
60	4.765	1.90×10^{-2}	2.55×10^{-1}
80	4.790	2.41×10^{-5}	3.44×10^{-1}

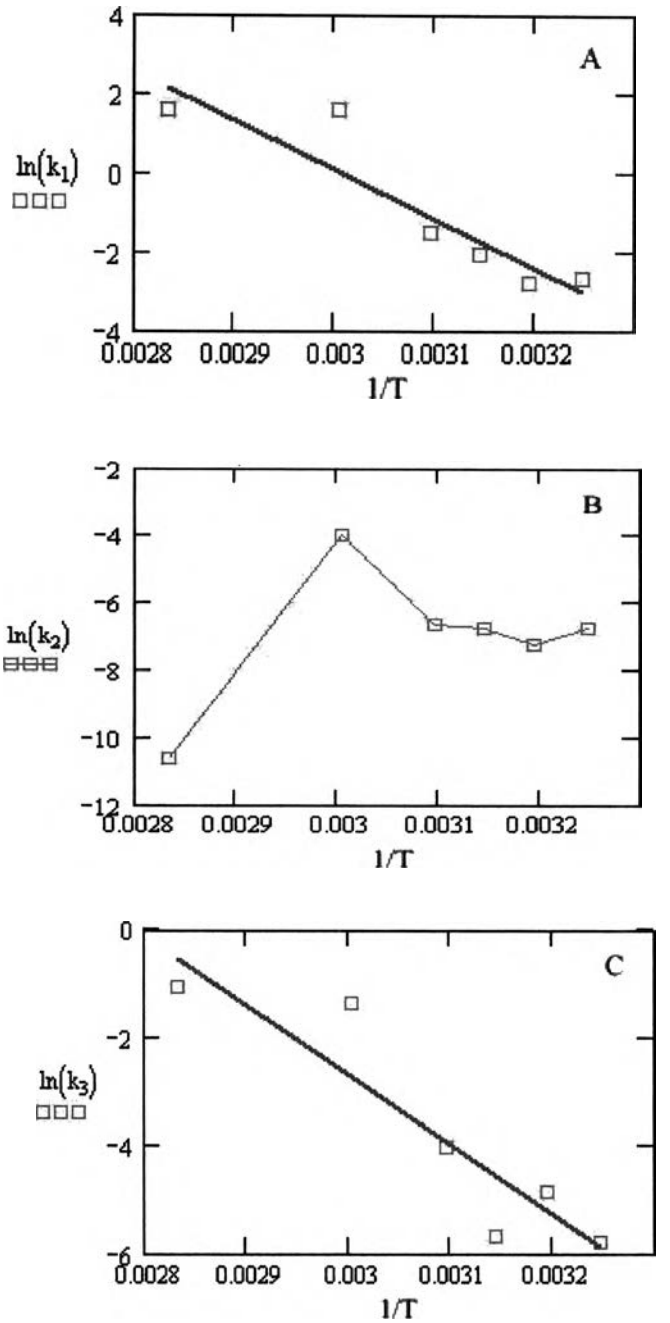

Figure 4.6 Effect of setting temperature on the rate constant of (A) textural formation (k_1), (B) textural degradation (k_2) and (C) fish protein degradation (k_3)

Figure 4.6A, 4.6B and 4.6C illustrate Arrhenius plots of ln k_1, ln k_2 and ln k_3 of gel strength versus the reciprocal of the absolute temperature. It is clear that the rate constant for texture formation (k_1) and fish protein degradation (k_3) follow Arrhenius equation, with values for ΔE_1 and ΔE_3 of 1.04 x 10^5 and 1.07 x 10^5 $kJ.kmol^{-1}$, respectively. However, the rate of gel degradation (k_2) does not follow Arrhenius kinetics: the results show that there are two different temperature regimes where the trend for degradation are markedly different. At the setting temperature between 35 to 60 °C, the rate constant increases; subsequently, it decreased in range 60-80 °C. This is because the activity of the proteinase enzyme was highest at 60 °C and was destroyed above 70 °C, as a result of thermal denaturation. The increase in k_1 is due to the heat-induced gel property of myofibrillar protein bringing about rapid network formation at higher temperatures. However, the gel needs an appropriate combination of temperature and time for complete network development. Thus cod surimi gel reaches its highest gel strength at a setting temperature of 40 °C in 20 minutes, which is a suitable condition for this gel. It is also clear that the time taken to reach the peak strength depends on the setting temperature.

As can be seen from the previous experiments that different setting temperatures in the initial step of gel formation provided cod surimi gels with different gel strengths and the gel strength passes through maximum area at a time depending on the setting temperature. This result emphasizes the significant role of temperature effects on the conformation of gel from myofibrillar proteins, myosin and actin.

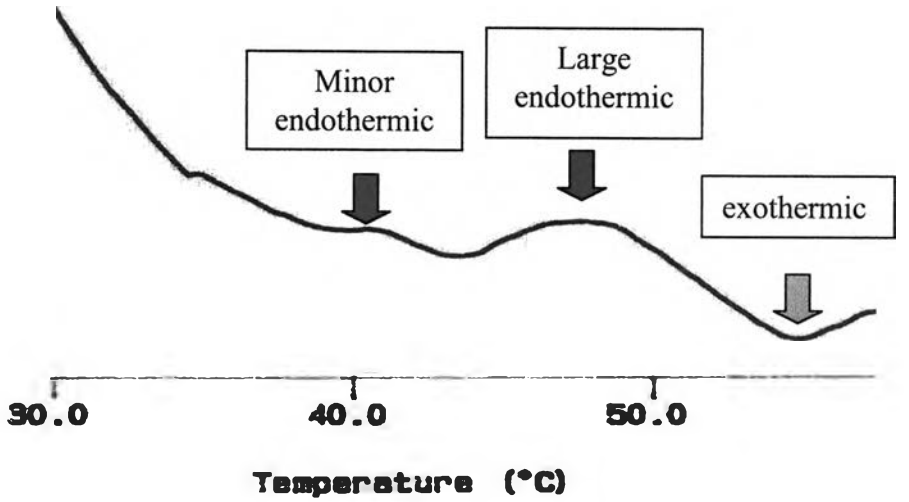

Figure 4.7 DSC thermogram of protein from cod surimi

The DSC analysis was therefore carried out to determine the thermal behavior of protein in cod surimi during thermal gelation and the results are shown in Figure 4.7. The events that make up the DSC thermo-profile are a net result of endothermic (protein denaturation) and exothermic (protein aggregation) processes occurring at the same time. It is possible that cod surimi protein undergoes thermal denaturation and localized exposure of hydrophobic residues as a result of unfolding. Next, the denatured protein begins to aggregate through intermolecular hydrophobic forces followed by clustering of the aggregates (Chan et al., 1992; and Gill et al., 1992).

The DSC thermograms in Figure 4.7 shows a minor endothermic region in the 37-43 °C range. This suggests that the natural actomyosin molecules undergo conformational changes. It is assumed that the conformation changes bring about active interaction between the molecules, such as the formation of intermolecular cross-linkages, leading to formation of the gel structure (Sano et al., 1988).

A large endothermic region in the range 43-55 °C shows that the conformation of the protein molecules changes further and the gel structure which begins to form between 35-45 °C advances to form a more dense state. These results demonstrate the mechanisms by which minced fish is converted into gel.

An exothermic region subsequently occurs showing the aggregation of unfolded protein. This step plays an important role in developing gel structure and strength. The net result of DSC thermogram suggests that protein denaturation step should occur first to allow the unfolded protein molecules to re-orient themselves, interact at specific points, and finally form an ordered three-dimentional network structure (Ziegler and Acton, 1984; Foegeding, 1988; Lin and Park, 1996). Similarly, Alvarez et al. (1999) suggested that the final structure of kamaboko networks was the outcome of denaturation-aggregation during setting of the surimi paste, and that this determines the possibility of reorganisation of the molecules to form the final network. All results suggest that high gel strengths should be obtained from the two-step heating method.

The present DSC results are in agreement with earlier DSC studies of fish natural actomyosin and myosin, which also report that the changes in viscoelasticity of natural actomyosin and myosin during thermal gelation relate to the conformation change of protein molecule (Sano et al., 1988). It also suggests that different gel strengths can be obtained by using different gel forming temperatures.

As mention previously, the different gel forming temperatures and times give different gel strength of surimi gel. Consequently, network structure examination by determine the volume fraction of solid (and void) and microstructural analyses using SEM were undertaken.

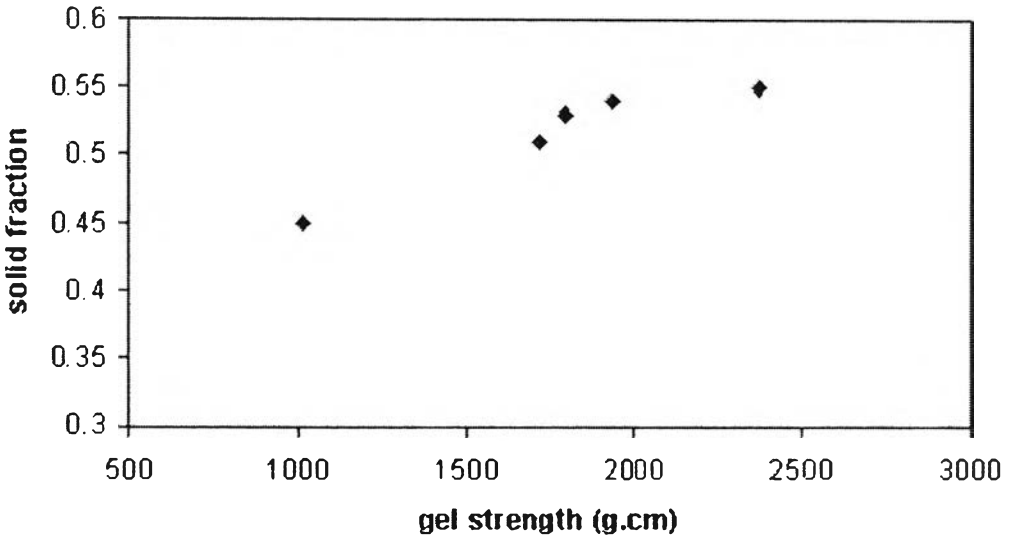

Figure 4.8 Relationship between solid fraction and gel strength for cod surimi gels setting at (A) without setting (gel strength 1015 g.cm); (B) 50 °C, 15 min (gel strength 1717 g.cm); (C) 35 °C, 35 min (gel strength 1796 g.cm); (D) 45 °C, 20 min (gel strength 1933 g.cm); (D) 40 °C, 30 min (gel strength 2373 g.cm).

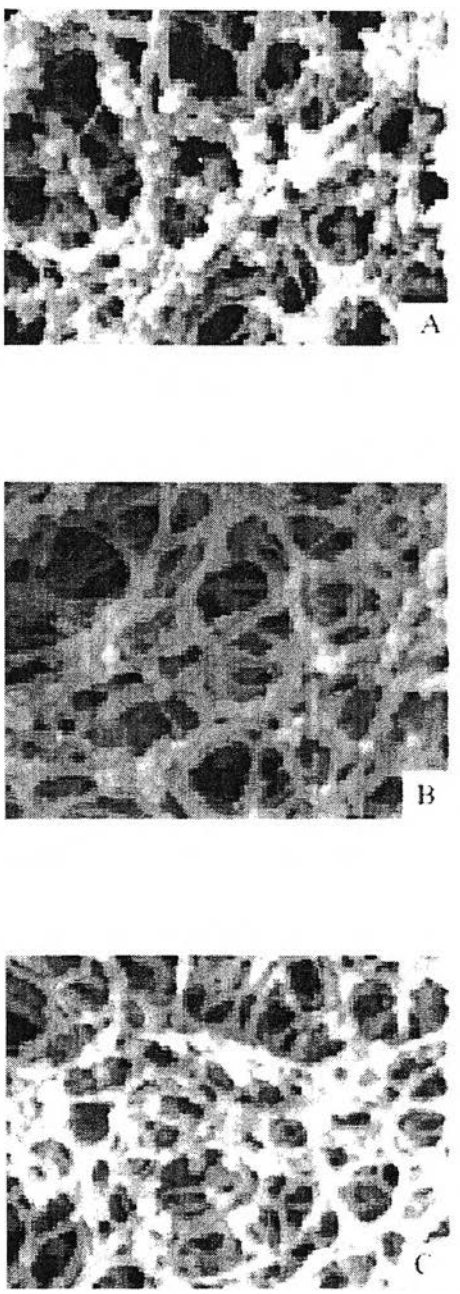

Figure 4.9 Scanning electron micrographs of gel network formed in cod surimi gels at gel strength of 1,015 g.cm (A), 1,717 g.cm (B) and 2,373 g.cm (C). Magnification = 10,000x.

Figure 4.8 shows that the gel strength changes can be correlated with the network structure as measured by the volume fraction of solid (and void). The solid fraction increased from 0.45 to 0.55 when the gel strength increased from 1,015 to 2,373 g.cm. Higher gel strengths were obtained with higher solid fraction (i.e. lower void fraction) gels. This can be explained by considering the effect of fineness and coarseness of the structure, in term of the thickness of the strands. The lower gel strength sample has thicker strands than the strands of the higher gel strength sample. However, the number density of strands (i.e. strands per unit area) for the low gel strength sample are lower than those for the high gel strength sample, as shown in Figure 4.9. This can explain why the lower gel strength sample has less solid fraction than the higher gel strength sample. Thinakorn (1998) found that increasing the void fraction (i.e. decreasing solid fraction) in the high density polyethylene (HDPE)/ natural rubber (NR) foam resulted in a decrease in hardness and tensile strength. Hug (2001) also reported that the compression force depended on the size of foam cell, normally expressed as pores per inch. It is evident that there is a strong relationship between the structure and the strength of the product. Similarly, cod surimi gel comprising of two parts —void and solid, also shows a correlation between gel strength and gel network including void and solid fraction, as revealed in Figure 4.8 and 4.9. Thus, the different structure of surimi gels can be represented by the different gel strengths.

4.3 Conclusion

Surimi gels were processed in two heating steps: setting at low temperature (35-50 °C for threadfin bream surimi and 35-80 °C for cod surimi) and then cooking at a higher temperature (90 °C). The mechanism of protein gelation, denaturation and aggregation are critical steps in forming heat-induced gels. The effect of different temperature-time histories experienced by surimi paste during the setting step on the final protein conformation results in different gel strengths and networks. Higher gel strengths were obtained with higher solid fraction gels and higher density of network fibres. A competitive-consecutive first order model of gel formation, enhanced by transglutaminase enzyme, and gel breakdown through proteolysis, has been developed to quantify the process of surimi gel development.