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Extraction and characterization of sodium alginates from *Sargassum polycystum* for manufacturing of tuna (*Thunnus* sp.) meatballs

M K Mokoginta^{1*}, N Indriati¹, N Dharmayanti¹ and S Z Nurbani¹

¹Jakarta Fisheries University, Jakarta, Indonesia

*E-mail: niken.stp@gmail.com

Abstract. *Sargassum* sp. is a type of seaweed that produces a high enough amount of alginate. Alginate is a natural anionic polysaccharide derived from the cell wall of seaweed. Alginate has several advantages such as sodium tripoliphospat, commonly used as one of the ingredients for meatballs. The purpose of this study was to identify brown algae used as samples, find out how to extract alginate, find out the quality of alginate produced, and apply alginate to the manufacture of tuna meatballs. The results showed that the sample of seaweed used was *Sargassum polycystum*. Alginate extraction process was done using Le-Gluahec-Herter method with ten stages of process. The resulting sodium alginate had a moisture content of 9.61%, ash content of 22%, pH level of 9.4 and a viscosity of 50 Cp. The character of the results met the 1981 Food Chemical Codex (FCC) standard for the food industry. The application of alginate to the manufacture of tuna meatballs can improve the texture of the resulting meatballs. The results of quality testing meatballs with chemical parameters and microbiology met the quality standards of fish meatballs, SNI 7266-2014.

Keywords: alginate, microbiology, sodium tripoliphospat, viscosity

1. Introduction

Alginate is a group of natural anionic polysaccharides derived from seaweed cell walls [1]. Although the potential for alginate-producing seaweed is quite high, it cannot be used optimally, considering the development of domestic alginate extraction methods that is still not going well [2]. *Sargassum* sp. is a type of seaweed that produces a high enough amount of alginate. The brown algae species from Indonesian coastal waters that have the potential to be processed into alginates are *Sargassum* sp., *Turbinaria* sp., *Hormophysa* sp. and *Padina* sp. The four alginophyte species (alginate-producing algae) are still obtained from natural preparations [3]. *Sargassum* is classified as one type of commercial seaweed, but not many know that *Sargassum* sp. is one type of seaweed currently in high demand because the amount is quite abundant but the utilization is less than optimal.

The biggest use of alginate is found in the textile, food, paper, pharmaceutical and cosmetic industries [1]. The paper industry uses alginate as an adhesive and preservative, while in fisheries alginate is used as glazing in fish freezing to avoid oxidation reactions. Alginate is also used to increase the viscosity of the medium in canning production [4]. Alginate from *Sargassum* seaweed has quite good viscosity when extracted with alginate calcium deposition process [5]. The processing of low-fat meat products can help maintain the stability of the emulsion and improve rheological properties. Alginate has several advantages, including other healing ingredients such as sodium tripoliphospat commonly used as



meatball paste. Sodium alginate produced by seaweed *Sargassum* sp. is a natural material that contains high fiber and important minerals. Therefore, *Sargassum* sp. sodium alginate seaweed is expected to be an alternative ingredient in making nutritious and safe meatballs. The purpose of this study was to identify the brown algae used as samples, to find out how to extract alginate from *S. polycystum* brown algae, find out how to extract alginate from *S. polycystum* brown algae, find out the quality of alginate produced, and apply alginate to the making of tuna meatballs. The importance of the application of alginate to the manufacture of tuna meatballs is that it can improve the texture of the meatballs.

2. Materials and Methods

Practical methods include explaining the tools and materials used during the research, sampling, extraction and characterization process of alginate, making and testing the quality of tuna meatballs.

2.1. Materials and tools

The main ingredients used in this extraction process are samples from brown algae that was identified by Oceanography Research Center - Indonesian Institute of Sciences (PPO-LIPI), aquadest, CaCl₂ 4%, HCl 2%, Na₂CO₃ 4%, CaCl₂ 10%, CaCl₂ 5%, HCl 5%, and alcohol 95%. The ingredients used in making meatballs were tuna meat, tapioca flour, garlic, pepper, salt, water and ice. The materials used in proximate testing were N-hexan, catalyst protein, Na₂BO₃, sodium carbonate, H₂SO₄.

The tools used during seaweed sampling in the field are the salinity refractometer, universal pH, thermometer, current drogue, GPS, sacks and scissors or knives. The tools used in the laboratory were Erlenmeyer flasks (Pyrex), measuring cups (Hokkai), beaker glasses (Pyrex), hot plates (Thermo Scientific), digital scales (Vibra), oven (Memmert), calico cloths, drop pipettes, blenders, a 40 mesh size filter, water baths, and cabinet dryers. The tools used in making meatballs were spoons, boiling pans, stoves, food processors, thermometers, basins, and digital scales. The tools used in the proximate testing of meatballs were ovens, furnaces, desiccators, fat slices, porcelain dishes, aluminum cups, filter paper, and digital scales. The tools used in microbiological testing were autoclaves, Erlenmeyer flasks, glass beakers, measuring cups, cotton, brown paper, rubber bands, digital scales, incubators, and water baths.

2.2. Sampling

The sampling location was in Lima Island, Serang Regency, Banten Province. It is located between 06°0'5.6376" south latitude and 106°09'15.3684" east longitude with an island area of 2.6 ha. Seaweed samples were taken by hand and cleaned using seawater to remove sticky dirt and then were put in a sack. The sampling location can be seen in figure 1.

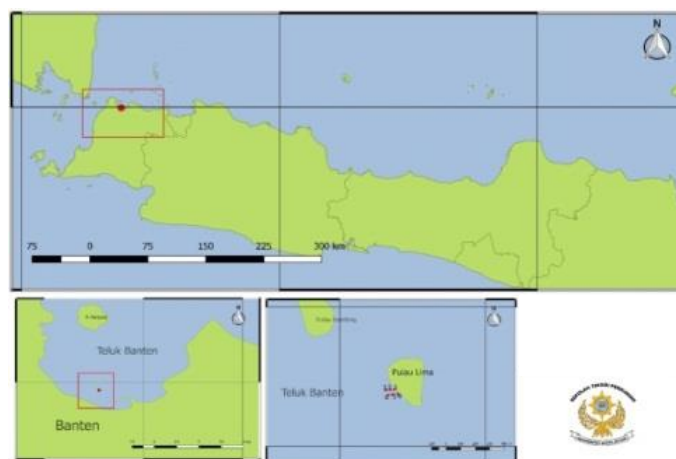


Figure 1. Sampling location, ● = station, ■ = island, ■ = sea.

2.3. *Algae extraction of brown S. polycystum*

Alginate extraction followed the Le-Gloahec-Herter method described in [6]. 25 grams of seaweed powder was mixed with 200 mL of distilled water and then soaked in 1% 200 mL CaCl_2 for two hours. The function of this process was to remove most of the laminarin, mannitol, salt and other follow-up components of carbohydrates contained in seaweed. The salts along with CaCl_2 were then removed by washing with clean tap water, while calcium alginate remained in the cell because it did not dissolve in water. This washing was stopped if the washing water was clean. Subsequently, immersion was carried out in 2% 200 mL HCl for thirty minutes in order to dissolve alkaline earth salt remnants. In addition, washing with clean water was carried out until the pH was neutral. The next stage of extraction by seaweed slurry was blended with 200 mL of 4% Na_2CO_3 solution then heated in a 90°C water bath for two hours while periodically being stirred. This process was carried out until all cellulose became fine particles and homogeneous paste was produced. Then the dilution was carried out by adding distilled water and 4% Na_2CO_3 in a ratio of 3:7. Then the resulting solution was filtered using a filter cloth to obtain the filtrate. The filtrate was then heated to a temperature of 40°C and then coagulated using CaCl_2 10% and filtrate in a ratio of 1:5, and stirred for fifteen minutes to obtain calcium alginate clots. The remaining filtrate was coagulated with CaCl_2 5% and filtrate solution in a ratio 1:5 to obtain a lump of alginate calcium.

Then, the calcium alginate obtained was acidified with 5% HCl until the pH of the calcium alginate 2-3 was obtained, then washed with 95% alcohol and alginate in a ratio of 1:1 by soaking while stirring periodically for 20 minutes and filtered. After that, Na incorporation was carried out using 1% Na_2CO_3 and calcium alginate solution in a ratio of 1:1.5 for one hour while stirring periodically, followed by re-washing using 95% alcohol two times. The last stage was drying with a temperature of 70-75°C for eight hours in a cabinet dryer. The final product obtained was dry sodium alginate. After obtaining dried sodium alginate, it was blended and sieved 80 mesh.

2.4. *Characterization of sodium alginate*

Characterization of sodium alginate includes parameters of moisture content, ash content, pH and viscosity parameters [7, 8].

2.5. *Making tuna meatballs*

The making of tuna meatballs began with the process of melting tuna meat. Then, 300 grams of tuna meat was mashed using a food processor by adding 75 grams of ice cubes (25% by weight of meat). Then, six grams of salt (2% by weight of meat) and seasoning (1 gram of pepper, 5 grams of garlic) were added into the mixture to blend again. After being evenly mixed, into the pulverized meat was added 45 grams of tapioca flour (15% by weight of meat) while stirred and pulverized until a homogeneous mixture was obtained. Next, 0.5 gram of sodium alginate powder (0.17% of the weight of the fish used) was added into the mixture and stirred evenly. Homogeneous dough was poured into meatball molds. The product maturation process was done by putting them into hot water with two stages of heating according to the temperature and the time specified. First, boiling was carried out at a temperature of 40°C-70°C for 10-20 minutes, then continue boiling at 90°C-100°C until the meatballs floated. In addition, the manufacture of tuna meatballs without the addition of alginate was also carried out.

2.6. *Testing the quality of tuna meatballs*

Fish meatball quality requirements followed the SNI 7266-2014 Fish Meatballs [9]. Chemical testing of fish meatballs with water content parameters ash and protein content was done according to SNI 01-2354-2006 [10]. Testing the physical properties of fish meatballs with folding test parameters and bite tests was done according to SNI 2372.6.2009 [11]. In addition, several other tests were performed: Total Plate Count (TPC) Microbiology Test (SNI 01.2332.3-2006), *Salmonella* test (SNI 01.2332.2-2006), *Escherichia coli* test (SNI 01.2332.1-2006) [10], and *Staphylococcus aureus* test (SNI 2332.9-2011) [12].

2.7. Data analysis

This research was carried out both qualitative (descriptively), and quantitative. The quantitative research data was processed using Excel for the sensory test average data. Folding test and bite test was analyzed with SPSS using the Wilcoxon test.

3. Results and Discussion

The sample used in this study was the genus *Sargassum* sp. The results of identification of the sample used obtained the following characteristics: yellowish brown algae, branching thallus resembling tree branches, shaped like leaves, rough and rather stiff surfaces, on the sidelines of the branch there was a small round that was hard and slippery like a fruit, short-stemmed, pointed leaves, and jagged edges. The algae grew attached to the coral reef. Seaweed found in the waters of Banten Bay has a main thallus length of 30-100 cm and a rock bottom substrate. Based on the characteristics above, it was suspected that the *Sargassum* obtained from the waters of Lima Island was a species of *S. polycystum*. *S. polycystum* has cylindrical, holdfast thallus characteristics such as discs and short stalks [13]. In addition, they can reach a height of about two meters; their small leaves are oval-shaped, 3 cm long by 1 cm wide, jagged or like a saw, flat or pointed curved end, without very clear veins, round ovoid air bubbles, sitting on branching trunks or short trunks, rounded edges, blunt sometimes there are like thorns, growing on stone substrates or other hard objects.

3.1. Characterization of sodium alginate

In this study, sodium alginate was characterized by describing the parameters of water content, ash content, viscosity and pH which aimed to determine the quality of alginate produced from *S. polycystum* seaweed from the Gulf of Banten beach waters. Characterization results of sodium alginate from *S. polycystum* seaweed can be seen in table 1.

Table 1. Results of characterization of Na-Alginate from *S. polycystum* seaweed originated from the waters of the Banten Bay Coast.

Characteristics	Na-alginate	FCC standards for food industry
Water content %	9.61	< 15
pH	9.4	3 – 10
Ash content %	22	< 23
Viscosity (cP)	50	10 – 5000

From this research, *Sargassum* sp. seaweed sodium alginate was 9.61%. The results obtained show that the water content produced met the Food Chemical Codex (FCC) standard where sodium alginate is <15% water content. Water content affects the quality and quality of a material. The water contained in the ingredients can affect the texture, storability, taste and appearance. The increase in water content in dry matter can cause damage, both due to chemical reactions and microbial growth of spoilers [14]. The results showed that the pH contained in the sodium alginate extracted from *Sargassum* sp. seaweed was 9.4. Alginate is stable at pH 5-10 [15], while pH higher than this value can cause the viscosity produced to be very small due to β -eliminative degradation. Food Chemical Codex [16] has set the food industry's sodium alginate quality standard at 3.5-10. When compared with these standards it can be concluded that the results of sodium alginate obtained are in accordance with the food industry quality standards. In this research, the extracted ash content of sodium alginate was 22%. In this study, extracted ash content of sodium alginate was 22%. These results are not much different from the ash content set by the Food Chemical Codex [16], which is 13-27%. Likewise, the ash content specified for food grade is 23% [17]. When compared with some of these standards, the resulting sodium alginate ash content meets the standards. The amount of alginate ash content was influenced by the amount of Na_2CO_3 solution added during extraction. The Na_2CO_3 contained in the sample was an organic salt mineral, so the greater the content in a material, the more the sodium ash content of alginate would be [6]. The alginate viscosity produced in this study was 50 cP (centiPoise). These results indicate that the resulting viscosity met the Food Chemical Codex (FCC) standard where sodium alginate viscosity ranges from

10 - 5000. Alginate can be characterized by the degree of viscosity in the form of a solution [18]. The viscosity of alginate was influenced by the strength of the gel, alginate concentration, degree of polymerization and molecular weight. The viscosity of alginate was also influenced by temperature, acid conditions and the ratio of M and G units of alginate molecules. Increased viscosity depends on the presence of high guluronic acid and manuronic acid [19].

3.2 Sensory test and physical test results

Assessment of the final product sensory test was carried out using 30 untrained panelists. Sensory tests for tuna fish meatballs without the addition of alginate and tuna fish meatballs with the addition of alginate include appearance, smell, taste and texture parameters. The results of the sensory test and physical assessment can be seen in table 2.

Table 2. Results of sensory test and physical assessment.

No	Treatment	Sensory assessment parameters				Physical test	
		Appearance	Smell	Taste	Texture	Folding test	Bite test
1	Alginate meatballs	7.0	7.0	7.0	9.0	5.0	9.0
2	Control meatballs	6.5	8.0	7.0	7.0	3.0	7.0

Sensory assessment of appearance is an overall assessment. The results of the standard deviation calculation showed that tuna fish meatballs with the addition of alginate got a specification value of 7.0, while meatballs without the addition of alginate got a 6.5 sensory value. In spite of these values, the appearance of each meatball was not much different. This was because the meatballs without the addition of alginate had a rough surface compared to those added with alginate. However, based on the nonparametric statistical test with the Wilcoxon test, there was no significant difference between the appearance of alginate meatballs and meatballs without the addition of alginate.

As for the sensory test results for smell parameters, meatballs with the addition of alginate got a sensory value of 7.0 while meatballs without the addition of alginate had a sensory value of 8.0. The difference in sensory value was possible because the addition of alginate in meatballs could reduce the specific odor of fish meatball products such as the smell of added spices and the smell of fish. However, the characteristics of alginate used did not smell. Chemically it is difficult to explain why compounds cause different scents, because compounds which have similar chemical structures and functional groups (stereoisomers) sometimes have very different scents. Conversely, compounds that are very different in chemical structure may cause a distinctive smell [20]. Based on the nonparametric statistical test with the Wilcoxon test, there was no significant difference in scent between the meatball added with alginate and meatballs without the addition of alginate.

Sensory test on taste parameters resulted in the same value of 7.0 for both meatballs with the addition of alginate and meatballs without the addition of alginate. This was due to the presentation of fish seasoning and meat used in each treatment equally. Acceptance of taste by panelists was influenced by several factors including chemical compounds, temperature of concentration and interaction with other flavor components. The results of data analysis with statistics using the Wilcoxon test on taste parameters between meatballs with the addition of alginate and meatballs without the addition of alginate showed that there was no significant difference in the taste of each meatball. In the texture test, tuna fish meatballs with the addition of alginate obtained 9.0 sensory values. Meatballs with the addition of alginate had a dense, compact, and chewy texture compared to meatballs without the addition of alginate, which got a 7.0 sensory value and which according to specifications had a compact and rather springy solid texture. Statistical data analysis using Wilcoxon test showed that there was a significant difference in texture between meatballs with the addition of alginate and meatballs without the addition of alginate. Therefore, it can be concluded that there was an effect of the use of alginate on the texture of the meatballs produced. Fat can be replaced with water and non-meat ingredients such as hydrocolloid (carrageenan, starch, maltodextrin, alginate) during the processing of low-fat meat

products that can help maintain the stability of the emulsion and improve rheological properties [21]. From the results of research conducted by [21], STPP and alginate could increase the elasticity level of beef meatballs.

The folding test was related to the gel strength test where the better the folding test result was, the better the elasticity of the fish meatball would be. The average value of folding tests on tuna fish meatballs with the addition of alginate was 5.0. The meatballs did not crack based on its specifications when doubled and included in AA grade [11], compared to fish meatballs without the addition of alginate, which only got an average value of 3.0 with the specifications of a little slack when folded once and included in grade B [11]. According to statistical data analysis using Wilcoxon test, there was a significant difference in the strength of meatball gel with the addition of alginate and meatballs without the addition of alginate. Therefore, it can be concluded that the addition of alginate affected the texture of the fish meatball produced.

A bite test was necessary to determine the level of consumer preference in knowing how much elasticity was produced by fish meatballs. The average value of the bite test results on meatballs with the addition of alginate was 9.0; their elasticity was very strong. Meanwhile, meatballs without the addition of alginate had an average value of 7.0, which means that the texture and elasticity of the meatball was rather strong. From the average value of the bite test it could be seen that meatballs with the addition of alginate had a very strong level of texture and elasticity compared to meatballs without the addition of alginate. The results of statistical data analysis using Wilcoxon test on texture and elasticity through bite testing between meatballs with the addition of alginate and meatballs without the addition of alginate showed that there were significant differences in the texture and elasticity of the fish meatballs produced.

3.3. Results of fish meatball chemical testing

In this study, a chemical analysis of the tuna meatballs was produced. Chemical testing was carried out on tuna fish meatballs with the addition of alginate and without the addition of alginate, which included testing protein content, water content, and ash content in accordance with the quality requirements of fish meatballs, SNI 7266-2014 [9]. The results of chemical analysis of tuna meatballs can be seen in table 3.

Table 3. Results of chemical testing of tuna meatballs.

No	Testing	Meatballs + alginate	Meatballs without alginate	SNI standard 4427-2014
1	Moisture (%)	44.45	44	Max 65
2	Ash content (%)	1.09	0.99	Max 2.0
3	Protein (%)	19.78	17.54	Min 7

From the table above it can be seen that meatballs with the addition of alginate had a higher water content with a value of 44.45% than meatballs without the addition of alginate, which had 44% moisture content. This was because alginate had water binding properties. The test results of the meatball water content in general met the SNI standard for the water content of fish meatballs. This was because the water content was less than 65%, while the water content in fish meatballs should be a maximum of 65% [9].

The average value of ash content in tuna meatballs with the addition of alginate was 1.09% higher than the value of meatball ash content without the addition of alginate, which was 0.99%. This was due to the alginate used, which contained a high amount of minerals, and sodium alginate, which had ash content of around 22%. However, the meatball ash content met SNI standard for ash content of fish meatballs nonetheless. This was because the ash content was less than 2.0%, while the ash content in fish meatballs should be a maximum of 2.0% [9]. Minerals in fish meat can be phosphate salts, calcium, sodium, magnesium, sulfur, and chlorine [22]. In sarcoplasm there is a lot of potassium, calcium, magnesium, and chlorine where potassium and calcium are often part of complex proteins. The ash

content of the product comes from the level of fish ash, NaCl, and flour. Ash content is composed of various types of minerals with various compositions depending on the type and source of foodstuffs [23].

The largest amount of protein found in meatballs is derived from protein from raw materials [24]. The protein content contained in tuna fish meatballs with the addition of alginate was 19.78% higher than that of meatballs without the addition of alginate, which was 17.54%. This is possible because seaweed was used as a raw material for making alginates in the form of *Sargassum* types which contain protein levels ranging from 5.53% [25,26]. In addition, the increase in protein levels was thought to be due to the added alginate which bound water and held the protein that was soluble in water during boiling [27]. Nonetheless, the protein content met the SNI standard for fish meatball products, which should be a minimum of 7% [9].

3.4. Microbiology testing results

In this study microbiological quality testing of fish meat was conducted to ensure that food was suitable for consumption. Microbiological meatball quality testing included TPC, *S. aureus*, *Salmonella*, and *E. coli*. Microbiology testing results can be seen in table 4.

Table 4. Results of microbiological testing of tuna fish meatballs.

No	Testing	Meatballs + alginate	Meatballs without alginate	Standard SNI-7266-2014
1	TPC (Col/g)	4.3×10^3	1.5×10^4	Max 1.0×10^5
2	<i>Staphylococcus aureus</i> (Col/g)	0	0	Max 1.0×10^2
3	<i>Salmonella</i> (Col/25 g)	Negative	Negative	Negative
4	<i>Escherichia coli</i> (APM/g)	< 3	< 3	< 3

In TPC testing, fish meat products with the addition of alginate obtained TPC 4.3×10^3 colonies/gram, while meatballs without the addition of alginate obtained TPC 4.3×10^3 colonies/gram. The TPC results still met the quality standards of tuna meatballs that were used as references in the processing of fish meatballs. According to SNI No. 7266-2014, the maximum TPC of fish meatball should be 1.0×10^5 colonies/gram.

S. aureus test results for each meatball sample at the identification stage of the suspect colony showed that there were no specific suspect colonies of *S. aureus*, namely circular, smooth, smooth, convex colonies, with diameters of 2 mm - 3mm, gray to black, around the edges of clear colonies (halo formed). Colonies have a fatty and sticky consistency when taken with a needle inoculation [12]. Therefore, it can be concluded that the results of testing the contamination of *S. aureus* bacteria on meatball products was 0 colonies/25 grams.

Salmonella testing on fish meatball products aimed to ensure that there was no contamination of *Salmonella* bacteria in fish meatball products. *Salmonella* test results on tuna meatballs were negative, in accordance with SNI standards for microbial contamination. These bacteria were not indicators of sanitation, but for food safety indicators. That is, because all known *Salmonella serotypes* in this world are pathogenic, the presence of these bacteria in food is considered harmful to health [26].

The results of testing of *E. coli* microbial contamination in tuna fish meatballs with the addition of alginate and without the addition of alginate showed that in 18 tubes tested using 3 tube series there were no positive tubes marked by media changes from clear to cloudy and gas bubbles forming in Durham tubes. Based on the test results it can be concluded that tuna meatball samples did not contain *E. coli* bacteria in accordance with SNI 7266-2014 [9] that the maximum contamination of *E. coli* bacteria in processed products for the protection of consumers regarding product quality is stated to meet the maximum threshold requirements for microbial contamination in food because the value is <3 MPN/g. *E. coli* is a gram negative group which is used as a food quality bio-inhibitor bacteria [27].

4. Conclusion

The result of sodium alginate had a moisture content of 9.61%, ash content of 22%, pH level of 9.4, and viscosity of 50 cP. Meatballs with the addition of alginate had a significant effect on the texture of meatballs. In the chemical tests, fish meatballs with the addition of alginate produced moisture content of 44.45%, ash content of 1.09% protein content of 19.78%, while meatballs without the addition of alginate obtained moisture content of 44%, ash content of 0.99%, and protein content of 17.54%. In microbiology tests, fish meatballs with the addition of alginate produced TPC 4.3×10^3 , while those without the addition of alginate produced TPC 1.5×10^4 , *S. aureus* 0 colonies/g, *Salmonella* negative, and *E.coli* <3 APM/gram. The results of chemical and microbiological testing met the SNI standard 7266-2014.

References

- [1] Szekalska M, Pucibowska A, Szymanska E, Ciosek P and Winnicka K 2016 Alginate: current use and future perspective in pharmaceutical and biomedical applications *Int. J. of Polymer Science* **16** 1-17
- [2] Husni A, Subaryono, Pranoto Y, Tazwir and Ustadi 2012 Pengembangan metode ekstraksi alginat dari rumput laut *Sargassum* sp. sebagai bahan pengental *Agritech*. **32** 1-8
- [3] Rasyid A 2003 Karakteristik natrium alginat hasil ekstraksi *Sargassum polycystum* Makalah disampaikan pada seminar RIPTEK Kelautan Nasional 30-31 Juli 2003 di Gedung BPPT (Jakarta: RIPTEK Kelautan Nasional)
- [4] Darmawan M, Tazwir and Hak N 2006 Pengaruh perendaman rumput laut coklat dalam berbagai larutan terhadap mutu natrium alginat *Bul. THP*. **9** 26-38
- [5] Rasyid A 2010 Ekstraksi natrium alginat dari alga coklat *Sargassum echinocarplum* *J. Oseanologi dan Limnologi di Indonesia* **36** 393 – 400
- [6] Wardani D 2008 Isolasi dan karakterisasi natrium alginat dari rumput laut *sargassum* sp. untuk pembuatan bakso ikan tenggiri (*Scromberomorus commerson*) (Surakarta: Sebelas Maret University)
- [7] Association of Official Analytical Chemist. 1995 *Official Method of Analysis of The Association of Official Analytical of Chemist* (Virginia: Published by The Association of Official Analytical Chemist Inc.)
- [8] Cottrell L W and Kovacks P 1980 Alginates In: Davidson R (ed). *Handbook of Water Soluble Gums and Resins* (New York: Mc-Graw-Hill.)
- [9] Badan Standarisasi Nasional 2014 Bakso ikan SNI 7266.2014 (Jakarta: Badan Standarisasi Nasional)
- [10] Badan Standarisasi Nasional 2009 Cara uji kimia- bagian 3: kadar lemak total pada produk perikanan SNI 2354.2006 (Jakarta: Badan Standarisasi Nasional)
- [11] Badan Standarisasi Nasional 2009 Cara uji fisika-bagian 6: penentuan mutu pasta pada produk perikanan SNI 2372.6.2009 (Jakarta: Badan Standarisasi Nasional)
- [12] Badan Standarisasi Nasional 2009 Cara uji *Staphylococcus aureus* pada produk perikanan SNI 2332.9-2011 (Jakarta: Badan Standarisasi Nasional)
- [13] Soekendarsi E, Juariah U, Setyobudiandi I and Soewardi K 2004 Jenis-jenis rumput laut di perairan indonesia (Bogor: Bogor Agricultural University)
- [14] Tambunan M P A, Rudiyansyah and Harlia 2013 Pengaruh konsentrasi Na_2CO_3 terhadap rendemen natrium alginat dari *Sargassum cristaeifolium* asal Perairan Lemukutan (Pontianak: Universitas Tanjungpura)
- [15] Bahar R, Arief A and Sukriadi 2012 Daya hambat ekstrak Na-Alginat dari alga coklat jenis *Sargassum* sp. terhadap proses pematangan buah mangga dan buah jeruk *J. Indonesia Chimica Acta* **2** 22-31
- [16] Food Chemical Codex 1981 *Food Chemical Codex* (Washington DC: National Academy Press)
- [17] Chapman V J and Chapman D J 1980 *Seaweed and Their Uses. Third Edition* (London: Chapman and Hall)

- [18] Eriningsih R, Marlina R, Mutia T, Sana A W and Titis A 2014 Eksplorasi kandungan pigmen dan alginat dari rumput laut coklat untuk proses pewarnaan kain sutra *J. Arena Tekstil* **6** 73-80
- [19] Amir H, Subaryono, Pranoto Y, Tazwir and Ustadi 2012 Pengembangan metode ekstraksi alginat dari rumput laut *sargassum* sp. sebagai bahan pengental *Agritech*. **5** 1-8
- [20] Winarno F G 1997 *Kimia Pangan dan Gizi* (Jakarta: PT Gramedia)
- [21] Rahardiyani D 2004 Bakso (traditional indonesian meatball) properties with postmortem conditions and frozen storage [Thesis] The Interdepartmental Program of Animal and Dairy Sciences, Faculty of the Louisiana State University and Mechanical College
- [22] Hadiwiyoto S 1993 *Teknologi Pengolahan Hasil Perikanan* (Yogyakarta: Penerbit Liberti)
- [23] Andarwulan N, Kusnandar F and Herawati D 2011 *Analisa Pangan* (Jakarta: Dian Rakyat)
- [24] Siswati J 2002 Kajian ekstraksi alginat dari rumput laut *Sargassum* sp. serta aplikasinya sebagai penstabil es krim [Undergraduate thesis] (Bogor: Bogor Agricultural University)
- [25] Amaliah S, Aris M and Haryati S 2016 Pengaruh penambahan bubuk rumput laut (*Kappaphycus alvarezii*) terhadap karakteristik bakso ikan payus (*Elops hawaiiensis*) *JPK*. **6** 40-50
- [26] Arifah N I 2010 Analisis mikrobiologi pada makanan (Surakarta: Sebelas Maret University)
- [27] Supardi and Sukanto 1999 *Mikrobiologi dalam Pengolahan dan Keamanan Produk Pangan* (Bandung: Penerbit Alumni)