Effect of freshness and salt on quality of white shrimp

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Abstract. The concentration of salt (NaCl) in white shrimp, which is one of the main export products of Thailand, is very important in quality control. In this research study, Na and Cl distribution in white shrimp was investigated using the X-ray fluorescence (XRF) technique to examine salt absorption and distribution within samples at two freshness levels, freshness levels 3 and 4. White shrimp of both freshness levels were soaked in a salt solution at 9 \% (w/v) for 90 min and boiled for 110-130 sec, in accordance with work instructions from a factory. Additional samples were cooked after soaking, and still other samples of raw shrimp (not soaked, not cooked) were examined by the XRF technique and the results obtained were compared with quantitative salt analysis by the titration method. The concentration of Na and Cl can be matched to a color scale to represent the level of Na and Cl absorption. The results show that white shrimp with freshness level 3 absorbed a higher concentration of salt solution (2.49±0.11 \%) than the samples with freshness level 4 (2.20±0.14 \%). The results were similar to those resulting from the titration method, although the samples at freshness level 3 indicate a higher salt concentration. However, white shrimp with freshness level 4 also indicated a higher TVB-N value than that of freshness level 3, but still meets the Thai standard for frozen shrimps and prawns.

1. Introduction

Frozen white shrimp is one of the main export products of Thailand to many countries, thanks to its quality and variety. White shrimp (\textit{Litopenaeus vannamei}), or \textit{vannamei}, is smaller than black tiger shrimp but is a strong breed and therefore popular for cultivation in many other countries, including the USA, Mexico, Colombia and Peru \cite{1}. White shrimp has 8 segments in the body, including 1 segment for the head, 6 segments for the body and 1 segment for the tail.

The production of frozen white shrimp consists of several steps, including raw materials receiving, cleaning, sizing, trimming, soaking, freezing, and packing, as well as cooking in the case that the product is the cooked type \cite{2}. One of the important steps in the production process, which increases productivity
and improves product quality, is soaking in a salt solution. Generally, salt and phosphate compounds are used to increase water holding capacity and improve product quality after thawing and cooking of the shrimp [3].

Salt or sodium chloride (NaCl) contains sodium ions and chloride ions that are bonded with ionic bonds. Salt is very important to the food industry, as it is inexpensive and can be used for various purposes, including cooking and preservation. The purity of salt used in industry is 99.9 % and its moisture content does not exceed 0.15 % [4]. Salt helps animal muscles absorb more water due to the increased strength of the ionic bond in the muscle protein [5]. Lopkulkiaert et al. (2009) [6] studied the effects of sodium bicarbonate containing traces of citric acid in combination with sodium chloride on yield and freezing loss of white shrimp. Fresh shrimp soaked in sodium bicarbonate containing traces of citric acid at 4 g and sodium chloride at 3 g per 100 mL leads to an increase of the yield. The opposite result was found, in that the freezing loss decreased 6.83-10.28 % and 6.41-12.4 % for uncooked and cooked products, respectively. The study of Wachirasiri et al. (2016) [7] reported that shrimps treated with arginine at 1 g per 100 mL with NaCl 3 g per 100 mL had increased cooking yields from 75.02 to 97.59 g per 100 g of fresh shrimp. And Wachirasiri et al. (2016) [8] studied changes in processing yield and physical properties of frozen white shrimp (Penaeus vannamei) treated with lysine and sodium bicarbonate. It was found that combinations of lysine, NaHCO$_3$, and NaCl increased both the thawing yield and cooking yield of shrimp, comparable to the use of sodium tri-polyphosphate (STPP), by providing more negative charge resulting in increased electrostatic repulsion between the myofibrillar proteins. Salt concentration in frozen shrimp, both cooked and uncooked products, is very important since it affects the product quality and taste, as well as the heath concerns for salt concentration. The control and monitoring of salt concentration in shrimp after soaking in salt solution is required in the frozen shrimp industry. The penetration of salt into shrimp depends on many factors such as soaking time and the size of the shrimp.

X-ray fluorescence technique (XRF) is used to study the composition of the elements in a sample, based on the difference of the energy layers of each element. The advantage of this technique is the ease of sample preparation without product destruction [9], and XRF research can be applied in various fields of study. The study of the composition of cigarette smoke and ash by Wavelength Dispersive X-Ray Fluorescence Spectrometer (WDXRFS) showed that heavy elements found in ash did not contaminate in the smoke [10]. The study of skin permeability of titanium oxide and zinc oxide nanoparticles, which are added to sunscreen, showed that two nanoparticles gathered in the stratum corneum with no permeation into the epidermis and dermis [11]. Pengthamkeerati et al. (2018) [12] reported on the content of silicic (95.6 %) extracted from biomass fly ash by using alkaline hydrothermal treatment and analyzed by the XRF technique. XRF was applied in many research projects and yielded reliable results for the component analysis [13]. The objective of the present research project was to study the effect of salt and freshness on the quality of white shrimp. The concentration of salt in the shrimp meat after soaking and cooking was determined by titration. The absorption and distribution of the sodium and chloride in the shrimp meats were investigated using the XRF technique.

2. Material and Methods

2.1. Materials and quality determination

White shrimp was obtained from Marine Gold Products Ltd. (Samut Sakhon, Thailand). Generally, the shrimp freshness could be divided into 5 levels, following the transportation or logistic period, as follows: level 1 is “just harvested” shrimp, level 2 corresponds to shrimp during transportation to the processing plant, levels 3 and 4 concern shrimp in the processing plant, and level 5 denotes shrimp with deteriorated quality that can still be used for the processing. Consequently, the shrimp arriving at the plant indicated their freshness mostly as level 3. The shrimp of freshness level 3 and 4 was evaluated by the specialists in the factory with a weight of 16 g per pc, as shown in figure 1. The samples were cleaned with cool water, washed with chlorine water, de-headed and de-veined with the skin on, and soaked in
a salt solution at 8-12 % (w/v) for 60-120 min. For the cooking, the samples were further boiled for 110-
130 sec by heated circulators. Finally, all samples were peeled and frozen.

The quality of shrimp in terms of freshness is judged by its appearance; head color, adhesion of head
and body, eyes, body and skin, meat texture, and the odor of the samples according to the factory
standard. Moisture content of the samples was determined in accordance with AOAC (2005) [14]. The
pH level of the samples was determined by weighing 5 g of shrimp meat in a beaker with distilled water
45 mL, homogenized and measured by a pH meter. Total volatile base nitrogen (TVB-N) of the samples
was measured in accordance with Siang and Kim (1992) [15] using 4 g of shrimp meat in a beaker with
4 % trichloroacetic acid (TCA), homogenized and filtered through a Whatman paper filter no 41 to
obtain clear extract. Subsequently, 2 mL of the extract and 2 mL of K$_2$CO$_3$ were added by pipette into
the outer ring of a Conway’s dish, while 2 mL of 1% boric acid with indicator (Bromocresol green +
Methyl red) was added by pipette into the inner ring. The dish was then placed in an incubator at 37 ºC
for 60 min and titrated with 0.02 N HCl to the endpoint indicated by the change of the color of the
indicator from green to pink.

![Figure 1. White shrimp with different freshness levels; (a) freshness level 3 and (b) freshness level 4.](image)

### 2.2. Na and Cl distribution by XRF technique

Four shrimp samples, comprising raw white shrimp (a); white shrimp with freshness level 3 after salt
solution soaking (b); white shrimp with freshness level 4 after salt solution soaking (c); and freshness
level 3 after salt solution soaking and cooking (d), were peeled and frozen. Frozen shrimp meat from
the third abdominal segment were cut into slices of 50 µm-thick in cross section with the use of a
cryomicrotome (CM1950 Cryotome from Leica, Singapore) and mounted on aluminium sample cups.
Samples were air-dried for 3 days prior to the XRF analysis. Samples used for these studies were
obtained without fixative media as shown in figure 2. For the XRF measurement, a wavelength
dispersive XRF (ZSX Primus IV XRF, Rigaku, Japan) was used. The XRF uses a Rh-anode X-ray tube
operated at 4 kW. Primary X-ray filter Ni400 excited the sample at two energy levels, 1.04 and 2.62
keV, and for analysis an area diaphragm of 0.5 mm was used. A gas flow proportional counter (F-PC)
was used as a detector. For analysis of the shrimp specimens, the samples were measured at an area scan
of 450 x 450 µm with a 50 µm step size in the x- and y-directions. Under field conditions, only one
replicate was analyzed.

### 2.3. % salt determination by titration method

Four samples as described above were peeled, ground, and measured for the salt concentration. The
whole shrimp was homogenized and 2 g of the homogenized sample was placed in a beaker, 2 % HNO$_3$
20 mL was added and stirred with a magnetic bar for 5 min, then titration was performed with 0.1 N
AgNO$_3$ using an automatic titrator.
Figure 2. White shrimp meat in different conditions after cutting with a cryomicrotome; (a): raw white shrimp materials, (b): white shrimp with freshness level 3 after soaking in salt solution, (c): white shrimp with freshness level 4 after soaking in salt solution, and (d): white shrimp with freshness level 3 after soaking in salt solution and cooking.

2.4. Statistical analysis
Analysis of variance was performed to analyze the statistical significance using SPSS 23, and the mean value comparison was carried out by Duncan’s multiple range test.

3. Results and discussion

3.1. Raw material quality
The freshness of white shrimp can be divided into 5 levels during the whole storage time starting with catching from the pond (the best quality, level 1) or natural sources to a level that is not acceptable for processing (level 5). However, the level of shrimp freshness used in the factory can be divided into 2 levels (corresponding to level 3 and 4 on the basis of each freshness level), by observing appearance such as color and adhesion of head and body (Table 1). The appearance and odor were different due to different storage times before processing, as summarized in Table 1. Shrimp are marine animals that display quality deterioration easily, and that constantly changes with time by the enzymatic reaction and/or bacteria [16].

| Appearance and odor | Freshness level 3 | Freshness level 4 |
|---------------------|-------------------|-------------------|
| Head color          | Blue-gray cheeks. Some pieces begin to be slightly black and begins to be yellow in the middle of the head | Orange and black head color Yellow in the middle of the head |
| Adhesion of head and body | Head and body begin to separate from each other | Head and body are separated |
| Eyes                | Opaque eyes       | Increased opaque eyes |
| Body and skin       | Slightly glossy skin | Slightly glossy skin |
| Meat                | Opaque meat color | Increased opaque meat color |
| Odor                | Very slightly fishy smell odor | Slightly fishy smell odor |
The moisture content of shrimp at freshness levels 3 and 4 were 78.60±0.01 and 80.06±0.01 %, respectively \((p<0.05)\), as shown in Table 2. Sriket et al. [17] reported the chemical composition of white shrimp with moisture content of 77.2 %, which might be different due to several factors such as the method of raw material handling after death of the marine animals, soaking with ice and cold water, and storage time.

The pH of white shrimp with freshness levels 3 and 4 were 7.05±0.04 and 7.74±0.06, respectively \((p<0.05)\). A slightly basic pH of the samples might be caused by autolysis by protease that generates amines and ammonia formation [3].

The TVB-N values of shrimp samples were 10.11±1.35 and 15.55±1.35 mg of N per 100 g of samples from freshness levels 3 and 4, respectively. The TVB-N values increased with storage time for marine products, due to the autolysis of the animals after death [3]. However, TVB-N values were less than 20 mg of N or 30 mg of N per 100 g of samples, which is still considered as fresh shrimp [18] and acceptable according to the Thai standard for frozen shrimps and fresh prawns [19].

| Parameters | Freshness level 3 | Freshness level 4 |
|------------|-------------------|-------------------|
| Moisture content (%) | 78.60±0.01<sup>b</sup> | 80.06±0.01<sup>a</sup> |
| pH         | 7.05±0.04<sup>b</sup> | 7.74±0.06<sup>a</sup> |
| TVB-N      | 10.11±1.35<sup>b</sup> | 15.55±1.35<sup>a</sup> |

*Means of shrimp at different freshness (n=3).
<sup>a,b</sup> mean with different letter in same row are significantly different \((p<0.05)\).

### 3.2. Na and Cl distribution by XRF technique

Figure 2. shows sample preparation for XRF analysis of four shrimp samples. The samples are of four different conditions; raw white shrimp material, freshness level 3 after salt solution soaking, freshness level 4 after salt solution soaking, and freshness level 3 after salt solution soaking and cooking. The samples were analyzed for Na and Cl distribution using µ-XRF. The intensity of the sodium and salt concentration shown in the XRF mapping was normalized for all shrimp samples, as shown in figure 3. The results of element distributions were in accordance with the quantitative salt analysis performed by automatic titrator as shown in Table 3. The color scale, blue and dark blue, was observed in the fresh raw shrimp (3a). The shrimp with better quality, freshness level 3 after soaking (3b) in the salt solution, absorbed more sodium chloride than the sample from lower qualities (the samples with freshness level 4, 3c). However, the salt concentration in the soaked and cooked shrimps decreased sharply, as can be seen from the titration method and exhibited clearly by the µ-XRF (3d). Shrimp at both freshness levels lost their salt content after cooking and cooling. Protein denaturation was apparent from the shrimp meat appearance. The intensity of the Na and Cl was high near the de-veining area, resulting from the penetration and distribution of Na and Cl through the shrimp meat. In addition, these Na and Cl hot spots were rarely observed in the cooked shrimp samples, a result of loss of salt content during the cooking and cooling process. Na co-localization with Cl was also observed in µ-XRF maps, inferring that NaCl salt was obtained, which arises from undissolved NaCl or recrystallization of NaCl during drying. Such co-localization of Na with Cl might be attributed to NaCl crystals, and additional research will be needed to resolve and characterize the implications for co-localization. A study by Chantharat et al. (2005) [20] reported a decrease of myosin in fresh fish with increased storage time. Myosin can hold the water in a sample. Therefore, more red area was found in the sample with freshness level 3 after soaking (3b), while that of freshness level 4 showed less red area.
Table 3. Salinity of white shrimp samples with different freshness levels (by titration method).

| White shrimp samples     | Freshness level 3 | Freshness level 4 |
|--------------------------|-------------------|-------------------|
| Raw shrimp\textsuperscript{A} | 0.25±0.02\textsuperscript{C} | 0.28±0.01\textsuperscript{C} |
| After soaking\textsuperscript{A} | 2.49±0.11\textsuperscript{A} | 2.20±0.14\textsuperscript{A} |
| After cooking\textsuperscript{A} | 1.50±0.07\textsuperscript{B} | 1.54±0.03\textsuperscript{B} |

*Mean of white shrimp samples salinity (n=3). \textsuperscript{A, B, C} and \textsuperscript{A, B, C} mean with different letter in the same column and row are significantly different (p<0.05), respectively. \textsuperscript{A} mean in same row is not significantly different (p<0.05).

![Figure 3. Concentration of Na and Cl by the X-ray fluorescence (XRF) technique on (a) raw white shrimp material, (b) white shrimp with freshness level 3 after salt solution soaking, (c) white shrimp with freshness level 4 after salt solution soaking, and (d) white shrimp with freshness level 3 after salt solution soaking and cooking. In the color scale, red represents a high concentration of the element while](image-url)
blue represents a low concentration of the element. The red box indicates the sample area for the XRF analysis, 450x450 μm.

4. Conclusion
In conclusion, the X-ray fluorescence (XRF) technique can be used to monitor the distribution of salt solution within the shrimp meat. It can give an accurate result and is very close to the results obtained by the titration method. The result of saline distribution analysis in the samples shows that differences of freshness level affect absorption and distribution of salt solution.

The freshness of white shrimp affected the absorption of salt and water holding capacity. The white shrimp at freshness level 3 showed a larger area of salt distribution, as investigated by XRF, and higher salt content by titration.

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