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ScienceDirect
Food Chemistry 108 (2008) 165–175
Food Chemistry
www.elsevier.com/locate/foodchem

Changes in protein compositions and their effects on physical changes of shrimp during boiling in salt solution

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Received 6 July 2007; received in revised form 25 September 2007; accepted 22 October 2007

Abstract

Dried shrimp is a high-value fishery product of Thailand. Boiling shrimp in salt solution is an important step during the production of dried shrimp and affects significantly the quality of dried shrimp. However, not much information is so far available on the effects of various boiling parameters on the quality changes of shrimp, especially in terms of the changes of shrimp protein compositions and their consequences on microstructural and physical changes of shrimp. The present work was thus aimed at studying the effects of boiling time and concentration of salt solution on the protein fractions, microstructural and physical changes of boiled shrimp. In addition, the relationships between protein compositions, cooking loss, texture and microstructure of shrimp were established using coupled image and fractal analysis. Boiling was investigated at various concentrations of salt solution and boiling times. Boiled shrimp was then evaluated in terms of its protein fractions, microstructure in terms of fractal dimension and physical changes (cooking loss and hardness). The relationships between all studied parameters were monitored and simple correlations between them were determined. The changes of cooking loss, hardness as well as the normalized changes of fractal dimension ($\Delta FD/FD_0$) highly correlated with the changes of protein compositions of shrimp.

Keywords: Boiled shrimp; Cooking loss; Fractal analysis; Image analysis; Protein fractions; Structure–protein fraction relationship; Texture

1. Introduction

Dried shrimp is a high-value fishery product of Thailand. Boiling shrimp in salt solution is one of the most important steps of dried shrimp production. The objectives of boiling shrimp in salt solution are to reduce the number of microorganisms in shrimp to an acceptable level and also to improve the flavor of shrimp. However, boiling affects several qualities of boiled shrimp, especially, cooking loss and texture due to changes of protein compositions, which are in turn affected by heat and salt content. Niamnuy, Devahastin, and Soponronnarit (2007) reported that the boiling conditions, namely, boiling time and concentration of salt solution, significantly affected the denaturation of protein in shrimp as well as the salt, moisture and protein contents of shrimp, which in turn influenced the various qualities of boiled shrimp, i.e., shrinkage and texture. Tapaneyasin, Devahastin, and Tansakul (2005) and Niamnuy, Devahastin, and Soponronnarit (in press) reported, through the use of sensory evaluation, that the boiling time and concentration of salt solution significantly affected the texture and overall acceptance of subsequently dried shrimp as well.

The protein in shrimp muscle can be divided into three main groups: myofibrillar (main protein in muscle fibre), sarcoplasmic (located in the interstitial region between individual muscle fibres) and stroma (endomysium, perimysium, epimysium connective tissue) protein. Several studies have indeed reported the relationship between protein compositions and property changes of marine foods

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doi:10.1016/j.foodchem.2007.10.058

during processing (Michalczyk & Surowka, 2007; Owusu-Ansah & Hultin, 1986; Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2007a; Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). However, no information is so far available on the effects of boiling in salt solution and its consequences on the changes of protein compositions and microstructure, which in turn affects many physical properties of shrimp.

It is well-known that microstructure affects many physical properties of a food product. Some studies have reported the relationship between microstructure and either chemical or physical changes of shrimp and other marine foods during processing (Beltran-Lugo, Maeda-Martinez, Aguilar, & Nolasco-Soria, 2006; Gomez-Guillen, Montero, Solas, & Borderias, 1998; Mizuta, Yamada, Miyagi, & Yoshinaka, 1999; Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2007b). However, it is not easy to describe changes of microstructure quantitatively without the use of other appropriate evaluation techniques. Recently, fractal analysis has been proposed as a tool to quantify microstructural changes of foods such as food powders, chocolate, bread, starch, among others (Barletta & Canovas, 1993; Pedreschi & Aguilera, 2000; Quevedo, Carlos, Aguilera, & Cadoche, 2002; Rahman, 1997). Moreover recently, relationships between various physical changes and microstructural changes of a food product have also been established successfully using fractal analysis. Kerdpiboon and Devahastin (2007) developed relationships between the fractal dimension of microstructural images and physical changes of carrot cubes during conventional hot air drying (HAD) and low-pressure superheated steam drying (LPSSD). The study showed that the apparent changes of carrot cubes, which were represented in terms of the percentage of shrinkage and rehydration behavior, correlated well with their normalized changes of fractal dimension ($\Delta FD/FD_0$) of the microstructural images.

Based on the above arguments the objectives of the present work were to establish the relationships between the concentration of salt solution on the protein compositions, cooking loss, texture and microstructural changes of shrimp during boiling in salt solution. In addition, the relationships between protein compositions and physical as well as microstructural changes of shrimp were established through the use of fractal dimension. Simple correlations between all studied parameters were also determined.

2. Material and methods

2.1. Materials

Raw fresh wild white shrimp (*Penaeus indicus*) was obtained from a local seafood wholesaler with a large size (150–160 shrimp/kg). The season only slightly influences the quality of raw shrimp used in this study because shrimp was caught from the source that has plentiful food and the climate is warm throughout the year. After grading and washing with tap water raw shrimp was weighed; 150 ± 0.5 g of raw deheaded shrimp was used in each boiling experiment. After capture and during the preparation shrimp was kept in a polystyrene box filled with crushed ice at 2 °C. The time that shrimp was stored in crushed ice (from capture to boiling) was around 5 h.

2.2. Boiling in salt solution

Raw deheaded shrimp was first equilibrated at room temperature for 10 min prior to boiling in salt solution (NaCl solution) that was contained in a 14 cm diameter stainless steel vessel at the boiling temperature of salt solution. The boiling conditions are as follows: concentrations of salt solution of 0%, 2%, 3% and 4% (w/v); boiling time of 1, 3, 5 and 7 min. The mass ratio of shrimp to salt solution was fixed at 1:2 because it has been reported that the mass ratio of shrimp to salt solution does not significantly affect any qualities of boiled shrimp, so the lower ratio is preferred to reduce the wastewater from the boiling process (Niamnuy et al., 2007). Boiling shrimp samples were just taken out from the vessel and then cooled down by storing the samples in crushed ice prior to quality analysis (within 30 min). All boiling experiments were performed in duplicate.

2.3. Determination of protein compositions

Muscle protein of shrimp was actionated according to the method of Hashimoto, Watabe, Kono, and Shiro (1979); the steps are shown in Fig. 1. Shrimp samples (20 g) were weighed into 10 volumes of buffer A (15.6 mM of Na_2HPO_4 and 3.5 mM of KH_2PO_4, pH 7.5), and homogenized fully with an Ultra-Turrax homogenizer (Kika Labortechnik, T25 basic, Staufen, Germany). The homogenate was centrifuged (Hitachi, CR21, Tokyo, Japan) at 5000*g* at 4 °C for 15 min. The extraction was performed three times. These three supernatants were combined and mixed with 10 volumes of 5% (w/v) cold trichloroacetic acid. The resulting precipitate was regarded as sarcoplasmic protein fraction and was collected by filtration. The supernatant was obtained as non-protein nitrogenous compounds fraction.

The above residue was homogenized with 10 volumes of buffer B (0.45 M of KCl, 15.6 mM of Na_2HPO_4 and 3.5 mM of KH_2PO_4, pH 7.5) in a Waring blender (Waring Commercial Blender, HGB2WP, Torrington, CT, USA) and centrifuged at 5000*g* at 4 °C for 15 min. The extraction was performed three times. All supernatants were combined and regarded as myofibrillar protein fraction. The residue was extracted overnight with 0.1 N NaOH under continuous stirring and then centrifuged at 5000*g* at 4 °C for 15 min. The supernatant was the alkali-soluble protein fraction. The final residue was regarded as stroma protein fraction (alkali-insoluble protein fraction). Total protein contents were determined by the Kjeldahl method with the conversion factor of 6.25 (AOAC, 2000) and the overall protein compositions of shrimp muscle were then calcu-

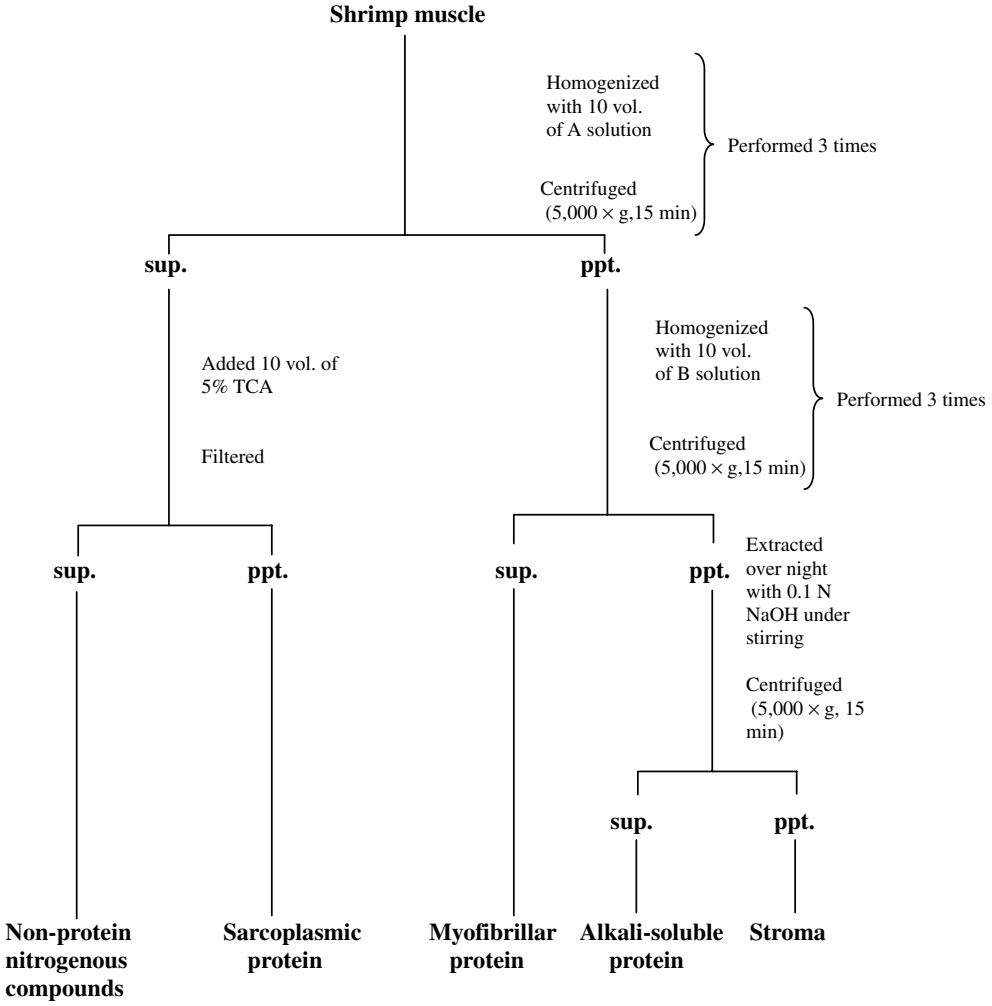

Fig. 1. Procedures for fractionation of shrimp muscle protein.

lated. The measurements were performed four times for each boiling condition and the average values are reported.

Protein loss was calculated from the differences in the protein content of shrimp before and after cooking (boiling) based on the dry basis. It is important to note that salt content in shrimp that increased during boiling was also included in the calculation

$$\%\ \text{Protein loss (d.b.)} = \%\ \text{Protein before cooking (d.b.)} - \%\ \text{Protein after cooking (d.b.)} \quad (1)$$

2.4. Cooking loss measurement

Cooking loss was calculated from the differences in the mass of shrimp before and after cooking (boiling)

$$\%\ \text{Cooking loss} = \frac{(\text{Mass before cooking} - \text{Mass after cooking})}{(\text{Mass before cooking})} \times 100 \quad (2)$$

The measurements were performed four times for each boiling condition and the average values are reported.

2.5. Texture analysis

A texture analyzer (Stable Micro Systems, TA.XT.-Plus, Surrey, UK) with 50 kg load cell was used to evaluate the texture of boiled shrimp. The 2nd (thickest) segment of deshelled shrimp was cut into a square with dimensions of $8 \times 8\ \text{mm}^2$; the thickness of the sample was dictated by the thickness of that particular shrimp sample. The cube was placed on a planar base in the direction of variable thickness and the force was then applied to the sample by a compression probe (Stable Micro Systems, P50, Surrey, UK) at a constant speed of 50 mm/min to a depth of 70% deformation. The maximum compressive stresses (maximum compressive forces during compression divided by the contact area between shrimp and probe) were indicated as the hardness values (Niamnuy et al., 2007). The measurements were performed on 20 shrimp samples per boiling condition and the average values are reported.

2.6. Measurement of microstructural changes and image processing

2.6.1. Microstructural imaging

The first step of microstructural imaging was fixation, in which each sample was preserved with Bouin's solution at room temperature for 24 h prior to passing through the remaining steps. The sample was subsequently soaked with flowing distilled water at room temperature for 20 min before removing the remaining moisture within the sample cells by flushing the sample with a series of isopropyl alcohol solutions at different concentrations starting with 50%, 70%, 95%, 95%, 95%, 100%, 100% and 100% (v/v), respectively. The sample was flushed with isopropyl alcohol from the lower to higher concentrations to prevent the damage of the cell structure, which might occur from the sudden loss of moisture (Kerdpiboon & Devahastin, 2007). Isopropyl alcohol within the sample was subsequently removed by flushing the sample with absolute xylene twice. The time used for flushing the sample with each concentration of isopropyl alcohol and absolute xylene was approximately 90 min per solution. Finally, the pores of the sample were replaced with paraffin by dipping the sample in melted paraffin at 60 °C (Aguilera & Stanley, 1999; Humason, 1979).

The treated sample was embedded in paraffin wax, which has a melting point in the range of 58–59 °C. The steps for preparing an embedded sample started from pouring heated paraffin wax into a stainless steel box with dimensions of $30 \times 24 \times 5\ mm^3$ to cover approximately one-third of the depth of the box. The sample was then placed at the middle of the stainless steel box and covered with a plastic embedded ring. Finally, additional liquid paraffin was poured to fully fill the box. The paraffin within the embedded sample could be set at room temperature and kept in a refrigerator at 4 °C. The cool embedded sample was easier to be sectioned than the sample at room temperature.

Each embedded sample was sectioned by a microtome (Jung, RM2025, Heidelberg, Germany) into a 8 μm thick slice. Shrimp was sliced at its 2nd segment. Sliced sample was placed on a glass slide and 95% ethanol was dropped indirectly to the sliced sample to expand the cell tissue. Ethanol was then eliminated by floating the sliced sample in water at 42–43 °C; the sliced sample was then immediately moved to the glass slide. The sliced sample was dried by placing at room temperature and then fixed on the glass slide with the use of a hotplate set at temperature of 48–50 °C for 8 h. The finished slide was then dyed using the Azan stain method (Humason, 1979); the shrimp muscle was highlighted in red and collagen was highlighted in blue. Since the structure of shrimp samples could be damaged by excessive temperature during the procedures, the temperature at each step had to be monitored carefully. Finally, the microstructural images were obtained using a light microscope (Olympus, BX51, Tokyo, Japan) at a 20× magnification level.

2.6.2. Image processing

A light microscopic image of the sample was captured by a digital camera (Olympus, C-5060, Tokyo, Japan) at image sizes of 520×520 pixels. The image was then transformed from a red, green and blue (RGB) format to a black and white format. The edge of the muscle was then detected to determine the perimeter of each muscle fibre, which was in turn used to calculate the fractal dimension.

2.6.3. Fractal dimension (FD) calculation

The fractal dimension of a black and white image was calculated using the box counting method (Quevedo et al., 2002) using MATLAB™ software (version 7.01). Ten light microscopic images were used for each sample. Cubic boxes with different sizes (r) were mounted into the images. The number of boxes (N_r), which were formed on the image was then counted. Fractal dimension was calculated by

$$FD = \frac{\log(N_r)}{\log(1/r)} \tag{3}$$

To obtain the changing values of FD of the sample undergoing boiling in salt solution, the normalized changes of FD are reported as (Kerdpiboon & Devahastin, 2007)

$$\Delta FD/FD_0 \tag{4}$$

$$\Delta FD = FD_t - FD_0 \tag{5}$$

where FD_0 and FD_t are the fractal dimension of the fresh sample and the fractal dimension of the sample at any instant during boiling in salt solution, respectively.

2.7. Statistical analysis

A full-factorial experimental design was applied to the experiments. All data were subjected to a statistical analysis. The effects of concentration of salt solution and boiling time on the various dependent variables were determined by the univariate full-factorial analysis of variance (ANOVA) using SPSS® software (version 10). All data were presented as mean values ± standard deviations (SD). Differences between mean values were established using the Tukey's multiple range tests at a confidence level of 95%.

The correlations between FD, protein composition changes and physical changes were determined by the Pearson's correlation coefficient. Pearson's correlation coefficient (R) is a linear correlation between two continuous variables. The value for the Pearson's coefficient can fall between 0.00 (no linear correlation) and ±1.00 (perfect linear correlation). The sign of this correlation coefficient (+, −) represents the direction of the relationship. A positive R indicates that the values of both variables change in the same directions while a negative R indicates that the values of both variables change in the opposite directions.

3. Results and discussion

3.1. Changes in shrimp protein compositions

Table 1 shows the detailed compositions of muscle protein, which varied with the boiling time and concentration of salt solution. For raw shrimp, the myofibrillar protein fraction (salt-soluble protein) was the major protein component; this was followed by sarcoplasmic protein (water-soluble protein) and stroma (alkali-insoluble protein). The alkali-soluble protein in raw shrimp, observed to be around 0.8% (dry basis), might exist due to the denaturation of protein during storage of shrimp in crushed ice prior to protein measurement.

Considering the effect of concentration of salt solution, myofibrillar, sarcoplasmic and stroma protein fractions significantly decreased but alkali-soluble protein fraction significantly increased with an increase in the concentration of salt solution. This is a well-known salting-out effect of protein (Fennema, 1996). However, it is important to note that shrimp protein was not really extracted in pure buffer A when salt was in the shrimp muscle; this might influence the protein compositions of shrimp. Increasing the NaCl content in the muscle has been shown to decrease the thermal stability of muscle protein at low salt concentration (Kijowski & Mast, 1988) leading to higher rates of transformation of myofibrillar and sarcoplasmic protein to alkali-soluble protein. However, protein that still did not

Table 1
Mean ± SD protein compositions of shrimp during boiling in salt solution ($n = 4$)

Parameter		% Protein (dry basis)				
Concentration of salt solution (w/v)	Boiling time (min)	Myofibrillar protein	Sarcoplasmic protein	Stroma	Alkali-soluble protein	Protein loss
Raw shrimp		54.58 ± 4.29^{f}	26.48 ± 2.56^{e}	3.72 ± 0.24^{d}	0.81 ± 0.07^{a}	0.00 ± 0.00^{a}
0	1	30.72 ± 2.51^{e}	13.71 ± 1.28^{d}	3.59 ± 0.21^{cd}	36.68 ± 2.84^{b}	0.89 ± 0.09^{ab}
	3	20.38 ± 2.14^{d}	8.51 ± 1.31^{c}	3.52 ± 0.28^{cd}	51.23 ± 3.63^{d}	1.95 ± 0.12^{b}
	5	15.76 ± 1.57^{c}	5.71 ± 0.86^{b}	3.49 ± 0.31^{cd}	58.02 ± 2.59^{d}	2.61 ± 0.52^{b}
	7	12.75 ± 2.34^{bc}	3.39 ± 0.73^{ab}	3.44 ± 0.20^{cd}	63.16 ± 3.52^{ef}	2.85 ± 0.23^{bc}
2	1	28.64 ± 3.68^{e}	13.05 ± 1.22^{d}	3.51 ± 0.33^{cd}	38.48 ± 2.88^{b}	1.31 ± 0.10^{ab}
	3	16.87 ± 2.55^{c}	6.63 ± 0.33^{bc}	3.40 ± 0.26^{cd}	55.17 ± 4.21^{d}	2.62 ± 0.16^{b}
	5	11.59 ± 2.28^{b}	3.83 ± 0.21^{ab}	3.28 ± 0.18^{c}	61.61 ± 3.26^{e}	4.08 ± 0.38^{c}
	7	9.01 ± 1.17^{ab}	1.87 ± 0.13^{a}	3.21 ± 0.29^{c}	65.28 ± 3.86^{f}	4.52 ± 0.34^{cd}
3	1	21.66 ± 1.86^{d}	10.37 ± 1.23^{cd}	3.63 ± 0.26^{cd}	46.78 ± 2.53^{c}	1.95 ± 0.33^{b}
	3	14.03 ± 1.45^{bc}	6.78 ± 0.655^{bc}	3.38 ± 0.20^{cd}	55.97 ± 3.24^{d}	3.83 ± 0.28^{c}
	5	9.20 ± 2.23^{ab}	3.43 ± 0.23^{ab}	3.28 ± 0.17^{c}	62.43 ± 3.72^{ef}	5.15 ± 0.34^{d}
	7	7.19 ± 1.71^{a}	1.49 ± 0.16^{a}	3.12 ± 0.21^{b}	65.34 ± 3.33^{f}	5.55 ± 0.29^{d}
4	1	13.99 ± 2.86^{bc}	8.10 ± 1.21^{c}	3.44 ± 0.22^{c}	54.94 ± 2.61^{d}	2.49 ± 0.31^{b}
	3	11.20 ± 1.23^{b}	4.95 ± 0.48^{b}	3.15 ± 0.23^{b}	60.34 ± 3.525^{e}	4.18 ± 0.30^{c}
	5	7.41 ± 1.12^{a}	2.19 ± 0.27^{a}	2.98 ± 0.14^{b}	63.92 ± 4.12^{ef}	5.49 ± 0.38^{d}
	7	5.58 ± 0.86^{a}	1.19 ± 0.194^{a}	2.59 ± 0.20^{a}	66.52 ± 3.42^{f}	5.61 ± 0.29^{d}

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

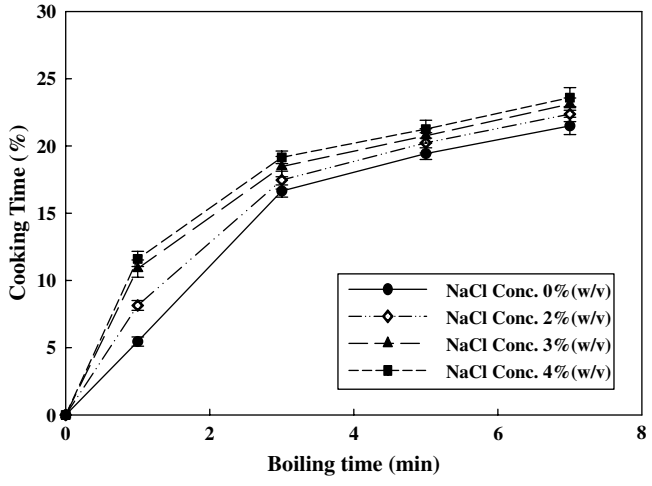

Fig. 2. Percentage of cooking loss of shrimp during boiling in salt solution.

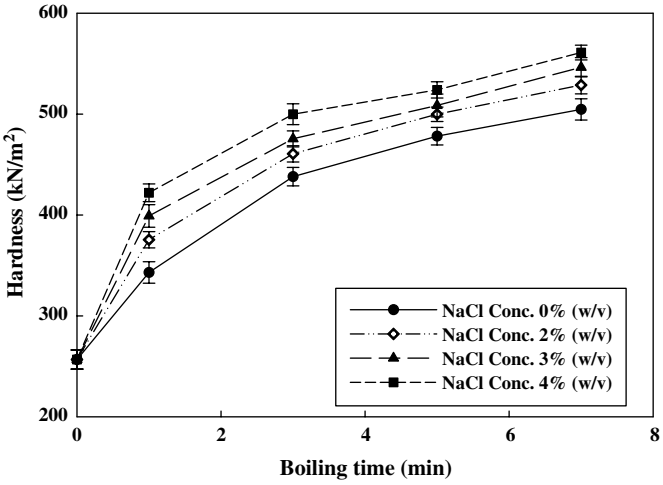

Fig. 3. Hardness of shrimp during boiling in salt solution.

denature could be dissolved in salt solution (Hashimoto et al., 1979) leading to higher protein loss during boiling. Within the concentration range of this study, all shrimp protein could be dissolved in salt solution and the amount of dissolved protein increased with an increase in the concentration of salt solution (Niamnuy et al., 2007; Regenstein & Stamm, 1980).

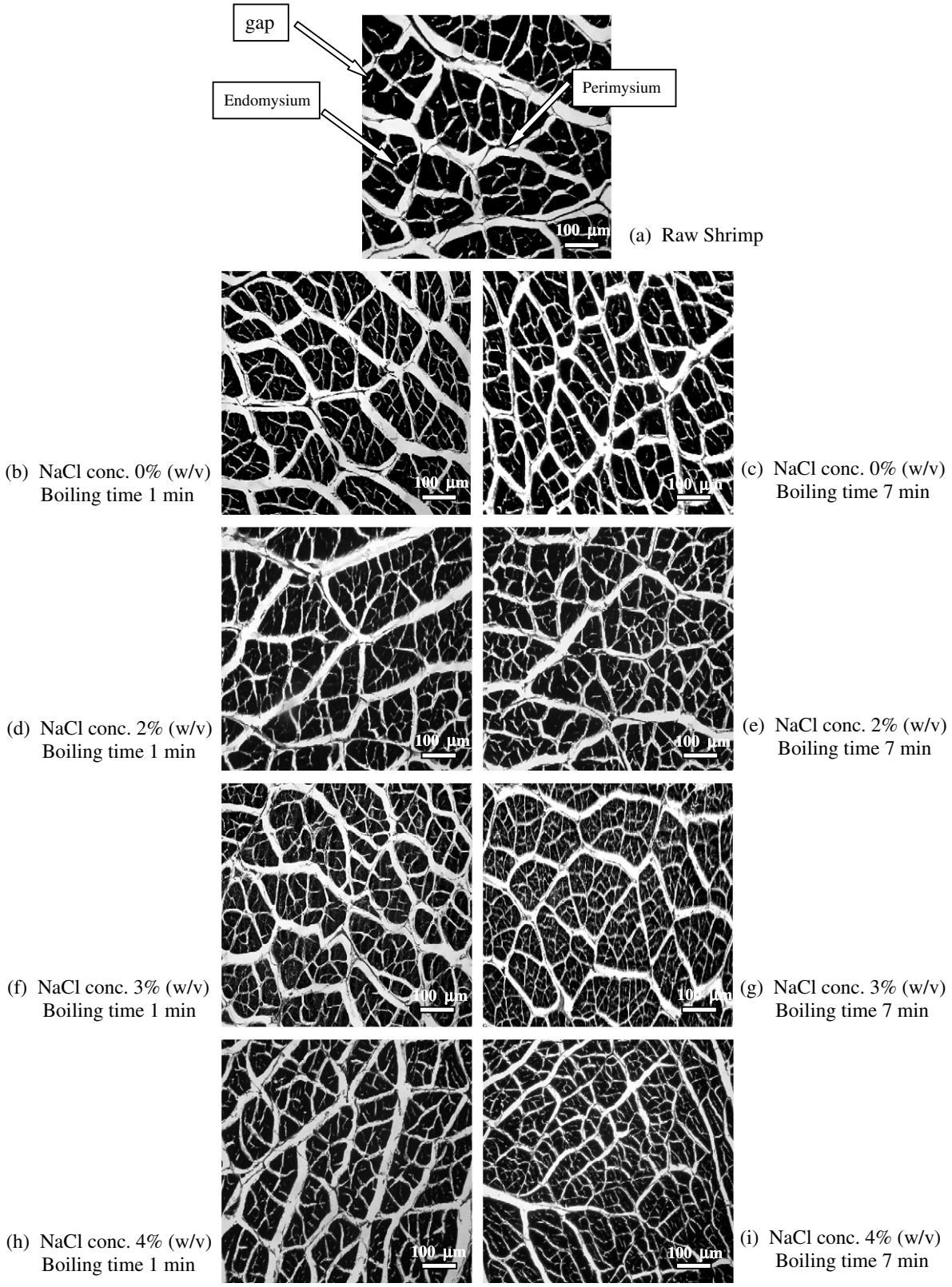

Fig. 4. Microstructure of shrimp during boiling in salt solution.

As the boiling time increased, changes in the protein compositions of shrimp were illustrated by a significant decrease in myofibrillar, sarcoplasmic and stroma protein fractions, accompanied by a significant increase in the alkali-soluble protein fraction and protein loss. During boiling in salt solution, when the temperature of shrimp reached the denaturation temperature of each protein (the denaturation temperatures of myosin, sarcoplasmic protein as well as of collagen and actin for fishery meat are 46–48 °C, 56–57 °C and 76–79 °C, respectively) (Michalczyk & Surowka, 2007; Paredi, Tomas, Crupkin, & Anon, 1996), denatured protein were detected as alkali-soluble protein. Again, protein that still did not denature could be dissolved in boiling salt solution leading to higher protein loss during boiling. In addition, accumulation of salt in shrimp meat during boiling accelerated dissolution of protein in solution. During the final period of boiling, however, protein loss was slowing down since most protein had already denatured. It was observed that the denaturation rates of protein were evidently higher than the rates of dissolution as shown in Table 1.

3.2. Physical changes of shrimp

Most of the water in muscle is held within the myofibrils, in the space between the thick filaments (myosin) and thin filaments (actin) (Offer et al., 1989) and some of the water is located in connective tissue (stroma). The moisture content of raw shrimp was around 80% (wet basis). As boiling proceeded, heat induced protein denaturation and aggregation leading to shrinkage of both the filament lattice and the collagen and also to exposure of hydrophobic areas of the myofibrillar structure, which allowed new intra- and inter-protein interactions that resulted in a more dense protein structure (Fennema, 1996; Straadt, Rasmussen, Anderson, & Bertram, 2007). Hence, water was pressed out of the muscle cells leading to water loss. It can be observed that the moisture content of boiled shrimp were around 75% (wet basis) and 72% (wet basis) for shrimp boiled in salt solution with salt concentration of 4% (w/v) for boiling time of 1 and 7 min, respectively. Water loss and protein loss were the main losses of the cooking loss.

In addition, hardness increased partly during boiling due to denser protein structure. Hence, the degrees of cooking loss and hardness of shrimp proportionally increased with the degrees of protein denaturation and protein loss. It can be seen that cooking loss (as shown in Fig. 2) and hardness (as shown in Fig. 3) significantly increased with an increase in the boiling time but insignificantly increased with the concentration of salt solution for the reasons mentioned earlier (Erdogdu, Balaban, Otwell, & Garrido, 2004).

3.3. Microstructural changes of shrimp

During boiling changes in meat structure such as destruction of cell membranes, shrinkage of muscle fibre, aggregation of sarcoplasmic protein and shrinkage and solubilisation of stroma protein occur (Niamnuy et al., 2007; Rowe, 1989). The changes of the microstructure of shrimp during boiling in salt solution were also investigated in this study. Light microscopic images of raw and boiled shrimp, focusing mainly on the transverse inner part of the shrimp meat, indicating muscle fibre, endomysium and perimysium, are shown in Fig. 4. The muscle fibre of raw shrimp was plump although there were gaps between muscle fibres (as shown in Fig. 4a). The gaps might occur due to small degradation in the muscle structure post mortem, especially in the case of endomysium connective tissue (Sotelo et al., 2004). As the boiling time as well as the concentration of salt solution increased, remarkable shrinkage of muscle fibres occurred due to protein denaturation and aggregation; the gap regions were also more frequently observed around the muscle fibres. Collagen, around the muscle fibres (endomysium) and around the muscle bundles (perimysium) reduced or even disappeared during boiling due to both thermal shrinkage and dissolution of gelatin (as shown in Fig. 4b–i).

However, it was rather difficult to define the microstructural changes of shrimp at various boiling conditions, especially at long boiling time and at high concentrations of salt solution. Fractal dimension (FD) was therefore used to represent the microstructural changes of the samples. FD of raw shrimp (Fig. 4a) was about 1.85 while FD of boiled shrimp samples varied between 1.87 and 1.96. The values of $\Delta FD/FD_0$, the significant changes of the fractal dimension of the samples compared with the fractal dimension of the raw shrimp ($p = 0.003$), are shown in Fig. 5. It was found that $\Delta FD/FD_0$ of the samples increased with the boiling time and concentration of salt solution. The shrinkage of muscle fibres significantly increased the fractal dimension, as expected. The boiling time had stronger influence on the fractal dimension changes than had the concentration of the salt solution, indicating that the effect of boiling time on the microstructural changes of shrimp was more obvious than the effect of salt concentration.

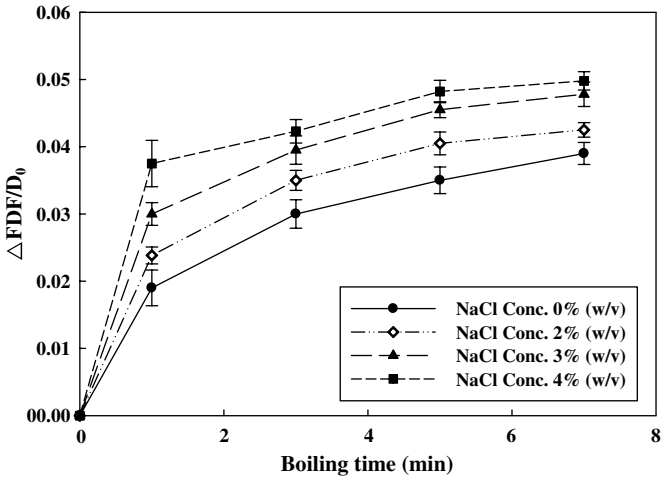

Fig. 5. $\Delta FD/FD_0$ of shrimp during boiling in salt solution.

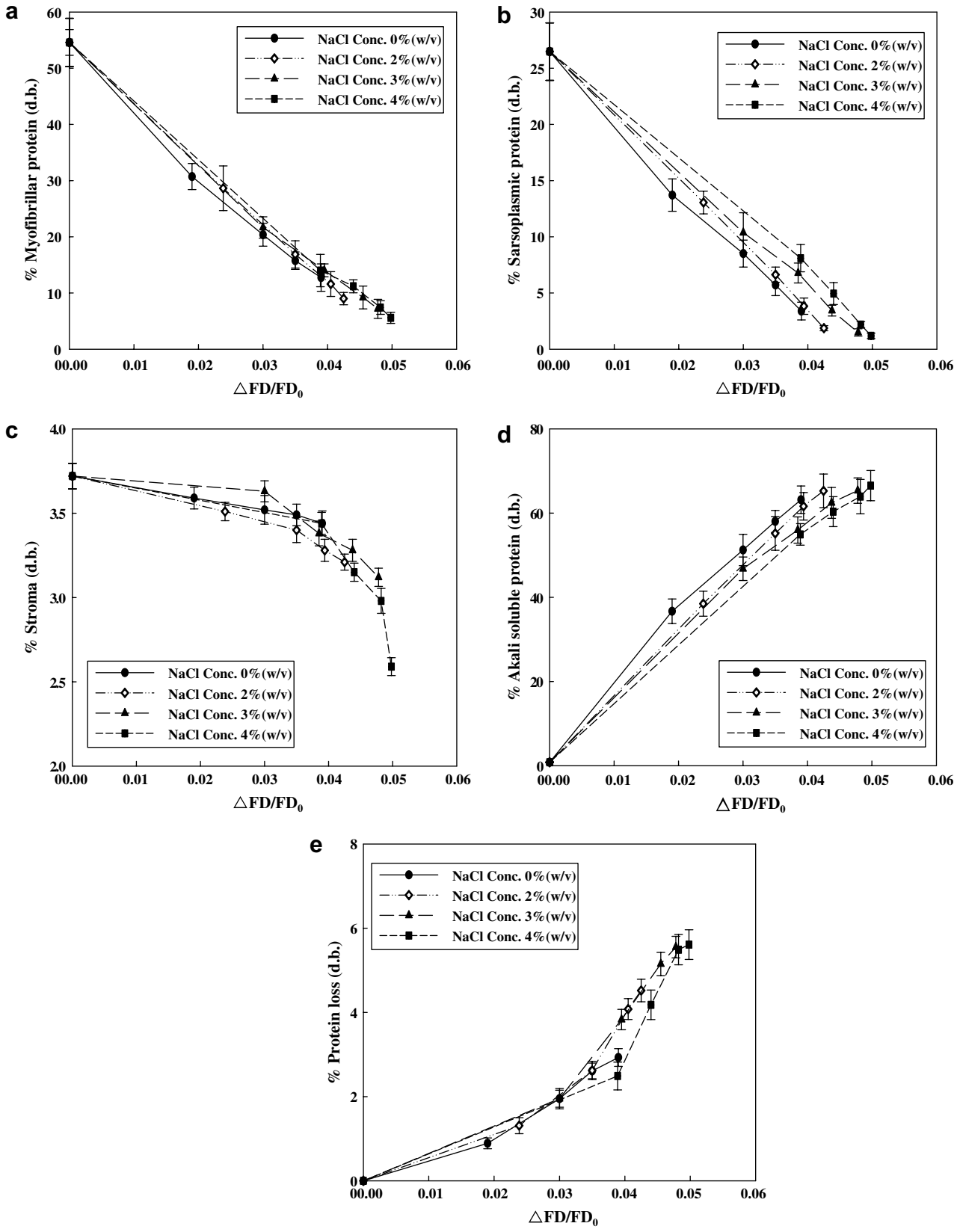

Fig. 6. Relationship between percentage of various proteins and $\Delta FD/FD_0$ of shrimp during boiling in salt solution.

3.4. Relationship between FD and protein composition changes of shrimp

Fig. 6 shows the relationship between protein fractions, namely, percentages (dry basis) of myofibrillar, sarcoplasmic, stroma, alkali-soluble protein as well as protein loss, and $\Delta FD/FD_0$. It was observed that there existed an inversely proportional relationship between the myofibrillar protein contents and $\Delta FD/FD_0$ at every concentration of salt solution (as shown in Fig. 6a). A similar trend existed in the case of sarcoplasmic protein fraction (as shown in Fig. 6b), whereas a proportional relationship between alkali-soluble protein and $\Delta FD/FD_0$ was observed (as shown in Fig. 6d). It was also observed that the Pearson's correlation coefficients showed high level of inverse relationship among fractal dimension (FD), myofibrillar protein, sarcoplasmic protein and alkali-soluble protein (Table 2). It is indeed known that transformation of myofibrillar and sarcoplasmic protein to alkali-soluble protein due to thermal denaturation and aggregation strongly affects microstructure of shrimp (Mizuta et al., 1999).

Fig. 6c illustrates the relationship between the percentage of stroma and $\Delta FD/FD_0$. It was found that the percentage of stroma decreased with an increase in $\Delta FD/FD_0$. However, the relationship was not quite good, probably because during boiling the collagen fibre shrank to only one-third of its resting length but it was still present in muscle as alkali-insoluble protein (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). Therefore, the stroma content might not well correspond to $\Delta FD/FD_0$.

The relationship between the percentage of protein loss and $\Delta FD/FD_0$ is shown in Fig. 6e. It can be observed that the percentage of protein loss increased with an increase in $\Delta FD/FD_0$. However, the relationship was not so obvious indicating that protein loss is not the main factor that induced microstructural changes during boiling. The normalized changes of fractal dimension could be used to monitor the protein composition changes of shrimp during boiling, especially in the cases of myofibrillar, sarcoplasmic and alkali-soluble protein (as shown in Fig. 6a, b and d). For example, at $\Delta FD/FD_0$ of approximately 0.03 the percentages of myofibrillar, sarcoplasmic and alkali-soluble protein of boiled shrimp were around 19–23%, 9–13% and 46–52% (dry basis), respectively, in all cases. This type of relationship was also observed at other $\Delta FD/FD_0$ and percentage of protein fractions in shrimp.

Some variations of the protein contents might be due to the effect of increased salt content in shrimp during boiling,

Table 2
Pearson's correlation coefficients of various parameters undergoing boiling in salt solution ($n = 4$)

	FD	Myofibrillar protein	Sarcoplasmic protein	Stroma	Alkali-soluble protein	Protein loss	Cooking loss	Hardness
FD	1.000	−0.991	−0.982	−0.726	0.981	0.872	0.940	0.968
Myofibrillar protein		1.000	0.991	0.643	−0.997	−0.820	−0.963	−0.978
Sarcoplasmic protein			1.000	0.642	−0.996	−0.812	−0.981	−0.989
Stroma				1.000	−0.627	−0.866	−0.684	−0.732
Alkali-soluble protein					1.000	0.785	0.963	0.978
Protein loss						1.000	0.834	0.862
Cooking loss							1.000	0.995
Hardness								1.000

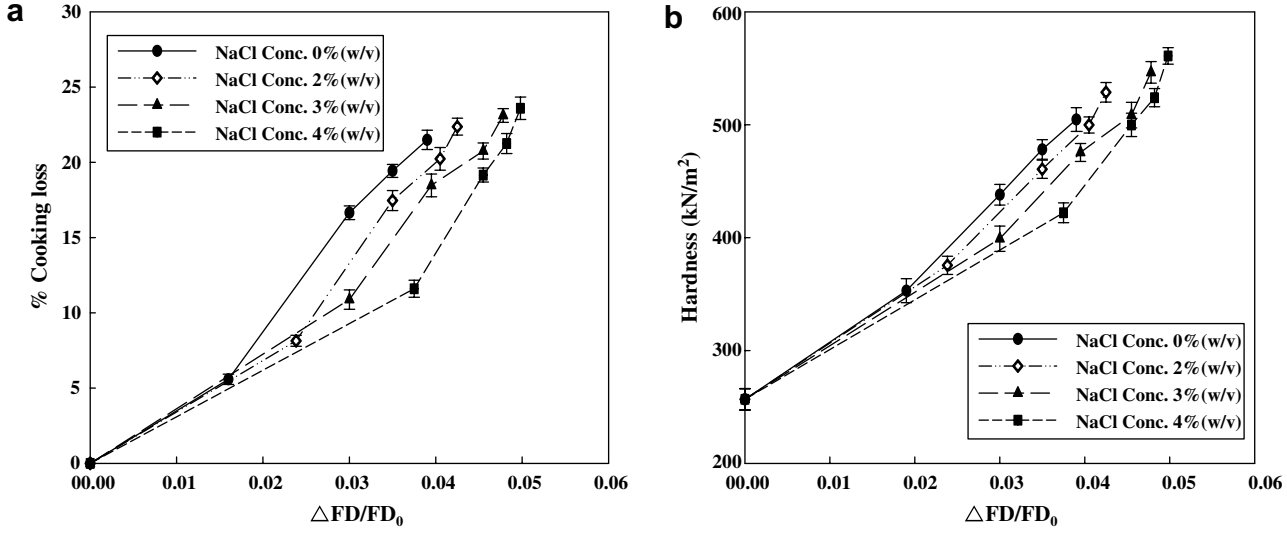

Fig. 7. Relationship between various physical changes and $\Delta FD/FD_0$ of shrimp during boiling in salt solution.

which in turn affected the protein fractions, but did not directly affect the microstructural changes of shrimp.

3.5. Relationship between FD and physical changes of shrimp

Fig. 7 shows the relationship between physical changes, namely, percentages of cooking loss and hardness, and $\Delta FD/FD_0$. It can be seen that well established relationships between both physical changes and $\Delta FD/FD_0$ existed. It can be observed that cooking loss and hardness of boiled shrimp increased with an increase of $\Delta FD/FD_0$. These could also be related to the relationships between protein fractions and $\Delta FD/FD_0$. The myofibrillar, sarcoplasmic and alkali-soluble protein contents of shrimp affected the water holding capacity, which was obviously related to the cooking loss and hardness of shrimp in the same way as in the cases of other meat products (Wilson & Van Laack, 1999).

The normalized changes of fractal dimension could also be used to monitor the changes in cooking loss and hardness of shrimp during boiling. For example, at $\Delta FD/FD_0$ of approximately 0.03 the percentage of cooking loss and hardness of boiled shrimp were around 8–13% and 370–420 kN/m^2, respectively, in all cases.

All results can also be used to explain the effect of boiling condition on the quality of dried shrimp. Boiling shrimp in salt solution of higher concentrations and for longer boiling time led to larger changes of microstructure and physical properties of boiled shrimp leading to harder and more shrinking dried shrimp (Niamnuy et al., in press).

The simple correlations between the changes of fractal dimension, changes of protein fractions and physical changes of shrimp during boiling are shown in Table 2. The results illustrate that the changes of fractal dimension correlated well with the changes in the protein fractions, namely, myofibrillar, sarcoplasmic and alkali-soluble protein and the physical changes, namely, cooking loss and hardness of shrimp. This trend is in good agreement with that of the previous studies in which high degree of correlations between protein denaturation, cooking loss and texture of various cooked meats were observed (Barbanti & Pasquini, 2005; Tornberg, 2005). However, the correlations of both the changes of stroma and protein loss with other parameters were not quite very well as shown in Table 2.

4. Conclusions

The effects of different boiling parameters on the protein fractions, microstructure and physical changes of boiled shrimp were investigated. The boiling time and concentration of salt solution affected the protein fractions in shrimp, which in turn influenced the microstructure, cooking loss and hardness of shrimp, as represented by the changes in $\Delta FD/FD_0$. An increase in boiling time and concentration of salt solution led to a decrease in the contents of myofibrillar, sarcoplasmic and stroma protein but to an increase in alkali-soluble protein and protein loss during boiling as well as to an increase in cooking loss, hardness and $\Delta FD/FD_0$ values. The changes of cooking loss, hardness as well as $\Delta FD/FD_0$ were highly correlated with the changes of myofibrillar, sarcoplasmic and alkali-soluble protein contents indicating that the muscle protein denaturation was an important factor influencing the microstructural and physical changes of shrimp during boiling in salt solution.

Acknowledgements

The authors express their sincere appreciation to the National Center for Genetic Engineering and Biotechnology (BIOTEC), the Thailand Research Fund (TRF), through its Royal Golden Jubilee scholarship to author Niamnuy, and the Commission on Higher Education for supporting the study financially. Our appreciation also goes to the Faculty of Fishery, Kasetsart University, Bangkok and the Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-ok, Chantaburi Campus, for their help with the microstructural imaging of the samples.

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