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Effectiveness of washing with *atung* (*Parinarium glaberimum* Hassk) solution on quantity and quality of dark meat yellowfin tuna (*Thunnus albacares*) surimi

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Abstract

Yellowfin tuna (*Thunnus albacares*) is the world's second-largest commodity with good nutrition. After processing, some wastes / residues / by-products can be used to produce surimi which still retains the tuna meat properties. The manufacturing of surimi requires safe preservatives, such as the natural preservative, *atung* (*Parinarium glaberimum* Hassk), which contains antibacterial substances. Therefore, the present work aimed to determine the effectiveness of 4% (w/v) *atung* solution on the quantity and quality of yellowfin tuna surimi produced from tuna by-products. The fish meat was treated with a washing agent of ice water and 4% *atung* solution with several washing frequencies. The parameters assessed were the yield and quality of surimi by analysing the protein content, myofibrillar protein content (salt soluble), water-holding capacity, *Salmonella* spp., and *E. coli*. In addition, the parameters included teeth-cutting, ashi strength, and folding. The 4% (w/v) *atung* solution effectively produced tuna fish surimi with 68.50% yield, 20.62% protein, 8.87% myofibrillar protein, and free from *Salmonella* spp. and *E. coli*. The water-holding capacity of the surimi was recorded at 68.9% and increased to 73.3% after three washes. The use of 4% (b/v) *atung* solution effectively produced surimi with a teeth-cutting value of 7.80 - 7.92, and an ashi strength of 7.97 - 8.08, whereas the folding test was B to A (3.80) after four washes.

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Introduction

Yellowfin tuna (*Thunnus albacares*) is a highly migratory fish in tropical and subtropical waters. The main food of this fish is pelagic fish, cephalopods, and crustaceans (Shi *et al*., 2022). Yellowfin tuna is one of the most important seafood commodities in the world. It is the world's second-largest tuna commodity, accounting for a quarter of the total global catch (Pecoraro *et al*., 2018; Le-Alvarado *et al*., 2021). Yellowfin tuna has high economic value, and is very popular among consumers, especially in Maluku. It contains high protein and low fat, at 23.2 and 2.4 g per 100 g of meat, respectively (Wahyuni, 2011). It also has high content of amino acids leucine and lysine. Leucine is an essential amino acid for protein synthesis, and can potentially treat and prevent obesity and diabetes mellitus. Meanwhile, lysine is essential for proper growth, converting fatty acids into energy, and helping to lower cholesterol. In addition, these fish also contain polyunsaturated fatty acids (PUFA) in the form of omega-3 fatty acids, and minerals such as calcium, sodium, magnesium, potassium, and iron. PUFA consumption is required for mothers to properly develop the foetal brain and retina during pregnancy (Singh *et al*., 2011; Swanson *et al*., 2012; Pedroso *et al*., 2015; Nurjanah *et al*., 2019). Capture fisheries commodities have grown from the first to the third quarter of 2015. The most significant increase in tuna commodities was albacore, bluefin, and bigeye tuna (MMAF, 2015). Notably, yellowfin tuna experienced significant growth, with an average production of 16.21% (Rahmantya, 2015).

The waste of fish processing continues to increase along with high level of food production. Moniharapon *et al*. (2019) reported the lack of waste treatment for tuna loin production in Parigi, Wahai Village, North Seram District. The waste can be raw meat, offal, and other fish parts dumped into the sea

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when producing tuna loin. For this reason, developing fishery products derived from raw tuna meat in Indonesia is necessary, one of them is producing surimi.

Surimi is an intermediate product of crushed fish meat which has undergone repeated washing, pressing, adding additional ingredients, packing, and freezing. The main focus in making surimi is to maintain the functional properties of the denatured protein, and increase the gelling ability (Wiradimadja *et al*., 2017). Surimi has several advantages, including having a stable supply and price as it has long shelf life, making it easier to plan further processed products, fewer waste disposal problems, and easier to handle (Moniharapon *et al*., 2014). As it originally had no smell or taste, surimi can be used as an artificial seafood product by mixing it with essence, aroma, and taste.

Surimi which uses dark meat, has higher fat content than white meat. Therefore, the surimi products stench faster, and the waste is more challenging to handle (Anggawati and Indriawati, 2011). Washing process is the most crucial step to producing high-quality surimi taste (Anggawati and Indriawati, 2011) without odour and with good gel strength (Mahawanich, 2008). Washing removes sarcoplasmic protein, blood, fat, and other nitrogen content from fish meat. Washing generally uses ice water. However, ice water alone is not able to eliminate microorganisms and pathogens. Therefore, an alternative washing with more leverage to kill spoilage and pathogenic microorganisms is necessary.

Surimi typically contains preservatives that are safe to eat which are derived from plants that provide antioxidants and antimicrobials. One of the plants that can be used as a natural preservative is *atung* (*Parinarium glaberimum* Hassk). Azelaic acid, an antibacterial substance found in the seeds of *atung* fruit, is capable of eliminating harmful germs that ruin foods, including *Staphylococcus aureus*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Bacillus subtilis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Escherichia coli* B and C, and *Pseudomonas aeruginosa* (Moniharapon, 2004; Moniharapon *et al*., 2005).

Until now, research that observes explicitly the effect of *atung* solution on yellowfin tuna surimi has not been found. Previously, *atung* had been used by the community as an efficacious prevention of diarrhoea, and to prevent premature bleeding in pregnant women. *Atung* is also used in handling fresh fish by fishermen to keep it fresh (Heyne, 1950). Moniharapon *et al*. (2019) demonstrated that tuna loin treated with *atung* solution produced meat with a bright red colour, and was free of *Escherichia coli* and *Salmonella* spp. Pattipeilohy *et al*. (2020) studied the quality of processed meatballs in Parigi, Wahai Village, North Seram which used yellowfin tuna surimi for meatballs, and washed with *atung* solution, and found that it had better quality compared to meatballs from surimi which were only washed with ice water. The present work thus aimed to determine the effectiveness of the *atung* solution on the quantity and quality of dark meat surimi of yellowfin tuna.

Materials and methods

Preparation of atung solution

The concentration of *atung* solution used was 4%, referring to previous studies and patents (Moniharapon *et al*., 2019). To prepare this, *atung* fruit seeds were grated into powder, and dried at room temperature for 2 - 3 d. The *atung* seed powder was sieved, the lumps were ground using a mortar, and air-dried overnight. *Atung* seed powder weighing 30 and 300 g was packed in plastic bags for storage. Preparation of a 4% (w/v) *atung* solution was as follows: 40 g of *atung* powder was soaked in 1 L of warm water (1:25) in a jerry can for 1 - 2 nights. The can was stirred occasionally while the water was still warm so the preservative components could come out. After soaking, the solution was filtered and put into another jerry can for further use (Pattipeilohy *et al*., 2020).

Production of surimi

Production of surimi was based on Tan *et al*. (1988) with modification using the dark meat of yellowfin tuna, which is a waste of tuna loin production. The meat was washed under running water, crushed, and soaked in various washing solutions for 10 min: ice water, 4% *atung* solution, and 4% *atung* solution with ice. After soaking, the meat was rinsed (two, three, and four times) to remove fat, skin, and blood, then filtered and squeezed. The results were then used as a sample for testing.

Treatment

The treatments implemented were as follows: A = washing material; A1: ice water; A2: 4% (w/v)

atung solution; A3: 4% (w/v) *atung* solution with ice; B = washing frequency; B1: two times; B2: three times; and B3: four times.

Yield

The yield calculation (%) was carried out by comparing the weight of surimi with the weight of whole fish (Radityo *et al*., 2014) using Eq. 1:

$$\text{Yield} = \frac{\text{weight of surimi (g)}}{\text{weight of whole fish (g)}} \times 100\% \quad \text{(Eq. 1)}$$

Protein

Determination of protein content was carried out based on the Kjeldahl procedure (AOAC, 2005; Rosaini *et al*., 2015). Surimi (0.5 g) and 1 g of selenium in 10 mL of concentrated H_2SO_4 were homogenised in a Kjeldahl flask. The homogenate was then digested in a fume hood until it was clear. The material was allowed to cool and put into a 100 mL volumetric flask while rinsing with distilled water. Distilled water was then added up to the calibration mark. In a 100 mL Erlenmeyer, the sample was mixed with 10 mL of 2% H_2BO_2, four drops of indicator solution, 5 mL of 30% NaOH, and 100 mL of distilled water. The mixture was distilled until the volume became approximately 50 mL. The tip of the distiller was rinsed with distilled water, and collected with its contents to produce a distillate. The distillate was titrated with a solution of GCL or 0.2 N H_2SO_4, and protein was calculated using Eq. 2:

$$\text{Protein level (\%)} = \text{V1} \times \text{Normality} \frac{\text{H}_2\text{SO}_4 \times 6.25 \times \text{p}}{\text{Material (g)}} \times 100\% \quad \text{(Eq. 2)}$$

Myofibrillar protein

Surimi (5 g) was added with 50 mL of 5% NaCl solution, then homogenised using a Waring blender for 2 - 3 min. The sample was centrifuged at 3,400 rpm for 30 min at 10°C, then filtered through Whatman filter paper No. 1. The filtrate (25 mL) was collected in an Erlenmeyer flask and stored at 4°C (Wahyuni, 1992). Analysis of myofibrillar protein content used the semi-micro Kjeldahl method (Eq. 3):

$$\frac{(\text{A}-\text{B} \times \text{N HCl} \times 14.007 \times \text{fp} \times 6.25 \times 100\%}{\text{Sample (mg)}} \quad \text{(Eq. 3)}$$

Water-holding capacity

The water-holding capacity test aims to calculate the ability of the meat to hold water. Sample (1 g) was added with 10 mL of distilled water in a centrifuge tube, and homogenised with a vortex for 30 s. The mixture was then centrifuged at 10,000 rpm for 30 min. The volume of the supernatant was recorded (Panpipat and Chaijan, 2016), and the yield was calculated using Eq. 4:

$$\text{Water} - \text{holding capacity} = \frac{\text{Amount of water retained}}{\text{Initial amount of water}} \times 100\% \quad \text{(Eq. 4)}$$

Salmonella

Salmonella analysis was performed following SNI-01-2332.2-2006. The analysis consisted of four stages.

i) Pre enrichment stage

In a stomacher container, 25 g of sample and 225 mL of lactose broth (1:9) were homogenised for 2 min. The sample solution was transferred aseptically into a sterile container, and incubated for 24 ± 2 h at 35 ± 1°C.

ii) Enrichment stage

For fishery products with a high level of contamination, 0.1 mL of sample solution was put into 10 mL of Rappaport-Vassiliadis (RV) medium, and 1 mL of sample solution into 10 mL of tetrathionate broth (TTB); for other types of fishery products, 1 mL of the sample solution was added to 10 mL of SCB and 10 mL of TTB, respectively. RV medium was incubated for 24 ± 2 h at 42 ± 0.2°C (water bath); TTB for 24 ± 2 h at 43 ± 0.2°C (water bath); and TTB and SCB for 24 ± 2 h at 35 ± 1°C (incubator).

iii) Isolation stage

The sample tube was shaken with a vortex. Samples from each broth were streaked onto Hektoen Enteric (HE), xylose lysine deoxycholate (XLD), and bismuth sulphite agar (BSA) media using a loop needle (3 mm). Streaked plates were incubated for 24 h at 35 ± 1°C.

iv) Salmonella morphology observation

On HE agar, *Salmonella* colonies were identified as bluish-green to blue, with or without a black core. On XLD agar, *Salmonella* colonies were pink, with or without a black core, whereas on BSA, the colonies were brown, grey,

or black. The identified colonies were then streaked on triple sugar iron (TSI) and lysine iron agar (LIA) media, and incubated for 24 ± 2 h at 35 ± 1°C. On TSI, *Salmonella* typically gave an alkaline (red) reaction on oblique streaks and acidic (yellow) on upright agar punctures, with or without H_2S (blackish colour on agar). Meanwhile, *Salmonella* culture would give an alkaline reaction (purple) in the entire tube of LIA agar. A completely yellow reaction on the puncture indicated a negative culture.

Escherichia coli

Escherichia coli analysis was performed following ISO 16649-2:2001 (ISO, 2001). Samples (20 g) and diluents (180 mL) were put into sterile stomacher bag, and homogenised using a stomacher for at least 30 s (10^{-1} dilution). The suspension (1 mL) was then mixed with 9 mL of diluents (10^{-2} dilution). With a sterile pipette, 1 mL of each dilution was transferred into sterile Petri dishes. After less than 15 min, a TBX medium (15 mL) at 44 - 47°C was added to each inoculum (pour-plating technique). Each Petri dish was carefully rotated and allowed to solidify by leaving it standing on a cool horizontal surface. After solidification, the Petri dishes were then incubated in an incubator at 44°C for 18 - 24 h. The number of colonies that appeared on the agar was counted. If the number of colony-forming units (CFU) growing was 15 and more, Eq. 5 was used:

$$N = Za/(V(n1 + 0.1n2)d) \quad \text{(Eq. 5)}$$

If the number of CFU growing was less than 15, Eq. 6 was used:

$$NE = Ec/(V(n)d) \quad \text{(Eq. 6)}$$

Folding test

Sample preparation was the same as in the gel strength test, only with a thickness/height of 4 - 5 mm. The quality of the folding test prescribed by SNI 2732.6:2009 (BSN, 2009) was: (i) does not crack when folded twice, "AA" quality with a rating of 5; (ii) does not crack when folded once, "A" quality with a value of 4; (iii) slightly cracked when folded once, "B" quality with a rating of 3; (iv) cracks when folded once, "C" quality with a value of 2; and (v) destroyed when pressed by finger, "D" quality with a value of 1.

Teeth-cutting test

The sample used was the same as in the folding test. The evaluation of teeth cutting test scored from 1 to 10 following SNI 2732.6:2009 (BSN, 2009).

Ashi strength test

The sample used was the same as in the folding and teeth cutting tests. The evaluation of ashi strength test scored from 1 to 10 according to Suzuki (1981).

Data analysis

A completely randomised design (CRD) with three replications followed by Tukey's test was used to examine the yield, protein content, myofibrillar protein, and water-holding capacity data (Steel and Torrie 1993; Gaspersz, 1994). Meanwhile, the folding, teeth cutting, and ashi strength tests were analysed using the Friedman and multiple comparison tests (Wayne, 1989). The CRD linear model used was as follows:

$$\text{Yi}jk = \mu + \acute{\alpha}i + \beta j + (\acute{\alpha}\beta)ij + \varepsilon\, ijk \quad \text{(Eq. 7)}$$

where, i: 1, 2, 3; j: 1, 2, 3; k: 1, 2, 3; Yijk: observation value of the *k*-th experimental unit that obtains the combination treatment of i-j (*i*-th level of factor A and *j*-th level of factor B); μ: population median; $\acute{\alpha}i$: additive effect of the *i*-th level of factor A; βj: additive effect of the *j*-th level of factor B; (άβ)ij: interaction effect of the *i*-level of factor A and *j*-level of factor B; and εijk: effect of error from the *k*-th experimental unit that obtained the combination treatment of i-j.

Subjective parameters (folding, teeth cutting, and ashi strength) were analysed following Wayne (1989) and Gaspersz (1994) using Eq. 8:

$$\text{S} = \frac{12}{b\text{t}(\text{t+i})}\sum \text{r}12 - 3\text{b}\,(\text{t} - 1) \quad \text{(Eq. 8)}$$

where, S: Friedman test (Xi^2); t: number of treatments; b: number of repetitions; and ri^2: sum of the squares of *i*-th rank.

The general formula for multiple comparison tests (Wayne, 1989) was as Eq. 9:

$$(\text{Ri} - \text{Rj})\ \text{VSZ} = \frac{\sqrt{bt(t-1)}}{b} \quad \text{(Eq. 9)}$$

where, Ri: *i*-th rank; Rj: *j*-th rank; b: number of group; and t: number of treatments.

Results

Objective parameters

The analysis results of the yield, protein, myofibrillar protein, and water binding capacity of surimi from the dark meat of yellowfin tuna are shown in Table 1.

Yield

The type of washing agent (A), washing frequency (B), and their interaction (AB) were indicated affecting the yield of surimi from the dark meat of yellowfin tuna (Table 1) ($F_{count} > F_{table}$), proceeded with Tukey's test (Table 2). Washing twice with ice water (A1B2) resulted in the highest average yield of surimi of 79.52%. However, washing with ice water four times (A1B4) yielded the lowest, 54.58%. Figure 1 shows the histogram of the yield of surimi.

Protein level

The analysis of variance (Table 1) and Tukey's test (Table 2) showed that the washing agent (A), washing frequency (B), and the interaction of both (AB) significantly affected the protein content of the surimi ($F_{count} > F_{table}$). Washing with 4% *atung* solution plus ice four times (A3B3) produced the highest protein content with a protein content of 22.25%, whereas washing twice with ice water only (A1B1) made the least protein content of 13.63%.

Table 1. Results of the analysis of variance and Tukey's test of surimi from dark meat of yellowfin tuna (*Thunnus albacares*).

Treatment	F_{Table}	F_{count}			
		Yield	Protein	Myofibrillar protein	Water holding capacity
A	6.28/5.72	6.36**	10.169.90**	6.87**	71.96**
B	6.28/4.82	191.98**	4.265.30**	16.34**	36.69**
AB	4.77/3.76	5.65**	32.37**	3.73**	2.82

**Significant different for the parameter if $F_{count} > F_{table}$.

Table 2. Analysis results and Tukey's test of surimi from dark meat of yellowfin tuna (*Thunnus albacares*).

	Mean value			
Treatment	Yield	Protein	Myofibrillar protein	Water holding capacity
A1				38.3^{b}
A2				68.9^{a}
A3				72.2^{a}
B1				46.7^{b}
B2				59.4^{ab}
B3				73.3^{a}
A1B1	85.27^{a}	13.63^{h}	8.79^{c}	
A1B2	79.52^{ab}	15.32^{g}	9.44^{abc}	
A1B3	73.62^{bc}	17.35^{f}	9.93^{ab}	
A1B4	54.58^{e}			
A2B1	83.37^{a}	17.52^{f}	8.73^{c}	
A2B2	73.87^{bc}	18.28^{e}	8.77^{c}	
A2B3	68.50^{cd}	20.62^{b}	8.87^{bc}	
A2B4	58.57^{e}			
A3B1	82.93^{a}	19.05^{d}	8.50^{c}	
A3B2	78.12^{ab}	20.38^{c}	9.22^{ab}	
A3B3	61.37^{de}	22.25^{a}	10.32^{a}	
A3B4	55.50^{e}			
Tukey's test value 0.05	7.72	0.22	1.11	13.92
Tukey's test value 0.01	9.30	0.28	1.38	18.23

The first number in the F_{table} is only for yield parameters; means followed by the same lowercase superscripts in the same column are not significantly different at $\alpha = 0.05$. A1: washed with ice water; A2: washed with 4% (w/v) *atung* solution; A3: washed with 4% (w/v) *atung* solution plus ice; B1: washed one time; B2: washed two times; B3: washed three times; and B4: washed four times.

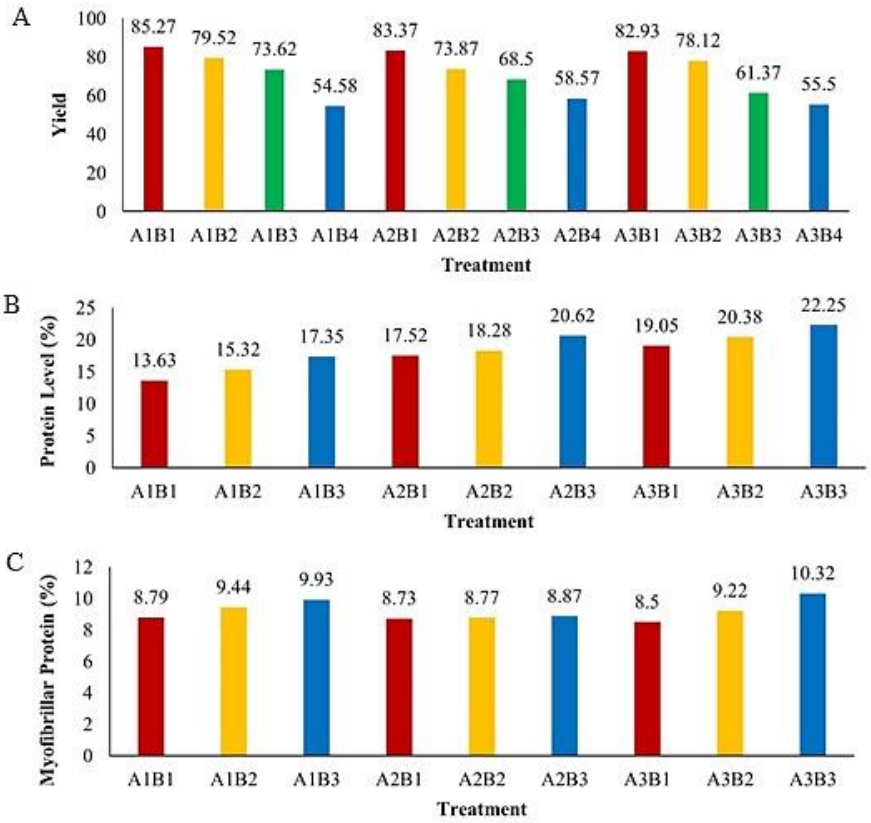

Figure 1. Histogram of **(a)** yield value, **(b)** protein content, and **(c)** myofibrillar protein content of surimi from dark meat of yellowfin tuna (*Thunnus albacares*).

Myofibrillar protein level

The analysis of variance indicated that the washing agent (A), washing frequency (B), and the interaction of both (AB) significantly affected the myofibrillar protein of the surimi ($F_{count} > F_{table}$). The results showed that washing four times with 4% *atung* solution plus ice (A3B3) had the highest myofibrillar protein content (10.32%). Meanwhile, washing twice with the same solution (A3B1) had the lowest myofibrillar protein content at 8.50% (Table 2).

Water-holding capacity

Surimi with the highest water-holding capacity was obtained with *atung* (A2) solution with 68.9%. This value was not significantly different from the *atung* solution plus ice (A3), but significantly different from ice water only (A1). Additionally, washing three times (B3) was quite effective in binding water (59.4%), as no statistically significant difference was found with the other washing frequencies (B1 and B2). Figure 2 shows the histogram of the average water holding capacity of surimi.

Salmonella spp. and Escherichia coli

All types of solutions and washing frequency used as treatments in this study resulted in surimi with negative content of *Salmonella* spp. and *Escherichia coli*.

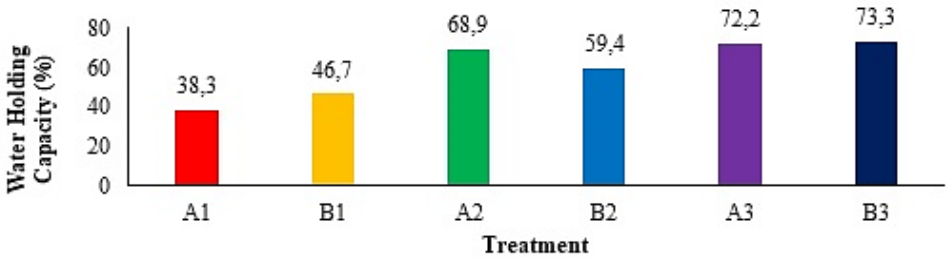

Figure 2. Water holding capacity of surimi from dark meat of yellowfin tuna (*Thunnus albacares*).

Subjective parameters

The summary of Friedman's test and multiple comparisons of the subjective parameters are presented in Table 3.

Folding test

Washing four times with ice water (A1B3) yielded the highest folding value, with a ranking and average of 25.5 and 3.99, respectively. Meanwhile, washing twice with 4% *atung* solution (A2B1) had the lowest ranking and average at 3.0 and 3.51 for each washing (Figure 3).

Teeth cutting test

Surimi folding test results ranged from 7.80 to 8.09. This indicated that the panellists considered the surimi as having moderate to strong resilience. The lowest teeth cutting value, which was included in the category of moderate resilience, was shown by surimi washed with *atung* solution two times (A2B1) with a ranking and average of 3.0 and 7.80, respectively. Meanwhile, washing four times with *atung* solution plus ice (A3B3) resulted in the highest teeth-cutting value with a ranking and average of 24.5 and 8.09. It was included in the strong resilience category (Figure 3).

Ashi strength value

Washing with *atung* solution plus ice four times (A3B3) obtained the highest ashi strength with a ranking of 26.0 and an average of 8.21. Meanwhile, washing three times with ice water only (A1B2) had the lowest value with a rank and average of 4.5 and an average of 7.91, respectively (Figure 3).

Discussion

Objective parameters

Yield

Surimi yield obtained in the present work ranged from 55.50 to 85.27%, thus indicating that the yield decreased after washing. The amount of surimi produced decreased with increasing washing frequency. This agreed with Endoma *et al*. (2022) where washing three to four times produced a low yield but a high water-holding capacity. This could have been due to the elements in the meat, such as dirt, fat, blood, and sarcoplasm dissolving in the water during the washing procedure with cold water at 10°C (5 - 10). Zhang *et al*. (2022) stated that washing can cause some of the immobilised water in fish to be transferred to free water, thus increasing the water's fluidity. The highest yield (68.50%) was obtained after three washes with 4% *atung* solution (A2B3). This result was corroborated by Priyadarshini *et al*. (2018) who stated that washing could remove dirt, fats, and protein in water. Anwar *et al*. (2013) further indicated that the decrease in yield of surimi was caused by the reduced water content during the pressing process, which decreased the surimi's weight.

Table 3. Results of Friedman's test and multiple comparison of surimi from dark meat of yellowfin tuna (*Thunnus albacares*).

Treatment	∑ Ranking and difference			Mean		
	Folding	Teeth cutting	Ashi strength	Folding	Teeth cutting	Ashi strength
A1B1	6.5^{bc}	8.0^{bc}	6.0^{c}	3.65	7.88	7.89
A1B2	12.5^{abc}	17.0^{abc}	4.5^{c}	3.80	7.95	7.91
A1B3	25.5^{a}	22.5^{a}	12.0^{bc}	3.99	8.01	7.97
A2B1	3.0^{c}	3.0^{c}	12.0^{bc}	3.51	7.80	7.97
A2B2	8.5^{bc}	15.0^{abc}	13.0^{abc}	3.71	7.88	7.98
A2B3	12.5^{abc}	13.5^{abc}	21.5^{ab}	3.80	7.92	8.08
A3B1	17.5^{ab}	12.0^{abc}	17.0^{abc}	3.89	7.90	8.02
A3B2	22.0^{a}	19.5^{ab}	23.0^{ab}	3.94	7.97	8.12
A3B3	24.5^{a}	24.5^{a}	26.0^{a}	3.97	8.09	8.21
X_i	23.1**	16.7*	19.8*			
X_i table 0.05	15.5	15.5	15.5			
X_i table 0.01	20.1	20.1	20.1			
Comparison number	13.1	13.1	13.1			

Means followed by the same lowercase superscripts are not significantly different at α = 0.05; A1: washed with ice water; A2: washed with 4% (w/v) *atung* solution; A3: washed with 4% (w/v) *atung* solution plus ice; B1: washed two times; B2: washed three times; and B3: washed four times.

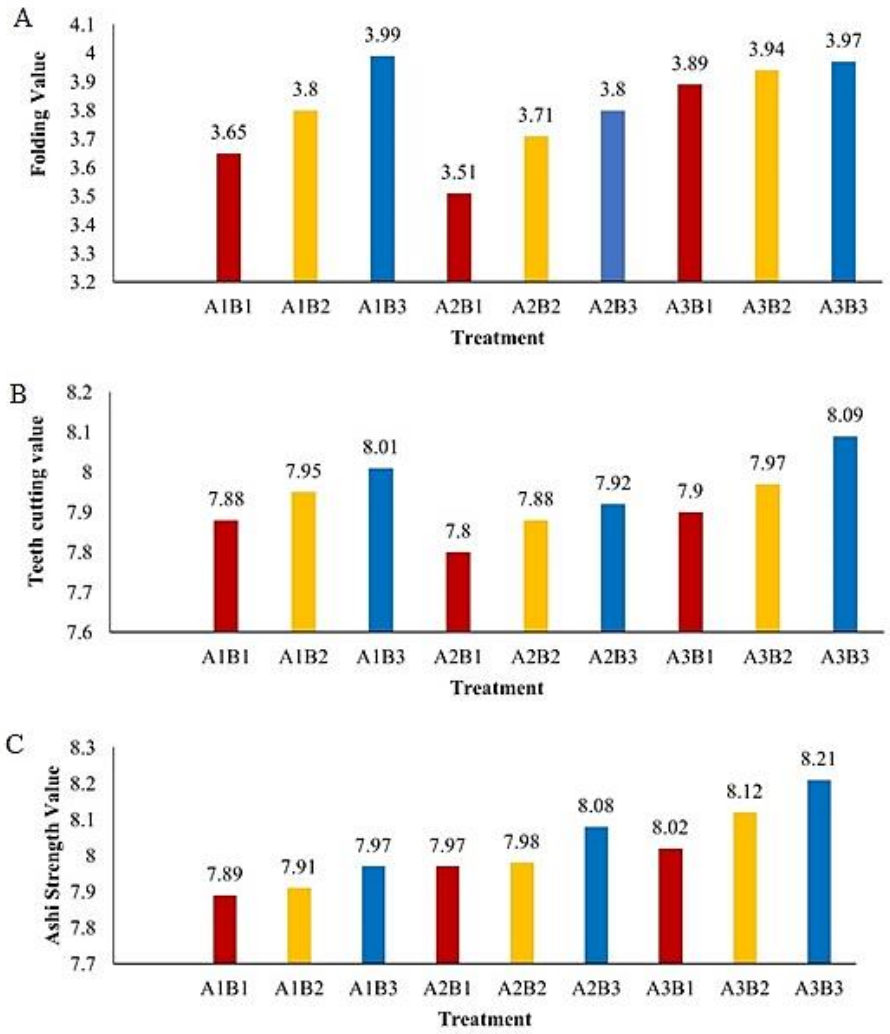

Figure 3. (a) Folding value, **(b)** teeth cutting test, and **(c)** ashi strength results of surimi from dark meat of yellowfin tuna (*Thunnus albacares*).

Rostini (2013) contended that the quantity of meat debris carried away by water while using gauze to extract surimi decreased the yield. Pattipeilohy *et al*. (2020) further explained that *atung* solution could maintain protein stability, as evidenced by the better quality of meatballs from surimi with *atung* solution compared to surimi produced by washing with ice water. Yield is the ratio of meat's weight to whole fish's weight. The calculation of fish yield is used to estimate the number of fish body parts that can be used as food ingredients (Radityo *et al*., 2014). Determination of yield is useful in analysing the benefits of the products to be marketed, where the profit will be greater as the yield increases (Pattipeilohy, 2005; Pattipeilohy *et al*., 2016; Moniharapon *et al*., 2016).

Protein

Protein has a major role in creating fish gels (Radityo *et al*., 2014). The protein value obtained is the protein value of the wet weight (wet basis). According to Suzuki (1981), protein in meat consists of sarcoplasm, stroma, and myofibrils. Sarcoplasmic proteins are soluble in water, whereas myofibril proteins are dissolved in a strong salt solution. In addition, the protein value is highly dependent on the additional ingredients added. In the present work, the value of surimi protein with additional ingredients was higher. This was shown by the protein value of washing four times with *atung* solution, which was 26.62% higher than washing two times with ice water, which was 13.62%. This was supported by Endoma *et al*. (2022) who stated that high washing frequency could result in high protein value. *Atung* can maintain the freshness of the fish so that it still has high protein value (Moniharapon and Pattipeilohy, 2018).

Pattipeilohy *et al*. (2020) stated that surimi cleaned with 4% *atung* solution had higher protein stability than ice water. The low percentage of surimi

protein in ice water could have been due to some of the protein being removed throughout the process (Erdiansyah, 2006). A low temperature (3 - 10°C) when washing is required to prevent protein denaturation and microbial growth (Surilayani *et al*., 2019). Additionally, as the temperature increases, protein activity rapidly decreases, and the gel-forming properties of myofibril proteins are lost. During the initial washing, a significant portion of the primary components that are water-soluble will be lost (Park, 1995). Each treatment in the present work had a protein content value that met the SNI 2694-2013 standard of 12%.

Myofibrillar protein

The salt-soluble protein content in each treatment of surimi ranged from 8.50 to 10.32%. The average results of the protein values increased differently. Myofibril protein is classified as a salt-soluble protein that can form tissue, and occupies around 70% of the total protein in ground fish meat (Park and Morrissey, 2000). This protein consists of actin, myosin, actomyosin, and regulatory proteins such as tropomyosin, troponin, and actinin (Suzuki, 1981; Nurfianti, 2007). In addition to salt content, the solubility of myofibril proteins is also affected by very low ions (Hennigar *et al*., 1988). Degradation of the myosin chain, particularly at low ionic strength or in the presence of proteases, causes the myofibril proteins to become soluble (Andini, 2006). Myofibril proteins' functional properties can affect food's character during processing, storage, and consumption (Subagio *et al*., 2004). Myofibril proteins often become more soluble during mixing, thus increasing the potential for gelling. These findings agreed with Hassan *et al*. (2017) who discovered an increase in the quantity of myofibril protein in catfish surimi along with an increase in washing frequency, as seen by the thickening of the myofibril protein marker band on the SDS PAGE test. Hamzah *et al*. (2015) also stated that the washing procedure could dissolve sarcoplasmic protein, fat, blood, and other components, thus increasing the amount of myofibril protein in the surimi. However, the present work discovered no statistically significant difference in the frequency of washing with 4% *atung* solution plus ice.

Water-holding capacity

Results indicated that the ability of surimi to hold water increased with washing frequency using 4% *atung* solution plus ice. Water-holding capacity is expressed as the quantity of water bound to protein. Santoso *et al*. (2008) also explained that this capacity refers to the ability of meat to absorb and retain water when subjected to mechanical processing, such as stirring, pulverising, mixing, printing, temperature treatment, storage, and transportation. Karthikeyan *et al*. (2004) reported that the water-holding capacity of sardine surimi increased with increasing washing frequency. Moreover, the myofibrils open more as the pH increases and trap more water (Ismail *et al*., 2010). However, the present work noted that washing two and three times with ice water yielded the same water-holding capacity. Anwar *et al*. (2014) stated that the ability to bind water is also influenced by the initial freshness of fish as the raw material.

In previous studies, adding natural additives to surimi could increase myofibril and gel strength due to its high water-holding capacity (Sousa *et al*., 2022). Sharma *et al*. (2023) also showed that adding a natural extract from *Citrus limetta* could manipulate the protein structure and its bonds to improve the gel and texture quality of surimi. The same result was also shown by the addition of *atung* in the present work. Results showed that using *atung* in the washing process yielded higher water-holding capacity value which significantly differed from washing with ice only.

Salmonella spp. and Escherichia coli

According to the World Health Organization (WHO, 2018), *Salmonella* is the major bacterial genus responsible for foodborne illness worldwide. Elizaquível and Aznar (2008) also mentioned that the most pathogenic bacteria in raw food are *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*. Therefore, the Food and Drug Supervisory Agency (BPOM, 2009) issued regulations about fish food and fishery products which must not contain *Salmonella* spp. This regulation was then strengthened by SNI 01-2897-1997 standards which states that the quality and food safety requirements for 25 g of fresh surimi must be negative for *Salmonella*. All surimi produced in the present work were negative for *Salmonella* spp., thus meeting the standard.

SSA is a selective medium for isolating *Salmonella* and *Shigella* bacteria. However, some strains of *Shigella* experienced stunted growth in this medium. *Salmonella* colonies on SSA media were marked red-black with a yellow zone around them (Zaraswati, 2006). No red-black colonies were

observed in the present work. Washing and using hygienic and sterile materials reduce the risk of contamination of the products made. Moreover, ice water and clean *atung* solution have been shown to act as a food preservative, as *atung* contains bioactive components that can kill several types of pathogenic bacteria (Moniharapon *et al*., 2019).

Escherichia coli is a bacterium that easily spreads by contaminating water and objects that come into direct contact, including fish processing. The presence of this bacterium in food or handling equipment is an indication of poor sanitation practices. *Escherichia coli* can cause enteropathogenic *E. coli* infection in children and adults (Nuraeni *et al*., 2000).

Various countries have started to pay serious attention to the effects caused by this bacterium in food derived from fishery products. Oscar *et al*. (2009) stated that a common cause of contamination is direct or indirect contact with the bacterium through contaminated products (cross-contamination). Based on the regulations of the Indonesian Food and Drug Supervisory Agency (BPOM, 2009) and SNI 2694:2013 (BSN, 2013), all kinds of products derived from fish shall not contain *E. coli*.

Atung seeds contain azelaic acid, which can kill pathogenic bacteria and food spoilers, namely *Staphylococcus aureus*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Bacillus subtilis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Escherichia coli* B and C, and *Pseudomonas aeruginosa* (Moniharapon, 2004; Pandyal *et al*., 2017). Indriyanto (2008) found that applying *atung* seeds in handling tiger prawns could extend their freshness. The use of natural preservatives has also been carried out by other studies, such as Kim *et al*. (2013), Krishnan *et al*. (2014), Kurćubić *et al*. (2014), and Elgadir (2015).

Subjective parameters

Folding test

The folding test was used to test the gel strength of the surimi. The test results were recorded using the organoleptic method based on SNI 2732.6:2009 (BSN, 2009), with the quality level consisting of AA (score 5), A (score 4), B (score 3), C (score 2), and D (score 1). The present work found that the combination of washing agents and frequency significantly affected the surimi folding test value. The folding test of red meat tuna surimi had an average value of 3.99 and 3.51, indicating that the surimi has little to no apparent cracks after being folded once. Widyaswari and Irlidiya (2019) further explained that the folding test results are directly related to the gel texture, where a high folding value denotes good gel quality.

The gel strength is influenced by the washing process, which can increase the myofibril protein concentration. Saliada *et al*. (2017) stated that the folding test value was directly related to the gel quality. Wijayanti *et al*. (2012) also explained that the folding test would increase with increasing washing time, and is proportional to pH, WHC, PLG, deformation, and gel strength. According to Sedayu (2004), values of 3 and 4 in the folding test indicate good elasticity. Therefore, it can be said that the surimi produced in the present work was still in good condition.

Teeth cutting

The teeth-cutting test was conducted by biting the surimi with molars, and focusing on its texture and suppleness. The highest value was found in surimi which was washed four times with 4% *atung* solution plus ice. Saliada *et al*. (2017) explained that the length of washing time could affect the concentration of myofibrillar proteins that make up the texture of surimi. Myofibril protein is crucial in gel formation, coagulation, and elasticity of processed meat products as it binds water and fat (Wibowo *et al*., 2015). The usually preferred teeth-cutting value is 5 - 6.

Ashi strength

The elastic property of surimi is called ashi. The ashi strength was tested and recorded by the organoleptic method with a value of 0 - 10 (Suzuki, 1981). According to SNI 2694:2013 (BSN, 2013), the minimum organoleptic value limit for ashi strength is 7. The taste assessment can be seen on the hedonic test assessment sheet. The highest ashi value was obtained in a four-time washing treatment with 4% *atung* solution plus ice. *Atung* solution acts as a humectant as it can bind water in food products (Amri, 2006). Furthermore, the elasticity of the gel is largely determined by the quality of the raw materials; thus, the freshness of the fish is the main requirement to obtain optimal elasticity (Santoso *et al*., 2008).

Fish meat washing can remove sarcoplasmic proteins, and increase the concentration of myofibril

proteins, which are important for the ability to form gels (Chaijan *et al*., 2004). The presence of sarcoplasmic protein and the temperature of the washing water can affect the strength of the surimi gel produced. The amount of water-soluble protein lost during washing depends on the temperature of the wash water. A higher water temperature will dissolve more protein. The best gel strength can be obtained if the crushed fish flesh is washed with water at < 10°C (Yusuf, 2012).

Conclusion

The use of 4% (w/v) *atung* solution was effective in producing yellowfin tuna surimi with 68.50% yield, 20.62% protein, 8.87% myofibrillar protein, and 73.3% water binding capacity, and free from *Salmonella* spp. and *Escherichia coli*. *Atung* 4% (w/v) solution also produced surimi with teeth cutting values of 7.80 - 7.92, ashi strength values of 7.97 - 8.08, and folding test values with grades B to A (3.80) at a washing frequency of four times. Results indicated that red tuna meat could have the potential as a surimi ingredient as an alternative food. In addition, using 4% *atung* solution in the washing process could produce good quality surimi. Therefore, it has the potential as an alternative to natural additives in producing good surimi. It is suggested to the final product producers based on surimi (intermediate product) to use 4% (w/v) *atung* solution. Moreover, the shelf life of the products produced should be further investigated. The utilisation of *atung* to improve the export quality of tuna loin and fish fillets also needs further research.

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