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Improving the Extraction Process of Sodium Alginate from Samu (*Sargassum piluliferum*) using the Plackett-Burman Design

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ABSTRACT

Alginates, which are mainly produced from kelp, improve and stabilize the texture of foods. Thus, they are important in the food and beverage industry. Brown seaweeds such as *Sargassum piluliferum* are abundant in the Philippines and could be potential sources of alginates. The Plackett-Burman screening was used as a tool to evaluate the importance of seven selected variables (formaldehyde solution, extractant ratio, Na_2CO_3 concentration, HCl concentration, extraction temperature, bleaching, and precipitant), which influence the extraction process of sodium alginates. The main factors that affected the extraction of sodium alginates were extraction ratio, Na_2CO_3 concentration, and bleaching. Extraction ratio and Na_2CO_3 significantly affected the production of sodium alginate by lowering the level of extractant concentration and obtaining a positive effect in terms of its yield. Bleaching with NaOCl did not significantly affect the yield. However, it had a positive effect on the purity of the alginate which is an essential physico-chemical parameter that reflects the proportion of the target compound. These factors could be further optimized to extract high yield and good quality sodium alginate from *Sargassum piluliferum*.

Keywords: extractant ratio, Plackett-Burman, *Sargassum piluliferum*, sodium alginate, sodium carbonate, sodium hypochlorite

INTRODUCTION

Macro algae are interesting sources of different bioactive polysaccharides, the uses of which range from industrial to novel food applications. These possess many different and often exotic polysaccharides that are currently explored for their functional properties in food and biomedicine (Kraan 2012). Alginates are the most abundant polysaccharides present in brown seaweeds (Fernandez et al 2009). There

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are specific tropical seaweeds that are potential sources of alginates. *Sargassum,* a brown alga, appears to be the most promising. It is usually the most ecologically dominant species along tropical and subtropical coasts (Prince et al 1979) and plays a remarkable economic role as a source of alginates (Blanshard & Mitchell 2013). It is one of the commonly found brown macro algae in the Philippines, with 72 recorded species (Ortis & Tromo 2000). The abundance of these species in the country is an opportunity to improve their marketing form into more expensive and value added products. One of the ways to do this is to extract their important polysaccharides, specifically alginates.

Alginates are used as organoleptic additives and stabilizers to improve, modify, and stabilize the texture of food (Nalamothu et al 2014). The extraction process should use suitable methods to obtain high yield and good quality alginates. This can influence the yield and chemical composition as well as rheological properties of the isolated alginates (McHugh 2003, Hernandez-Carmona, Freile-Pelegrin & Hernandez-Garibay 2013; Gomez et al 2009 as cited in Peteiro 2018).

In order to determine the effect of numerous factors affecting the alginate extraction process, a screening design should be performed. According to Vanaja and Shobha (2008), a 'screening design' refers to an experimental design which can be applied when a large number of potential causative factors have to be examined, to identify the most important ones that may have an effect on one or more responses of interest. This will reduce the number of factors to be investigated in further experimentation. When there are many factors to be tested, the Plackett-Burman design is an excellent option, since it allows the understanding of the effects of various physico-chemical, biochemical, and sensory variables using a minimum number of experiments. Plackett-Burman is a design widely used in food research because it allows the screening of the main factors from a large number of variables that can be retained in the further optimization process (Siala et al 2012). The projective property of the Plackett-Burman design is that it allows the experimenter to follow up an initial design with runs that allow an efficent separation of main effects and interactions (Wass 1997; Nijhuis et al 1999; Plackett & Burman 1946; Yannis 2001 as cited in Siala et al 2012).

Hence, this study explored the possibility of expanding the use of *Sargassum*, which is largely underused despite its abundance, to a more profitable value added form through the production of sodium alginate. Generally, this study aimed to extract and use sodium alginate from locally found *Sargassum piluliferum;* identify the effects of the different variables that influence the extraction of sodium alginate using Plackett-Burman design; and evaluate the physical and chemical characteristics (moisture content, ash content, pH, alginate purity, and percent yield) of the extracted sodium alginate.

MATERIALS AND METHODS

Procurement of Materials

The *Sargassum piluliferum* was procured from Higatangan Island, Biliran. The chemicals and reagents used were purchased from Far Eastern Drug and Yana Commodities in Cebu City, Philippines.

Preparation of Sargassum piluliferum

The *Sargassum piluliferum* underwent different preparation methods based on the defined variables used. *Sargassum* was dried in a cabinet drier for 8-10 hours at 60^0C. Then it was milled to powder using a grinder. The powdered *Sargassum* was kept in a clean container, ready for use.

Extraction Process of Sodium Alginate

The extraction of sodium alginate was done using different methods based on the concentration and temperature of extractants and conditions used. The *Sargassum* was soaked for 24 hours in 0.1% (Reyes et al 2005) and 0% formaldehyde solution and washed with distilled water. The leaching process was done by soaking *Sargassum* in 0.5 N HCl (Kasim et al 2017) and 0.3 N HCl for 2 hours. Afterwards, it was washed with distilled water until the pH was neutral. It was then bleached using 100 mL 5% hypochlorite (NaOCl) for 30 minutes and washed with distilled water. It was extracted by heating with 7.5% (Zailanie 2015) and 3% (Mazumder et al 2014) sodium carbonate (Na_2CO_3) for 3 hours at a temperature of 90^0C (Mazumder et al 2014) and 60^0C (Mushollaeni 2011). Then, it was filtered with a fine mesh cloth and the filtrate was collected. The filtrate was washed three times with distilled water. It was slowly added with 10% HCl while stirring until pH 2 was attained. Alginic acid, characterized by the deposition in the form of gel, was obtained (Kasim et al 2017). The precipitated alginic acid was then collected and washed with distilled water. The alginic acid was added with 10% sodium carbonate (Na_2CO_3) (Mushollaeni 2011) and stirred until its pH reached less than 8 (Reyes et al 2005) and finally precipitated with 95% isopropanol (Zailanie 2015) and 94% ethanol (Mazumder et al 2014) to obtain a fiber sodium alginate. The sodium alginate precipitate was then filtered and dried in an oven at a temperature of 45^0C for 15 hours. Figure 1 shows the process flow diagram in extracting sodium alginate.

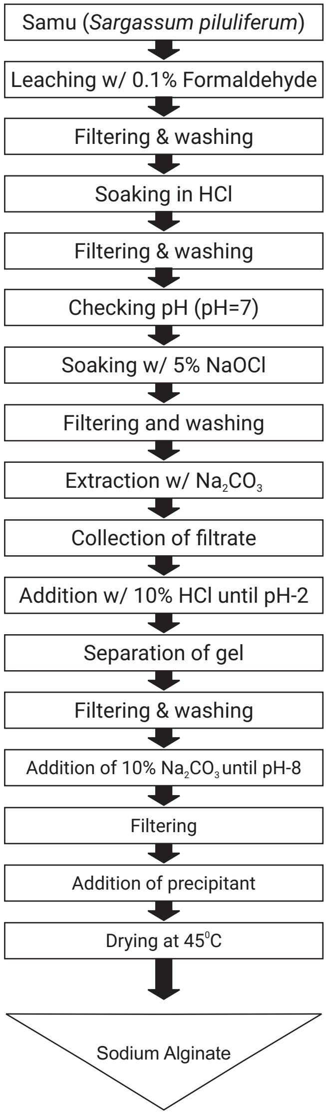

Figure 1. Process flow diagram of sodium alginate extraction

Experimental Design for Screening

The screening experiment used Plackett-Burman design to identify the most important factors that affected the sodium alginate extraction. Table 1 shows the design set-up for 7 variables having 8 runs, and Table 2 shows the 7 variables-8 runs used in the experiment.

Table 1. Plackett-Burman Design for 7 variables using 8 runs

Run	A	B	C	D	E	F	G
1	+	+	+	-	+	-	-
2	+	+	-	+	-	-	+
3	+	-	+	-	-	+	+
4	-	+	-	-	+	+	+
5	+	-	-	+	+	+	-
6	-	-	+	+	+	-	+
7	-	+	+	-	-	+	-
8	-	-	-	-	-	-	-

Table 2. Plackett-Burman 8-run design with 7 variables at high and low values used in the extraction of sodium alginate from Samu (*Sargassum piluliferum*)

Extractant Ratio	Formaldehyde Concentration (%)	HCl Conc. (N)	NA_2CO_3 Conc. (%)	NA_2CO_3 Temp (^{0}C)	NaOCl concentration	Precipitant
40:1	0.1	0.5	7.5	60	unbleached	94% ethanol
40:1	0.1	0.3	3	60	bleached	95% isopropanol
40:1	0	0.5	3	90	unbleached	95% isopropanol
20:1	0.1	0.3	7.5	90	unbleached	95% i sopropanol
40:1	0	0.3	7.5	90	bleached	94% ethanol
20:1	0	0.5	7.5	60	bleached	95% i sopropanol
20:1	0.1	0.5	3	90	bleached	94% ethanol
20:1	0	0.3	3	60	unbleached	94% ethanol

Physico-Chemical Analysis

Moisture Content

The empty crucible (plain) with cover was first dried in an oven set at 105^{0}C for 3 hours, then transferred to a desiccator for cooling. Afterwards, its weight was taken. This was repeated until a constant weight of the crucibles was obtained. About 3 g of the sodium alginate sample was weighed on a dish. Then, this was placed in an oven to dry for 3 hours at 105^{0}C. After drying, the crucible, partially covered with a lid, was placed in the desiccator for cooling. Its weight was taken and the moisture content was determined using the equation below:

$$\text{Moisture} = \frac{W_1 - W_2}{W_1} \times 100$$

where:
W1 = weight (g) of the sample before drying
W2 = weight (g) of sample after drying

Ash Content

The method used by Gholamipoor et al (2013) was followed in determining the ash content of the extracted sodium alginate. Five grams of sodium alginate sample was placed in a crucible and dried in an oven at 105^{0}C for 30 minutes. The dried sample was weighed again and then calcined in the furnace at 450^{0}C for 3 hours. Calcined samples were cooled in a desiccator, and then weighed. The ash content was determined using the equation below:

$$\text{Ash content (\%)} = \frac{\text{Weight of the ash}}{\text{Weight of the dry algae}} \times 100$$

pH Measurement

The pH measurement of the alginate was done by dissolving 0.5 g of sodium alginate in 50 mL distilled water and heated at 60-70^{0}C for 20 minutes while stirring occasionally to make the solution uniform. This was allowed to cool. The pH of the alginate solution was measured using a HANNA digital portable pH meter HI 98108.

Alginate Purity Determination

The method of Hernandez-Carmona et al (1999) as cited by Reyes-Tesnado et al (2005) was adopted in determining the purity of the alginate produced. One gram of calcium chloride was dissolved in 100 mL of methanol-water solution (40-60%).The resulting solution was added to 100 mL of 0.5% alginate solution, while gently stirring. Then the precipitate was removed using a fine filter and then washed with 20-80% methanol-water solution. Second washing was done using 40-60% methanol-water solution. The precipitated alginate was dried in an oven set at 105^{0}C for 2 hours. Then, the alginate was maintained in a desiccator for one hour and weighed. The alginate purity was computed using the formula:

$$\text{Alginate purity} = \frac{\text{Weight of the precipitate}}{\text{Weight of the initial alginate on dry basis}} \times 100$$

Percent Yield

The yield of the extracted sodium alginate was computed using the formula:

$$\text{Yield of sodium alginate (\%)} = \frac{\text{Weight of sodium alginate}}{\text{Weight of milled seaweed}} \times 100$$

Statistical Analysis

The result of the physico-chemical tests from the Plackett-Burman design was analyzed using Statistica 8.0 software to determine the variables which significantly affected the sodium alginate extraction process.

RESULTS AND DISCUSSION

Variable Screening

The results of the variable screening showed the effect of the seven variables on yield, moisture content, ash content, pH, and purity of the extracted sodium alginate. Results indicated that the variables having the most number of significant effects were extractant ratio and Na_2CO_3 concentration. The variables with the least number of significant results were formaldehyde solution, HCl concentration, extraction temperature, bleaching, and precipitant (Table 3).

Table 3. Summary of the effects of different parameters on the extraction of sodium alginate using Plackett-Burman design

Parameter	Parameters				
	Yield (%)	pH	Moisture Content (%)	Ash Content (%)	Purity (%)
Mean/Interc.	17.23	10.26	15.90	67.18	81.80
Extractant Ratio	13.77**	0.08 ns	-8.02 **	-24.98 **	5.04 **
Formaldehyde Sol.	-1.82 ns	- 0.08 ns	-4.69 **	-4.94 **	-0.10 ns
HCl Conc.	0.43 ns	-0.41 *	-4.83 **	34.20 **	0.32 ns
Na_2CO_3 Conc.	-10.62 **	0.01 ns	-5.54 **	-4.91 **	-7.41 **
Extraction Temp.	0.05 ns	0.23 ns	-4.79 **	-26.51 **	2.10 ns
Bleaching	3.56 ns	0.11 ns	-2.82 *	-2.58 **	17.61 **
Precipitant	8.32 **	0.38 ns	-3.19 *	-25.89 **	-3.70 ns

ns not significant (p=0.05) *significant(p≤0.05) **significant (p≤0.01)

Effect of Formaldehyde, HCl, and NaOCl

Formaldehyde concentration, HCl concentration, and bleaching with NaOCl were the pre-extraction parameters used in the variable screening. The method used in extracting sodium alginate from *Sargassum piluliferum* resulted in solid, fibrous (unmilled), brown alginates (Figure 2). In a similar study conducted by Indrani and Budianto (2013), alginate powder produced by *S. crassifolium* was dark brown, which was probably a reflection of fucoxanthin pigment often contained in certain algae that probably covered the other pigments. This is in contrast with the commercially available alginate, in which color treatment or bleaching was done to produce white alginates. Though bleaching was applied in the study as pre-treatment to the milled seaweeds, the percentage of the bleaching agent (NaOCl) used was not enough to produce a white end product. Bleaching with NaOCl does not significantly affect the yield because its primary purpose is to produce a white end product. However, it has a positive effect on the product's purity (Table 3) which is an essential physico-chemical parameter that reflects the proportion of the target compound. Results of the statistical analysis showed that the influence of HCl on the yield was at the maximum level, hence, pre-soaking with HCl was held constant at maximum level. HCl breaks down the cell walls of seaweed which will facilitate the dissolution of the cell wall to facilitate the extraction (Winarno 1990 as cited by Zailanie 2015). Pre-soaking in HCl solution hydrolyzed the alginofit cell walls, reduced impurities, and made the alginate easier to extract (Mushollaeni 2007; Truss et al 2001). Hence, bleaching with NaOCl was the variable considered for optimization.

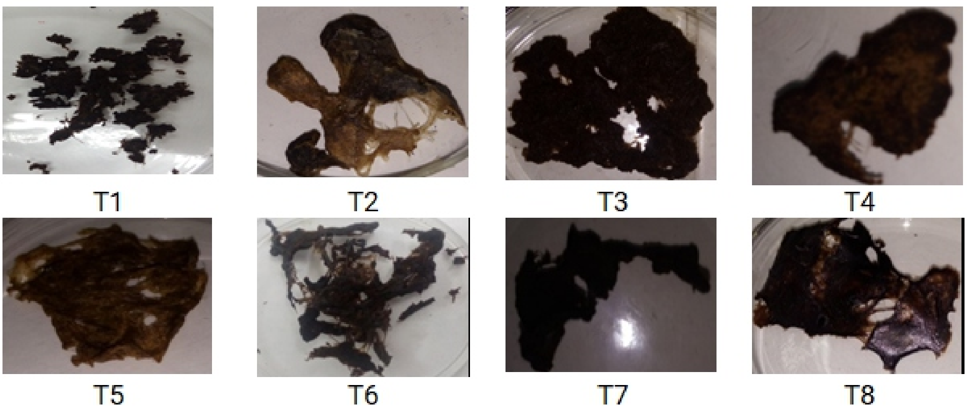

Figure 2. Samples of sodium alginate from each run in Plackett-Burman

Effect of Na_2CO_3 Concentration, Extractant Ratio, Temperature and Precipitant

Statistical analysis results showed that the variables, namely: Na_2CO_3 concentration, extractant ratio, temperature, and precipitant, greatly affected the alginates' moisture and ash content. All the treatments conformed to the standard moisture value of alginate which is <15% (Food and Chemical Codex 1993). On the other hand, the variability in the ash or mineral content of the alginates was due to the different treatments and purification process used (Jayasinghe et al 2016).

In this study, the effect of sodium carbonate concentration and extractant ratio significantly affected the yield of the sodium alginate. It was evident that a lower level of extractant concentration produced a positive effect in terms of its yield. This result is supported by the study of Chou and Chiang (1976) as cited by Haerunnisa (2008) which suggested that high concentrations of Na_2CO_3 (3% to 5%) reduced yield and viscosity of sodium alginates because the polymer chains of alginic acid degrades into oligosaccharides. In addition, pectin, the adhesive material between the cell walls of brown seaweed, is unstable in alkali solution; it creates a network in the cells of *Sargassum sp*. to facilitate alginate release. Therefore, Na_2CO_3 is a specific solvent to extract alginates from brown seaweed. The maximum level of extractant ratio was also set for optimization since it significantly affected the yield of extracted alginates. Therefore, extractant ratio, Na_2CO_3 concentration, and % NaOCl were the three variables considered in determining the optimum condition in producing sodium alginate.

Results showed that the extraction temperature had no significant effect on the yield of the alginates. It was evident that at high temperature, the yield increases. Mohamed (2017) states that high temperature increases the yield of extraction. However, high temperature can also lead to the breakdown of uronic acid chains, and, consequently, lower the viscosity of the extracted sodium alginate (Swee-Yong et al 2009). Hence, a temperature of 60^0C was held for optimization. Statistically, 95% isopropanol showed a positive effect in terms of yield, which is in accordance with the study conducted by Mairamo (1977), Yunizal et al (1999), and Yani (1998) as cited by Mushollaeni (2011) in which a 75 to 95 % concentration of isopropanol was the finest concentration to get a higher yield of sodium alginate. Moreover, Mushollaeni (2011) states that the use of 95% isopropanol could increase the yield because of its ability to bind water in the alginate solution so that sodium alginate could be separated. Hence, for optimization, 95% isopropanol was used as precipitant.

Results showed that the alginate extraction process can influence the yield and chemical composition as well as rheological properties of the isolated alginates (McHugh 2003; Hernandez-Carmona G et al 2013; Gomez et al 2009). Thus, optimization of the process is necessary to maximize the extraction of sodium alginates from *Sargassum piluliferum*.

CONCLUSION AND RECOMMENDATIONS

The variable screening Plackett-Burman was used to identify the variables that significantly affected sodium alginate extraction. A 7-variable, 8-run screening experiment was conducted with the following variables: formaldehyde solution, extraction ratio, Na_2CO_3 concentration, HCl concentration, extraction temperature, bleaching, and precipitant. The mean results of the physicochemical analysis, namely: yield with 17.23%, 10.2 pH value, moisture content of 15.90%, ash of 67.18% ash content, and purity of 81.80% were significantly influenced by the extraction ratio,

Na_2CO_3 concentration and NaOCl concentration as important factors in sodium alginate extraction. To produce sodium alginate with highest quality and yield, these factors should be optimized. Values for Na_2CO_3 concentration at low level and at high level extractant ratio showed a positive effect, whereas NaOCl showed a negative effect on yield, pH, moisture, and ash content, but showed a positive effect on purity at maximum level.

Optimization of extractant ratio, Na_2CO_3 concentration, and NaOCl concentration is suggested to produce the best quality sodium alginate from *Sargassum piluliferum*.

REFERENCES

Blanshard JMV and Mitchell J. 2013. Polysaccharides in Food. Elsevier.

Chou HN and Chiang YM. 1976. Studies on Algin from Brown Algae of Taiwan. I. Estimation of Yield and quality of Algin. Acta Oceanographica Taiwanica, No. 6. 134-13.

FCC. 1993. Food Chemical Codex. 434: National Academy Press Washington, .

Fernandez-Martin F, Lopez-Lopez I, Cofrades S & Jimenez Colmenero F. 2009. Influence of adding sea spaghetti seaweed and replacing the animal fat with olive oil or a kinjac gel on pork meat gelatin batter gelation. Potential protein/alginate association. Meat Science, 83, 209-217.

Gholamipoor S, Nikpour-Ghanavanti Y, Oromiehie AR & Mohammadi M. 2013. Extraction and Characterization of Alginate from Sargassum angustifolium collected from northern coasts of Persian Gulf, Bushehr. International Symposium on Advances in Science and Technology, 1-5.

Gomez CG, Perez Lambrecht MV, Lozano JE & Villar MA. 2009. Influence of the extraction–purification conditions on final properties of alginates obtained from brown algae (Macrocystis pyrifera). Int J Biol Macromol, 44(4):365–371.

Haerunnisa. 2008. Analisa Kualitas dan Formulasi Alginat Hasil Ekstraksi Sargassum filipendula Untuk Pembuatan Minuman Suplemen Serat Dalam Bentuk Effervescent. [Skripsi]. Program Studi Kimia Fakultas Sains dan Teknologi.

Hernandez-Carmona G, Freile-Pelegrin Y & Hernandez-Garibay E. 2013. Conventional. In D. H., Functional ingredients from algae for foods and nutraceuticals (pp. 475–516). Cambridge: Woodhead Publishing Limited.

Hernandez-Carmona G, Mchugh DJ, Arvizu-Higuera DL & Rodriguez-Montesions YE. 1999. Manual de tecnicas de control de calidad para el acido y sus derivados. Mexico: CICIMAR-IPN.La Paz.

Indrani D and Budianto E. 2013. A study of extraction and characterization of alginates obtained from brown macroalgae Sargassum duplicatum and Sargassum crassifolium from Indonesia. Dental Journal Vol.2 , 65-70.

Jayasinghe PS, Pahalawattaarachchi V & Ranaweera KKDS. 2016. Effect of Extraction Methods on the Yield and Physiochemical Properties of Polysaccharides Extracted from Seaweed Available in Sri Lanka. Poultry, Fisheries and Wildlife Science, 1-6.

Kasim S, Marzuki A& Sudir S. 2017. Effects of Sodium Carbonate Concentration and Temperature on the Yield and Quality Characteristics of Alginate Extracted from Sargassum sp. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 8(1) 660-667.

Kraan S. 2012. In Carbohydrates-Comprehensive Studies on Glycobiology and Glycotechnology. In Algal Polysaccharides, Novel Applications and Outlook (pp. 518 (489-532). Accessed from INTECH open access chapter, Creative Commons Attribution Licensed.

Mariamo AL. 1977. Sulfated Seaweed Polysaccharide Food Colloids. AVI Wesport, Connecticut, 80-95.

Mazumder A, Holdt SL, De Francisci D, Alvarado-Morales M & Mishra HN. 2014. Optimization of extraction process of crude alginate from Sargassum muticum by response surface methodology. Poster session presented at 5th Congress of the International Society for Applied Phycology. Sydney, Australia, Technical University of Denmark.

Mchugh DJ. 2003. A guide to seaweed industry. FAO fisheries technical paper no. 441. FAO Rome.

Mohamed F, Ahmed B, El Montassir D, Moha T & Francois B. 2017. Extraction and characterization of sodium alginate from Moroccan Laminaria digitata brown seaweed. Arabian Journal of Chemistry, 1-8.

Mushollaeni W. 2007. Extraction of Alginate from Brown Seaweeds Sargassum, and Turbinaria. Young Lecturer Experiment Paper, 20-33.

Mushollaeni W. 2011. The physicochemical characteristics of Sodium Alginate from Indonesian brown seaweeds. African Journal of Food Science, Vol. 5 (6) 349-352.

Nalamothu N, Potluri A & Mupalla M. 2014. Review on Marine Alginates and its Application. Indo American Journal of Pharmaceutical Research, 1-10.

Nijhuis A, Van Der Knaap HCM, Jond SDE & Vandeginste BGM. 1999. Strategy for ruggedness tests in chromatographic method validation. Anal chim Acta, 391: 187-202.

Ortiz AT and Trono GC. 2000. Growth and reproductive pattern of intertidal and subtidal Sargassum (Sargassaceae, Phaeophyta) populations in Bolinao, Pangasinan. Science Diliman, 12(2): 45-50.

Peteiro C. 2018. Alginate Production from Marine Macroalgae, with Emphasis on Kelp Farming. In B. &. Rehm, Alginates and Their Biomedical Applications (pp. 1-41). Singapore: Springer Series in

Biomaterials Science and Engineering 11.

Plackett RL and Burman JP. 1946. The design of optimum multifactorial experiments. Biometrika, 33: 305-325.

Prince JS and O'neal SW. 1979. The ecology of Sargassum pteropleuron Grunow (Phaeophyceae, Fucales) in the waters of Sourth Florida: I. Growth, reproduction and population structure. Phycologia, 109-114.

Reyes-Tisnado R, Hernandez-Carmona G, Montesinos YE, Higuera DL & Gutierrez FL. 2005. Food Grade Alginates Extracted from the Giant Kelp Macrocystis pyrifera at Pilot-Plant Scale. Rev. Invest Mar., 26(3): 185-192.

Siala R, Frikha F & Mhamdi S. 2012. Optimization of acid protease production by Aspergillus niger II on shrimp peptone using statistical experiment design. The Scientific World Journal, 1-11.

Swee-Yong C, Ping-Keong W & Ching-Lee W. 2009. Extraction and characterization of alginate from brown seaweeds (Fucales, Phaeophyceae) collected from Port Dickson, Peninsular Malaysia. Journal of Applied Phycology, 23:191-196.

Truss V, Taure D & Grasdalen H. 2001. Algal biomass from Fucus vesiculosus (Phaeophyta): Investigation of the Mineral and Alginate Components. Proc. Estonian Acad. Sci. Chem, 50: 95-103.

Vanaja K and Shobha Rani RH. 2008. Design of Experiments: Concept and Applications of Plackett Burman Design. Clinical Research and Regulatory Affairs, 24:1, 1-23.

Wass JA. 1997. Formal experimental design and analysis for immunochemical product development. Part I. IVD Technology Magazine.

Winarno F and G. Seaweed. 1990. Processing Technology. Pustaka Sinar Harapan, Jakarta.

Yani M. 1998. Modification and Optimation Processing of Alginate Extraction from Jenis Turbinaria sp. Final Paper. Agricultural Faculty of Bogor Agricultural Institute. Bogor, 20-35.

Yannis LL. 2001. A Plackett-Burman screening design directs the efficient formulation of multicomponent DRV liposomes. Journal Pharm Biomed Anal, 26: 255-263.

Yunizal J, Murtini T, Basmal J, Nasran S, Marsiana E & Abdulrokim I. 1999. Technology of Extraction Alginate from Brown Seaweeds. Technical Paper, Jakarta, 25-40.

Zailanie K. 2015. Study of Alginate Sargassum Filipendula with FTIR Confirmation. Journal of Life Science and Biomedicine, 5(6): 167-170.