

Salt reduction in salted jellyfish (*Lobonema smithii*) using a mechanical washing machine

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ABSTRACT

Edible salted jellyfish sold in local markets need proper rehydration before cooking. Multiple washing steps with water is a common practice but time consuming and labor-intensive. In the industrial, use of a washing machine for desalination is an alternative approach. This research aimed to determine the effect of water wash cycles in a jellyfish washing machine on salt reduction of rehydrated jellyfish. Salted jellyfish with an irregular sheet were mechanically washed for 1, 2, and 3 cycles, each for 15 min and with new wash water. Results showed that changes in the percentage of rehydrated jellyfish calculated as washing yield (%) and salt content determined by three analysis methods (titration, atomic absorption spectroscopy, and X-ray fluorescence spectrometry) significantly decreased with increasing numbers of wash cycles. The salt reduction may be due to the agitation and dilution effect during wash cycles. However, extended wash cycles caused soft texture and generated broken pieces, resulting in a higher loss of desalted product. Therefore, the selected wash cycle of 2 times yielded acceptable flesh quality and remaining salt content (1.02%). Using a mechanical washing machine could be an appropriate procedure for jellyfish rehydration, saving time, and increasing productivity.

Keywords: salted jellyfish; salt content; washing machine; wash cycles

1. INTRODUCTION

Foodstuffs made from jellyfish are generally known as delicacies in Asian countries, including Thailand, Japan, China, and South Korea (Hsieh et al., 2001). Recently, jellyfish-based foodstuff has got growing attention in European countries (Torri et al., 2020) due to its unique jelly and crunchy texture, low-fat content, low-calorie level, and its richness in protein, especially collagen (Hsieh et al., 2001; Pedersen and Vilgis, 2019). Among 38 reported edible species (Brotz et al., 2017), in Thailand, two main species of edible

jellyfish, *Lobonema smithii* and *Rhopilemma hispidum*, are produced (Wongsa-ngasri et al., 2008) and exported in a dried, salted form to several countries, including Japan, South Korea, Malaysia, China, and Taiwan (Omori and Nakano, 2001). The Thai Fishery Annual Report presented exports of salted jellyfish in 2017 at 57,900 tons with a value of greater than 200 million baht (Department of Fisheries, 2019). The demand and preference for jellyfish as a foodstuff has increased worldwide.

In general, freshly caught jellyfish are perishable at room temperature because they have a high water

content of approximately 95% throughout the whole body (Hsieh et al., 2001; Pedersen and Vilgis, 2019). Therefore, dehydration with salt (NaCl), potassium alum sulfate ($KAl(SO_4)_2^-$), and sodium bicarbonate ($NaHCO_3$) are used to preserve jellyfish flesh products (Pedersen and Vilgis, 2019; Hsieh and Rudloe, 1994; Pedersen et al., 2017; Hu et al., 2019). However, the type and quantity of salts used have reported varying among jellyfish producers (Wongsa-ngasri et al., 2008), and no sodium bicarbonate permitted for the salting process in European countries (Pedersen et al., 2017). Huang (1988) and Wongsa-Ngasri et al. (2008) reported that the salt content in commercial salted jellyfish ranged from 23.18-25.43% and 21.95%-23.33%. After the dehydration process, the salted jellyfish are separated by size into five grades, including AA, A, B, C, and CD grades depending on the diameter and shape of the jellyfish sheet. The lowest grade, CD, has a diameter of less than 30 centimeters and an irregular shape that will be subjected to shredding according to the customer's request. Therefore, this grade of salted jellyfish was used in this study.

Due to the enormous amount of salt used in the jellyfish dehydration process, the product must then be subjected to several wash steps for salt removal. The salty taste of rehydrated jellyfish is not suitable for cooking and, also, the recommendation for salt intake is less than 2,000 milligrams per day (World Health Organization, 2013). A person with high sodium levels may be at risk of high blood pressure, heart disease, and stroke (Frankowski et al., 2014).

To cook for food, the traditional wash method for local sale or home cooking is by washing the salted jellyfish with water several times and with overnight soaking (Hsieh and Rudloe, 1994; Thumthanaruk et al., 2011). This method takes a long time with a labor-intensive operation and also generates lots of wastewater. The resulting rehydrated jellyfish may have uneven salt content. To make them more convenient to use, this jellyfish factory offers the desalted product by operating

the wash step in the process using the washing machine funded by the Thailand Research Fund (TRF). This machine has a rotating impeller for circulating the sample in water, reducing the salt content in salted products. The method benefits to shorten the time of washing and to increase productivity with minimal maintenance. However, no data of salt reduction has been collected after washing by this process.

According to the vast quantity of NaCl used during salting, the salt content can be determined by different instruments and analysis methods by quantifying the concentration of Na^+ and Cl^- . For quality control in the jellyfish factory, the refractometer is generally used to measure the salt content. This measurement offers several advantages, such as the ease of use and maintenance, short time required for measurement, multi-usable, and inexpensive analysis equipment. However, the drawback of using a refractometer is the accuracy of the reading. The titration method used is a back titration of silver nitrate with potassium thiocyanate (KSCN). This measurement is to quantify the chloride ions (Cl^-), which react with silver ion (Ag^+) (Shahine and El-medany, 1979). The sophisticated analytical instruments are atomic absorption spectroscopy (AAS) by quantifying the concentration of free atoms of chemical analytes in a sample based on the absorption of light (Skoog et al., 2017), or the X-ray fluorescence spectrometry (XRF), which uses for quantitative and qualitative measurement by determining the energy or wavelength of the X-ray light emitted by an element (Presle et al., 2017). No determination of remaining salt after washing by this machine and by different methods has reported so far.

Therefore, this research aimed to study the effect of wash cycles of jellyfish, size CD, using a jellyfish washing machine on reducing the salt content of jellyfish. Also, three analysis techniques of salt in samples, including titration, AAS, and XRF, were compared. The outcome of this study could benefit in setting a guideline for the washing process in the jellyfish

factory as well as supporting research data of advanced jellyfish washing machine.

2. MATERIALS AND METHODS

2.1 Materials

The umbrella portion of grade CD salted jellyfish (*Lobonema smithii*) kindly obtained from Chockdee Sea Products Co., Ltd., Samut Songkhram, Thailand. Grade CD salted jellyfish samples used were of an irregular sheet in which a diameter and thickness of the sample were 23.83 ± 5.91 and 0.33 ± 0.057 cm, respectively. The samples were stored in a plastic box and kept at room temperature until used.

2.2 Washing of salted jellyfish

Samples of 10 kg (M_1) were subjected to the jellyfish washing machine developed by Mr. Samak Rakmae and Chockdee Sea Products Co., Ltd. Tap water was poured into the machine at a ratio of 1:40 (w/v). This machine is a cylindrical tank with a diameter and a height of 80 and 100 cm equipped with a rotating impeller operating at 100 rpm to circulate the sample in the water tank. Each cycle was set for 15 min and after finishing each cycle the wash water was drained and new water was used. In this study, three cycles of washing were compared. After that, the desalted samples were set for draining for 5 min and the samples were weighed (M_2). The resulting washed samples were stored in sealed polyethylene (PE) bags, transported to the laboratory, and kept at 10°C until analyzed.

2.3 Analyses

2.3.1 Washing yield

The washing yield of desalted jellyfish was derived from the ratio of the weight of washed jellyfish to the original weight of the sample (M_1 ; 10 kg) and calculated by Equation (1):

$$\text{Washing yield (\%)} = \frac{M_2}{M_1} \times 100 \quad (1)$$

2.3.2 pH and total soluble solids

The pH of jellyfish samples was measured using a pH meter (Eutech, pH 700, Singapore) by blending 5 g of the sample and 45 mL of distilled water and measuring the filtrate portion. The jellyfish sample's total soluble solid was measured using a salinity refractometer (Atago, Master-S28M, Japan).

2.3.3 Salt content

For titration method (AOAC, 2000), a 1 g of sample was mixed with 25 mL of 0.1 N silver nitrate and 10 mL of concentrated nitric acid. Then, the mixture was boiled on a hot plate for 10 min. After that, 50 mL of distilled water and 5 mL of 5% ferric alum indicator were added before being titrated with a standard 0.1 N potassium thiocyanate (KSCN) solution until the solution color changed to brownish-orange color. The percentage of salt (sodium chloride) was then calculated by Equation (2):

$$\text{Salt (\%)} = 5.8 \times \frac{(X_1 \times N_1) - (X_2 \times N_2)}{W} \quad (2)$$

where X_1 = volume of AgNO_3 (mL), X_2 = volume of KSCN (mL), N_1 = concentration of AgNO_3 (N), N_2 = concentration of KSCN (N), and W = weight of sample (g).

For AAS, the jellyfish sample of 1 g was prepared in 1% concentrated nitric acid. The sample was boiled on a hot plate for 10 min and then filtered. After that, the sample was subjected to the atomic absorption spectrophotometer (Agilent, 240FS AA, USA) to determine the sodium (Na^+) level at a wavelength of 330.3 nm. The sodium concentration in the sample displayed in mg/L after extrapolation from the standard curve.

For element analysis by XRF, the jellyfish sample prepared was cut into 6×6 cm pieces. Then the sample was subjected to quantifying the element content by XRF (Horiba, XGT-5200WR, Japan) with operating conditions of X-ray tube voltage at 30 kV,

X-ray current 1.0 mA, and processing time of 100 s.

2.3.4 Chemical composition

Protein, ash, fat, and moisture content were determined according to the AOAC method (AOAC, 2000). The salted jellyfish before and after washing was measured protein content using the Kjeldahl method. The protein was calculated using the conversion factor of 5.55 (James, 1995). The ash content determined using the muffle furnace method, and the fat content was analyzed using the Soxhlet extraction method. The moisture content was determined using the oven drying method.

2.4 Statistical analysis

All experiments were carried out in triplicate. The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for statistical significance ($p < 0.05$) using the SPSS (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL USA)

3. RESULTS AND DISCUSSION

3.1 Effect of washing cycles on washing yield, pH, and total soluble solids

The washing yield of desalted jellyfish washed after 1, 2, and 3 cycles was $93.75 \pm 0.98\%$, $90.49 \pm 0.69\%$, and $81.23 \pm 0.52\%$, respectively. This significant reduction could be the effect of the stirring blade as the rotation of the blade aided dissolving of salt by the circulation of the jellyfish flesh in water, thereby diluting salt from the flesh. However, the rehydrated soft gelatinous jellyfish tissue was destroyed by the mechanical force of the stirring blade, resulting in the generation of broken and small pieces that can slip out through the sieve holes of the washing machine.

The pH value of the salted jellyfish was 3.43 ± 0.06 , which slightly differed from other research work, which reported pH of salted jellyfish at 4.46–4.64 (Omori and Nakano, 2001). Depending on the salting chemicals used in the dehydration process (sodium

chloride, potassium alum sulfate, and sodium bicarbonate), the salted jellyfish sample is a highly acidic food with a pH level of less than 4.6 due to the synergistic effect of sodium chloride, potassium alum sulfate, and sodium bicarbonate (Huang, 1988). This salting helped to prevent microbiological growth. The pH levels of desalted jellyfish washed after 1, 2, and 3 cycles were 6.80 ± 0.60 , 7.70 ± 0.10 , and 7.73 ± 0.06 . The increase of wash cycles caused changes to the pH value of the desalted jellyfish that were close to the pH of tap water of 7.76 ± 0.06 .

The value of total soluble solids of salted jellyfish measured by a salinity refractometer was $23.20 \pm 0.40\%$. For salted jellyfish, the soluble solids were from the salt chemicals. The use of water in the wash cycles solubilized salt ions of NaCl (Na^+ , Cl^-), potassium alum sulfate (K^+ , Al^{3+} , SO_4^{2-}), and sodium bicarbonate (Na^+ , HCO_3^-). After using the jellyfish washing machine, each of the resulting jellyfish samples showed undetectable salt content after the wash cycle. The traditional commercial wash method reported the value of soluble solid at less than 1°Brix after several washing cycles and overnight soaking (Chancharen et al., 2016; Rodsuwan et al., 2016; Silaprueng et al., 2015). These results showed that the significant loss of salt in jellyfish flesh was obtained starting from the first cycle of washing with no measurable values of salt indicated by the salinity refractometer. However, by human tasting, the flesh of washed jellyfish from 1, 2, and 3 cycles of washing still had some salty taste because the human sensitivity to the salty taste is at least 10 millimolar or 0.064% of salt (sodium chloride) (Amerine et al., 1965), which is more sensitive than refractometer. Hence, an alternative method for salt determination should be used.

3.2 Effect of washing cycles on salt content as determined by three different methods

Salt content is one of the measured qualities of the desalted jellyfish. As regards to salt chemicals used

in salting jellyfish, the various dissolving of salts in water yields sodium ion (Na^+), aluminium ion (Al^{3+}), potassium ion (K^+), chloride ions (Cl^-), and bicarbonate ion (HCO_3^-). This research compared three analysis techniques for the salt content determination, including titration, AAS, and XRF.

According to the indirect measurement from the titration method by quantifying the chloride ions (Cl^-) in the salted jellyfish reacted with silver (Ag^+) ions (Shahine and El-medany, 1979), the results revealed the salt content in this salted sample was $14.21 \pm 0.00\%$ (Figure 1), which is not consistent with other reports which show the salt content varying from 23.33-25% (Wongsa-ngasri et al., 2008; Huang, 1988). The differences in salt content with other studies could be due to the procedures used by different salted jellyfish producers. However, the salt content in salted jellyfish determined with titration method is not consistent with the value determined by a salinity refractometer in which the total soluble solid may be varieties of salts (Na^+ , Al^{3+} , Cl^- , HCO_3^-) and small soluble peptides that impart the index of refraction. As a result, the salt content obtained from the titration method lowered than that from the refractometer. In the washing step, the measurement was recorded in both the washed flesh and the drained (or washed) water samples. The salt content in washed flesh was significantly reduced from $3.38 \pm 0.06\%$ to $1.02 \pm 0.01\%$ and $0.81 \pm 0.03\%$ as the wash cycles proceeded. The drained water of cycles 1, 2, and 3 had a salt content of $2.72 \pm 0.00\%$, $0.94 \pm 0.04\%$, and $0.66 \pm 0.00\%$. The tap water had a salt of $0.33 \pm 0.08\%$. The titration results confirmed that by applying the wash cycle to the salted jellyfish, some salt contents remained in the washed sample with a minimal salty taste.

Different salt determinations delivered different values of salt content. Given the variety of salt chemicals used for salting jellyfish, the selection of this measurement was only for determining the concentration of Na^+ analyte, the free atom in the

gaseous state, by absorption of light using atomic absorption. Results showed a similar trend of salt reduction in washed jellyfish and washed water as the number of wash cycles increased (Figure 2). The percentage of sodium ion in salted jellyfish was $15.30 \pm 0.35\%$, which is not much different from the result obtained from the titration method. The sodium ion content in desalted jellyfish after 1, 2, and 3 wash cycles reduced significantly from $12.71 \pm 0.33\%$ to $4.73 \pm 0.17\%$ and $2.05 \pm 0.14\%$, respectively. The sodium content in washed water was $0.71 \pm 0.01\%$, $0.14 \pm 0.02\%$, and $0.05 \pm 0.01\%$ and the sodium content of tap water was $0.00 \pm 0.00\%$. According to the quality standard of drinking water in Thailand, the acceptable maximum concentration of chemicals including Fe, Mn, Cu, Zn, CaCO_3 , Mg, SO_4 , Cl, F, and NO_3 is in the value of 0.3, 0.05, 1.0, 3.0, 100, 0.05, 200, 250, 0.7, and 4 mg/L (Thai Industrial Standard Institute, 2006). In this determination, only the sodium ions were quantified. Concerning the washing step, the higher content of sodium ions detected in the flesh and the washed water due to the direct proportional concentration of sodium element in the samples to the adsorption of free sodium atom in gaseous state. The results from this method deliver a precision value of sodium content; however, the cost of determination is a concern for this small jellyfish factory.

XRF is widely used for elemental analysis based on the exciting emission from a material when bombarded with high-energy X-rays. The results showed ten elements of salted jellyfish: Na, Al, Si, P, S, Cl, K, Ca, Fe, and Br (Table 1). Concerning the salts used in jellyfish, the individual elements were Na, Cl, K, Al, S, H, C, and O derived from sodium chloride, aluminum potassium sulfate, and sodium bicarbonate (Wongsa-ngasri et al., 2008). However, the pronounced elements of the results were Na and Cl, which were higher than the results of the titration method and the AAS method. The XRF technique can only identify elements heavier than Na (Presle et al., 2017), thus in

the jellyfish sample, the elements of H, C, and O were not detected. Focusing on the quantifying Na and Cl elements, as the number of wash cycle increased, the contents of Na⁺ and Cl⁻ in washed jellyfish and washed water significantly decreased, while the quantities of remaining elements increased.

After washing, some salts remained in the jellyfish flesh due to the chemistry of the protein fiber, mostly collagen, having both negatively and positively charged amino acids in the protein structure (Belitz et al., 2009). Thus, the binding of Na⁺ and Cl⁻ can bind to the jellyfish protein. At the wash step, the water

molecules having H⁺ and OH⁻ can bind to Cl⁻ and Na⁺ (Belitz et al., 2009; Damodaran et al., 2008), which helps to remove Na⁺ and Cl⁻ from the jellyfish protein as a result of reducing salt content. The binding of H⁺ and OH⁻ to jellyfish proteins will rehydrate and turn the collapsed protein fiber during salting to swollen protein.

Upon three different salt detections, a decreasing trend of salt reduction in washed jellyfish similarly appeared but differed in values due to different principles of detection and limitations of each measurement.

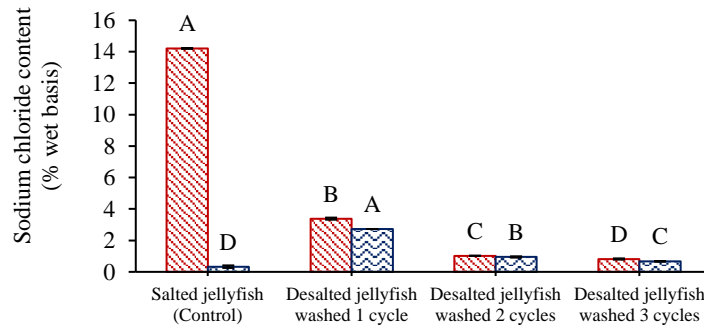


Figure 1 Salt content in salted jellyfish before and after washing obtained by titration method; bars with the different superscripts were significantly different ($p < 0.05$)

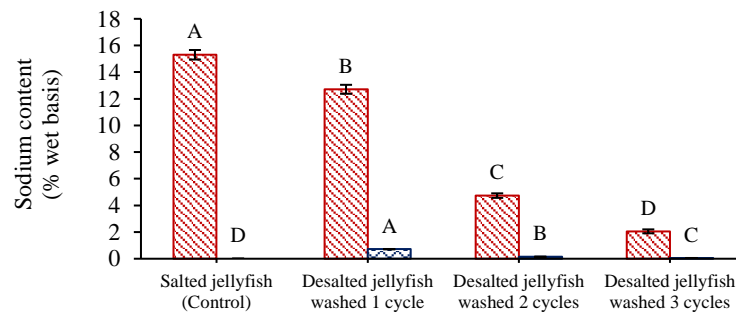


Figure 2 Sodium percentage in salted jellyfish before and after washing using atomic absorption spectroscopy; bars with the different superscripts were significantly different ($p < 0.05$)

Table 1 Element percentage in salted jellyfish before and after washing obtained by X-ray fluorescence spectrometry

Element	Concentration (Mass %)			
	Salted jellyfish (Control)	Desalted jellyfish washed 1 cycle	Desalted jellyfish washed 2 cycles	Desalted jellyfish washed 3 cycles
Na	28.04±0.50 ^d	23.24±0.83 ^a	11.65±0.32 ^b	4.33±0.34 ^c
Al	0.37±0.09 ^c	1.15±0.22 ^c	7.87±0.92 ^b	9.93±0.29 ^a
Si	0.13±0.01 ^d	0.96±0.20 ^c	1.32±0.10 ^b	2.95±0.06 ^a
P	0.09±0.02 ^c	0.16±0.02 ^c	1.59±0.49 ^b	3.56±0.14 ^a
S	1.40±0.20 ^d	5.32±0.08 ^c	9.17±0.19 ^b	15.91±0.29 ^a
Cl	68.26±0.49 ^a	66.35±0.76 ^b	65.14±0.87 ^b	56.43±0.24 ^c
K	0.31±0.18 ^c	1.30±0.10 ^b	1.46±0.03 ^b	3.36±0.11 ^a
Ca	1.21±0.18 ^b	1.26±0.13 ^b	1.29±0.22 ^b	2.92±0.61 ^a
Fe	0.05±0.04 ^b	0.11±0.03 ^b	0.21±0.04 ^a	0.27±0.03 ^a
Br ^{ns}	0.11±0.01	0.12±0.01	0.27±0.23	0.30±0.06

Note: Different superscripts (a, b, and c) in the same row mean that the values significantly different ($p < 0.05$), ns = not significant ($p > 0.05$)

3.3 Proximate composition of salted jellyfish before and after washing

The protein, ash, fat, and moisture of salted jellyfish were 3.28±0.07%, 21.18±0.58%, 0.98±0.01%, and 74.53±0.09%, respectively (Table 2). As the number of wash cycles increased, the moisture increased due to the water absorption in jellyfish protein. The reduction

in salt content, in turn, increased the quantity of protein content slightly, but not the fat content. These results were slightly different from others studies, which reported protein, ash, fat, and moisture content of desalted jellyfish (*Lobonema smithii*) of 2.34-3.49%, 0.21-0.59%, 0.84-3.63% and 92.75-95.78% (Kromfang et al., 2009; Thumthanaruk and Lueyot, 2014).

Table 2 Proximate composition of salted jellyfish before and after washing

Sample	% Protein	% Ash	% Fat ^{ns}	% Moisture
Salted jellyfish (Control)	3.28±0.07 ^D	21.18±0.58 ^A	0.98±0.01	74.53±0.09 ^D
Desalted jellyfish washed 1 cycle	3.96±0.06 ^C	4.77±0.61 ^B	0.97±0.08	90.45±0.26 ^C
Desalted jellyfish washed 2 cycles	4.79±0.07 ^B	1.39±0.17 ^C	0.95±0.04	91.86±0.06 ^B
Desalted jellyfish washed 3 cycles	5.63±0.04 ^A	1.05±0.06 ^C	0.95±0.06	92.38±0.04 ^A

Note: Different superscripts (a, b, and c) in the same column mean that the values significantly different ($p < 0.05$), ns = not significant ($p > 0.05$)

4. CONCLUSION

The washing machine can be applied for desalting jellyfish. The increase in the number of wash cycles decreased the washing yield, total soluble solids,

and salt content of desalted jellyfish. Compared to the titration method, AAS and XRF, the AAS gave an in-depth value on only sodium ion content. In contrast, XRF yielded precisely the elements of Na and Cl, but

both are sophisticated and expensive analyzers. Therefore, apart from the refractometer, from a commercial perspective, the choice used for salt determination could be the titration method, which gives quick response, valid results and requires no sophisticated instrumentation.

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