ISSN: 2063-5346
Section A-Research paper

Extraction Process Optimization of Sodium Alginate from Samu (Sargassum piluleferum) and Evaluation of its Stabilizing Property for the Production of Sweetpotato (Ipomoea batatas L) Beverage

[1*]Aguanza, MA., [2]Lauzon, R. and [2]Emnace, I.

[1]College of Technology and Allied Sciences, Bohol Island State University-Calape Campus, San Isidro, Calape,Bohol, Philippines;
[2]Department of Food Science and Technology, Visayas State University, Visca, Baybay City, Leyte, Philippines

*Corresponding author E-mail: maaezel.aguanza@bisu.edu.ph

Abstract

Alginates are used as stabilizers in food and beverages industry. Previous study on the variable screening of extraction process showed a significant result of alginates extracted from *Sargassum sp.* This study aimed to optimize the extraction process, utilize and characterize Sodium alginate from locally found *Sargassum piluleferum*. Response Surface Methodology using Central Composite Design (CCD) of Experiment was employed in order optimize the extraction process. The optimum condition identified for the NaOCl concentration, Na_2CO_3 concentration and Extractant Ratio (Na_2CO_3:Samu) were 5.1%, 1.8% and 50:1 respectively. Having a yield of 19.43% in dry basis, 12% moisture, 90.14% purity, pH of 8.4, crude fiber of .16% and 210 mpa s viscosity. FTIR analysis shows similar results with the commercial sodium alginate. In order to test its stabilizing property, the optimum extracted Sodium alginate was added on a sweetpotato beverage and was able to hold and make the solutions stable at 1-1.5% Sodium alginate. Hence, the results of optimized extraction process demonstrated in this study, proves that alginates can be extracted from locally found *Sargassum piluleferum*.

Keywords: Central Composite Design, Fourier Transform Infrared (FTIR) Spectra, *Sargassum piluleferum*, Sodium alginate, Sodium Carbonate

Introduction

Marine plant resources are the major source of raw materials for the production of marine seagrass derived products such as Alginic acid, alginates, agar, carrageenan, iodine and the like, this products are widely used in food and beverage industry, pharmaceutical and textile industry. Alginates was used in the food industry to stabilize mixtures dispersion and emulsions and consequently increase the viscosity and forms gel (Toft *et al*.,1986;Venkatesan *et al*., 2017). At present, countries like Argentina, Australia, Canada, Chile, Japan, China, Mexico, Norway, South Africa, United Kingdom and United States of America are the leading producers of alginates (Kran, 2012). The main commercial sources of phaeophytes for alginates are *Ascophyllum, Laminaria,* and *Maycroystis* other minor sources include *Sargassum, Duvillea, Eklonia, Lessoni*a and *Turbinaria* (Bixler and Porse, 2010). One of the mostly found brown macro algae in tropical countries like, Philippines is the *Sargassum* sp. with 72 species recorded (Ortis and Tromo, 2000) and *Sargassum* and *Turbinaria* are exported to Japan for animal feed and fertilizer (Laksmono *et al*., 2013). The abundance of these species in our country poses a challenge to shift its marketing form into a more expensive and value added products through the process of extracting its important polysaccharides specifically the alginates. *Sargassum sp*. is one of the brown algae species which produces alginic acid that is widely used in pharmaceutical, textile and food industry.

A way to maximize the extraction process of Alginates from *Sargassum* species is through process optimization. According to Granato and Calado (2014) in order to improve the process and to obtain a product with a certain characteristics is essential to companies whose target needs to meet with low energy and production cost but produces higher yield, the use of optimization procedures improves the process conditions. The main objective of optimization study is to determine the effects of independent variables. Optimization achieves the best quality characteristics of a certain product without excessively extending the experiment time or lengthy experimentation assays.These procedures can be performed using a Response Surface Methodology approach. The primary goal of Response Surface Methodology (RSM) is to identify the optimal values for control variables that result in a specific area of interest, either maximizing or minimizing the response. Response Surface Methodology (RSM) is a statistical approach that aims to optimize intricate process parameters while minimizing the need for conducting numerous experiments. RSMs are commonly analyzed in two ways, either by Box-Benken design (BBD) or Central Composite Rotatory design (CCRD). This methodology has frequently been used in developing various extraction parameters for polysaccharides, phenolic compounds, pigments and proteins from different biomasses (Bezerra *et al*., 2008) . Myers *et al*., (2009) states that for food processes, and especially for food development, optimization is a way to obtain ideal conditions to achieve a desired quality (physicochemical, chemical and sensory). Hence, optimization is being employed to optimized processing conditions (such as temperature, pressure, concentration, moisture and energy input) in order to obtain an improved product.

Sodium alginates, plays a vital role not only in the fields of pharmacy and medicine but also a gateway for new and exciting research application in the food industry. Alginates serve as sensory-enhancing additives and stabilizers in food, employed to enhance, alter, and stabilize the texture of food products (Aguanza, MA., et.al., 2018).

Because of its natural chemical properties such as viscosity enhancement, gel forming ability and stabilization of aqueous mixtures and emulsions it is widely used in food industries.

One of the natural phenomenon observed with non-alcoholic beverages developed from rootcrops specifically the sweetpotato is the observable separation of the solid and liquid material. Sweetpotato is a popular rootcrop in tropical countries, it is by nature starchy. This beverage is processed by boiling first in order to soften the sweetpotato and then blended together with water and mango (flavorant) to come up with a characteristic solid-liquid beverage. The use of sweetpotato for drink preparation is a viable option for processing sweetpotato into a valuable product that increases its economic value. Hence, a stabilizer aids the problem of solid separation in sweetpotato beverages. Furthermore, this study was conducted to evaluate the potential of the optimized extracted Sodium Alginate as stabilizers in the production of non-alcoholic sweetpotato beverage. Furthermore, this study was conducted to determine the optimum extraction conditions that yields the highest value of alginate based from its physic-chemical properties.

Materials and Methods

Preparation of Samu (Sargassum piluleferum)

The Sargassum piluleferum was collected from Higatangan Island, Biliran, Philippines. The preparation for Samu (*Sargassum piluleferum*) was based on the results of the previous study on variable screening. Variable screening specifically Plackett-Burman Design is an experimental design that is used to filter out insignificant variables that might affect the optimization and selects the most relevant variable that will be needed to come up with the optimum condition. It was dried first in a cabinet drier at 60^0C for 8 hrs. Afterwhich, the dried form was milled in grinder to obtain a powdered dried Sargassum. The powdered Sargassum was kept in clean container ready for extraction process.

Extraction Process of Sodium Alginate

The variables that showed significant effect (NaOCl concentration, Na_2CO_3 concentration and extractant ratio) in Plackett Burman Screening were used in the extraction process of alginate. Plackett-Burman is a screening design developed by R.L. Plackett and J.P. Burman, it was designed to study the initial effects of the factors and factors level in order to improve the quality control process so that intelligent decisions can be made before further optimization. It is used in selecting the most significant variables for optimization. The reagents and chemicals used during the experiment are all laboratory grades. The milled samu was soaked first in 0.5 N HCl then washed with distilled water until pH reached neutral value. Then it

ISSN: 2063-5346
Section A-Research paper

was soaked for 30 minutes in NaOCL at levels of 2.5, 5 and 7.5 %, filtered and washed. Extraction process then followed where three levels of extractant (Na_2CO_3) concentration was used (1.5 %, 3.0 % and 4.5%) with extractant ratio of 35:1, 40:1 and 45:1. The solution was heated at 60^0C for 3hrs with constant stirring. After heating, the melted samu was filtered and the filtrate was collected. Then it was added with 10% HCl until pH reached 2. The formed alginic acid characterized by deposition of gels was collected and washed until pH was neutral. It was added with 10% Na_2CO_3 with constant stirring until pH reached 8 and was precipitated with 95% Isopropanol. The formed Sodium Alginate was dried in an oven set at 45^0C until dried.

Table 1. Experimental combinations for extraction process optimization of Sodium Alginate from Samu (*Sargassum piluleferum*)

TREATMENT	Na_2CO_3 CONCENTRATION (%w/v)	EXTRACTANT RATIO(w:v)	NAOCl CONCENTRATION (v/v)
1	1.50	35:1	2.5
2	1.5	45:1	7.5
3	4.5	35:1	7.5
4	4.5	45:1	2.5
5	3	40:1	5
6	1.5	35:1	7.5
7	1.5	45:1	2.5
8	4.5	35:1	2.5
9	4.5	45:1	7.5
10	3	40:1	5
11	.49	40:1	5
12	5.50	40:1	5
13	3	31.63:1	5
14	3	48.36:1	5
15	3	40:1	.816
16	3	40:1	9.18
17	3	40:1	5

Legend*Extractant Ratio (Na_2CO_3 : dried alga)

Physico-Chemical Analysis

Samples of the experimental treatments were analysed for their physico-chemical qualities that include Moisture Content, Ash Content, pH and crude fiber. The Moisture Content was determined following the standard method of drying the samples at 105^0C for 3 hrs, and obtaining its Moisture content following the standard equation of AOAC, 1980. Then the ash content was determined by following the methods established by Gholamipoor *et al*., (2013). On the other hand, the pH was measured using a laboratory pH meter digital portable pH meter HI 98108. The crude fiber was determined using the procedures set by the Association of Official Analytical Chemists (AOAC, 1980).

Alginate Purity Determination

The method of Hernandez-Carmona *et al*., (1999); Reyes-Tesnado *et al*., (2005) was adopted in determining the purity of the Alginate produced. A solution was prepared by dissolving one gram of calcium chloride in a 100 mL methanol-water solution (40-60% concentration). Afterwards, the resulting solution was carefully mixed with 100 mL of a 0.5% alginate solution while being gently stirred. Subsequently, the precipitate was eliminated by passing it through a fine filter and subsequently washed with a solution containing 20-80% methanol and water. Second washing was done using 40-60% methanol-water solution. The precipitated alginate was dried in an oven set at 105^0C for 2 hrs. Then the alginate was maintained in a desiccator for one hour and weighed. The alginate purity was computed using the formula;

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

Alginate Viscosity Test

The viscosity of alginate was determined using the method of James (1995); Mushollaeni (2011). A 250 ml aliquot of a 1% alginate solution was heated to 50°C until it transformed into a gel-like state, indicating its readiness for viscosity determination using a viscotester.

Percent Yield

The percent yield of the extracted Sodium Alginate was computed using the formula:

$$\text{Yield of sodium alginate (\%)} = \frac{\text{Weight of Sodium Alginate}}{\text{weight of milled seaweed}} \text{ x } 100$$

Statistical Analysis and Modelling

Results of the analysis was subjected to Response Surface Regression (RSREG) analysis using Statistica 8.0 the Statistica 8.0 s was also used for the graphical presentation of the response surface plots.

Fourier Transform Infrared (FTIR) Analysis

The determination of the spectra as confirmatory test for Sodium Alginate was conducted in the Department of Pure and Applied Chemistry (DOPAC). The optimized dried sample was analysed using Fourier transform-infrared (FT-IR) spectrophotometry (Perkin Elmer RX1 FT-IR). The spectra was recorded in the absorbance range from 4000 to 650 $^{cm-1}$ (mid-infrared region).

Preparation of Sweetpotato Beverage

The preparation of sweetpotato beverage followed the method and formulation developed by Lauzon (1995). The sweetpotato root was washed and sanitized, then cooked and peeled. It was cut into smaller size to ease the blending process. The water (4L), cooked sweetpotato (1/2 kg), sugar (1/2 kg), 2 pcs mangoes and Sodium alginate was mixed together (0, 0.5, 1.0, and 1.5,). Then the mixture was pasteurized at 80^0C for 1 minute. It was allowed to cool down and calamansi juice was added. Finally, it was filled in sanitized bottles and stored.

Test for Liquid-Solid Separation

The liquid-solid separation was determined employing the method used by Lauzon (1993). The different treatments were allowed to stand undisturbed overnight to allow the liquid-solid separation in the product. The distance from where the clear liquid started to develop up to the point where the solid layer began to appear was measured and considered for solid formation. The same method was used in measuring the liquid formation in the product and will be noted as blurred and clear.

Results

Determination of Optimum Condition of Sodium Alginate Extraction

Sodium alginate was produced using 17 treatments for experimental combinations. All other variables was set constant and subjected to different physico-chemical analysis for optimization. Yield, Moisture Content, pH, ash and Purity are the dependent variables considered for optimization, these are the factors that greatly measures the quality of the extracted alginates.

Yield

Alginates extracted from *Sargassum sp* is influence by its environmental condition and extraction process, hence optimization on its extraction process increases the yield of extracted alginates. Alginates obtained from *Sargassum piluleferum* has a mean yield of 15.82% (Table 2) the result is comparable to the results obtained by Davis *et al*., (2003) reported that the percent yield values of alginates were 3.35%, 12.4% and 17.7%, respectively for *Sargassum dentifolium, Sargassum asperifolium* and *Sargassum latifolium*.

Table 2. Parameter estimate of physico-chemical tests for Sodium alginate

PARAMETER	PHYSICO-CHEMICAL PROPERTIES				
	YIELD (%)	MOISTURE (%)	pH	ASH (%)	PURITY (%)
Mean/Interc.	15.81509	10.81709	10.01650	47.8182	81.29921
(1)Na_2CO_3	-4.29240^{**}	0.81319^{ns}	0.16461^{ns}	-15.3901^{**}	-5.54197^{*}

ISSN: 2063-5346
Section A-Research paper

Conc.(L)					
Na_2CO_3 Conc.(Q)	-2.65693*	-0.22848 ^{ns}	0.08044 ^{ns}	-2.8682 ^{ns}	-1.50596 ^{ns}
(2)Extractnt Ratio(L)	-4.58168**	0.56848 ^{ns}	-0.35067 ^{ns}	2.5139 ^{ns}	4.73172*
Extractnt Ratio(Q)	1.84454 ^{ns}	0.81172 ^{ns}	0.15187 ^{ns}	-0.7109 ^{ns}	2.10521 ^{ns}
(3)NaOCL Conc.(L)	0.57096 ^{ns}	2.35101**	-0.19135 ^{ns}	12.1877*	0.49752 ^{ns}
NaOCL Conc.(Q)	0.69142 ^{ns}	1.09398 ^{ns}	-0.50857**	11.1159*	1.94913 ^{ns}
1L by 2L	-0.37875 ^{ns}	0.68500 ^{ns}	0.31250 ^{ns}	1.8175 ^{ns}	-1.63750 ^{ns}
1L by 3L	-1.09625 ^{ns}	0.06750 ^{ns}	-0.31250 ^{ns}	-2.1175 ^{ns}	5.36000 ^{ns}
2L by 3L	5.07375**	-0.66000 ^{ns}	-0.13750 ^{ns}	9.3925 ^{ns}	-9.43250**

^{ns} not significant ($p>0.05$) *significant ($p\leq 0.05$) **significant ($p\leq 0.01$) (Q)- Quadratic Effect, (L)- Linear Effect

The Response Surface Regression analysis shows that Na_2CO_3 concentration has a significant negative linear and quadratic effect to the yield of Sodium alginate which means that increasing the concentration of the Sodium Carbonate decreases the yield regardless of the extractant ratio of 36.50:1 and NaOCl should not be less than 8.34% to achieved a predicted yield value of 17.92% (Table 2 and Appendix Table 1) which is higher than the obtained mean (15.81%). It is evident that at lower concentration of Na_2CO_3 (1.5 -3.5%), higher extractant ratio and higher NaOCl concentration gives a better yield. This observation is in agreement with the study of Kasim, *et al*., (2017) in which higher yield was obtained at 2% concentration than 6% concentration of Na_2CO_3. This is mainly because polysaccharides are more susceptible to degradation when isolated with higher sodium carbonate concentration (Whyte and Englar, 1976). Moreover, alginate yield is affected by the increase in alkali concentration as the average molecular weight start to decrease due to change in pH of the media (Hernandez-Carmona *et. al*., 1999 and Mazumder et al.,2016). On the other hand, basically the purpose of NaOCl is for bleaching purposes. In this study the NaOCl, was one of the pre-treatments used prior to extraction, NaOCl might have the same action with the acid pre-treatment HCl, Peteiro, (2018) states that acid pre-treatment is effective in removing potential contaminants or impurities (fucoidans, laminarins, proteins and polyphenols), leading to a higher yield and purity of alginates.

Moisture Content

The permitted moisture content of Sodium alginate by Food Chemical Codex (FCC) is less than 15% (FCC, 1993). The overall response mean for moisture is 10.81% (Table 2). The obtained moisture content has already fulfilled the permitted condition by FCC.

The predicted moisture content mean value is 9.90 at saddle point (Appendix Table 1). Saddle point means a closer value with the obtained mean value, hence the obtained mean moisture value 10.81% is slightly higher than the predicted mean value. Analysis of variance and parametr estimates (Table 2) shows a linear effect of the NaOCl concentration to the moisture content which means that exceeding the amount of NaOCl than the predicted value which is 1.57 % (Appendix Table 1) would also mean an increase of its moisture content. Increasing the individual variables namely extractant ratio, Na_2CO_3 and NaOCl concentrations causes moisture content to increase at greater than the allowable moisture content of < 15%.

Ash

Each type of seaweed shows a different ash content depending on the the amount of mineral salts that attached to their surface (Salasa**,** 2002). The overall response mean for ash is 47.81 (Table 2). Parameter estimates (Table 2) shows that NaOCl has a positive linear and quadratic effect on the ash content and a negative linear effect is shown in the influence of Na_2CO_3 concentration. The negative significant linear effect of Na_2CO_3 means increasing the amount of Na_2CO_3 will cause a decrease of the ash, this is only true when NaOCl should not be less than the critical value of 5.14%. However, the amount of mineral salt could be different in each alginofit and the conditions of hydrology and hydrochemistry on the habitat also influence the ash content (Salasa, 2002; Truss *et al*., 2001). The experiment shows that ash content is lowest at higher values of the independent variables (NaOCl, Na_2CO_3, and Extractant Ratio). Hence, an increase of the independent variable causes a decrease on the ash content, implying that it is not advisable during extraction process to increase the amount of extractant as it will also affect the ash content of the alginates. Moreover, naturally the mineral salt amount of seaweed depends on the type, age and condition of hydrology and hydro-chemical nature, where the habitat of sea grass (Salasa, 2002).

pH

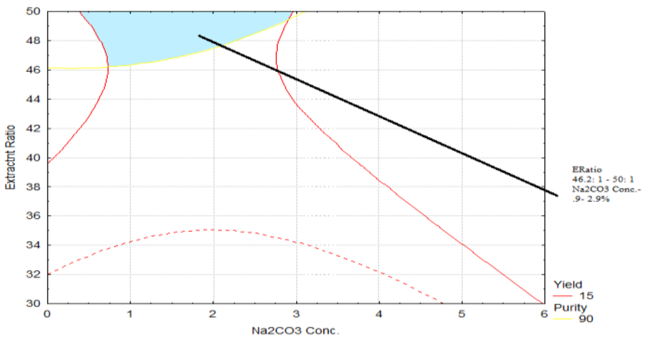
The overall response mean for pH is 10.81 (Table 2). McDowell (1977) and Arvizu-Higuera (2002) states that pH of dry alginates should be between 5 and 10 for more stability. The experiment showed that at lower concentration of Na_2CO_3, and lower concentration of NaOCl but at higher extractant ratio will favor the pH of less than 10 which is the acceptable limit for alginate pH. Parameter estimates states that the pH of alginate is affected by the quadratic interaction of the bleaching agent NaOCl, in which an increase in its concentration would also mean an increase of its pH. The NaOCl natural pH of about 11 (Rowe, 2013) might also contribute to the alginates pH. Apparently the pH of the alginate was influenced by the addition of Sodium Carbonate during the process of conversion of alginic acid to sodium alginate (Kasim, 2017).

Purity

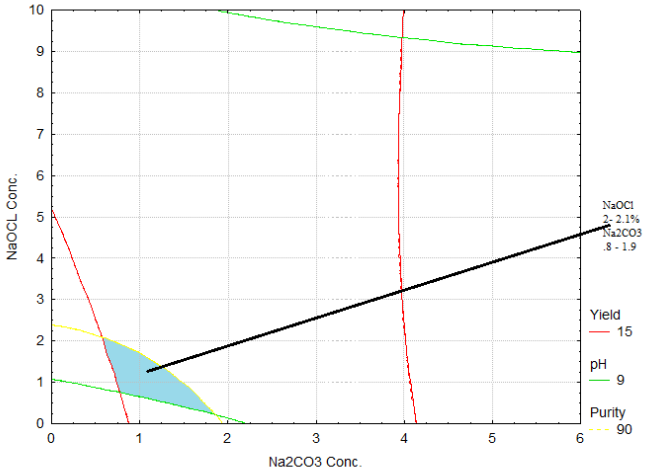
The purity of the Sodium alginate should be 90.8-100% (FCC, 1981). However the overall response mean for purity is 81.29% (Table 2) this might be because brown algae contain varying amounts of phenolic compounds which are extracted together with the alginate and represent a contamination in most alginates (Smidsrod, 1980). Parameter estimates (Table 2) shows a negative significant effect on the Na_2CO_3 concentration and a positive effect with extractant ratio only when paired with NaOCl concentration. When Na_2CO_3 concentration increases more than 1.8% (Appendix Table 1) it will result a decrease on its purity. The presence of excess Na_2CO_3, the alginic acid is converted to a Sodium alginate, causing polymer dissolution (Kleinubing *et al*., 2013). An increase of purity will be observed if extractant ratio is at maximum39.31:1(Appendix Table 1) only when interacted with NaOCl at greater than 6.42%. Lower concentration of Na_2CO_3, higher extractant ratio with higher NaOCl concentration will possibly remove impurities and produces a possible yield of >95% purity.

Optimized Region of the Experiment

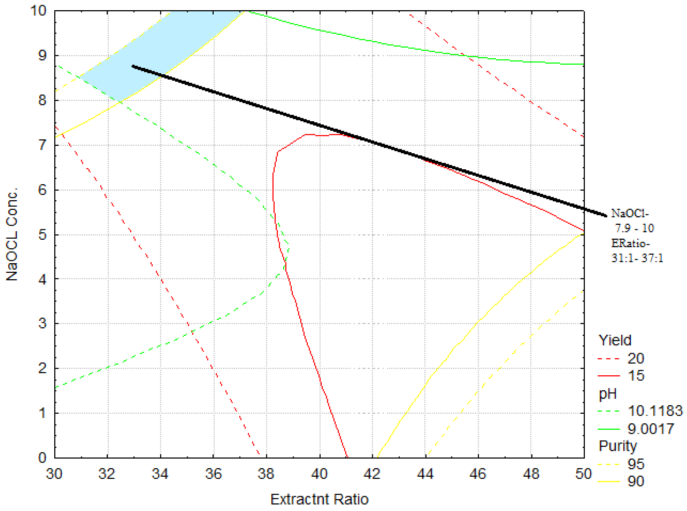
The optimum region for the extraction process was established by superimposing the contour plots in the results of the evaluation of physico-chemical characteristic of Sodium alginate from *Sargassum piluleferum* with constant extractant ratio, NaOCl and Na_2CO_3 concentration. The optimum region was generated by limiting the yield not less than 9% with purity ≥ 90% and pH averaging from 8-10. The resulting optimum region was found at high extractant ratio and NaOCl with low concentration of Na_2CO_3. The higher extractant ratio the more raw material for alginate extraction is present. On the other hand, high level of Na_2CO_3 reduces yield of alginates but significantly improve its purity. The limiting factor which determines the optimum region is generally bounded by the purity, hence higher NaOCl was used. That's why the chosen optimum point was balanced between a high yield, low purity processing condition and low yield, high purity processing condition. This is why the optimum values used for extractant ratio, NaOCl and Na_2CO_3 concentration were 50:1, 5.1% and 1.8%, respectively.



a.



b.



c.

Figure 1. Optimum region at (a) constant NaOCl concentration, (b) constant extraction ratio, and (c) constant Na2CO3 concentration.

Physico-chemical property of the optimized product

Yield

The algae species influence the quantity and quality of the alginates (Haug *et al*.,1974) and most of the commercially available alginates are extracted from seaweeds found in cold waters within a temperature range of 20^{0}C or below are having a good yield and viscosity(Viswanathan and Nallamuthu, 2014). Results of the study show (Table 3) that *Sargassum piluleferum* though found in temperate water having a temperature of 29^{0}C (Cordero *et al*., 2018) still yields a higher value than the expected yield of most *Sargassum* species. The obtained yield of 19.43% (Table 3) is comparable to the obtained optimum yield of alginates from *S. muticum* which is 13.57% (Mazumder *et al*., 2016), 22.56 % with *Sargassum sp* and 14.77% from *Turbinaria* (Kusumawat *et.al*., 2018).

Moisture, Purity and Fiber Content

Results of the moisture content and purity of the optimized product conforms to the standard allowable or acceptable physico-chemical characteristics of alginates, which is <15% moisture and 90.8-100% purity (FCC, 1981). The obtained crude fiber (0.16%) as shown in Table 3 was low. However according to Reyes-Tisnado, *et al*., (2005), small amounts of cellulose and hemicellulose may have passed through the filter aid retaining small amounts after filtration.

Viscosity and pH

A viscosity of 10-500 cP in 1% solution is the specified viscosity value for alginates (Chou, 1976; Winarno, 1996 and Kasim**,** 2017), hence the obtained viscosity is within the acceptable range. Viscosity of sodium alginate is connected to the alginates pH. The products pH-8.4 (Table 3) was sufficiently enough to give a very viscous solution at 1% Sodium alginate. This is because the viscosity is influenced by the degree of acidity (pH). The pH 5-10 has stable viscosity, but pH below 4.5 and above 11 viscosity will easily degrade (labile) (Laksomono, 2013). The extracted Sodium Alginate conforms to the standard acceptable physico-chemical properties. Though alginates do not have any nutritional value, its most important properties are the ability to form a gel and the stabilization of aqueous mixtures and emulsions (Moe, 1995). The physico-chemical properties of Sodium Alginate are relevant to their uses. As such, sodium alginates acts as moisture barrier in breaded or batter-covered products which come in contact with a sauce or filling (Earle and Mckee, 1986) and because of its high viscosifying property it has the ability to retain colloidal suspension. Moroever, aside from its inherent physical properties, functionality of alginates may also be affected by the interactions with other components of the food product such as proteins, fat or fiber (Stephen et al., 2006).

Table 2. Physico-chemical property of the optimum condition

YIELD	MOISTURE	PH	PURITY	CRUDE FIBER	VISCOSITY
19.43%	12.81%	8.4	90.14%	0.16%	210 mpa s

Fourier Transform Infrared (FTIR) Spectra of Sodium Alginate

Fourier Transform Infrared (FT-IR) analysis offers excellent information on the nature of the present bands, allowing identification of different functionalities on the cellular surface (Kleinubing et al., 2013). This technique was used in order to analyze if the extracted Sodium alginate presented the same signalling with the commercial sodium alginate. The absorbance range was recorded from 4000 to 650 cm-1, mid-infrared region. It can be observed that the commercial and extracted Sodium

Alginate has the same characteristic bands. Alginates have a characteristic bands at 3400-3250 cm^{-1} and is associated with stretching vibrations of O-H bonds (Sartori et al., 1997 and Garcia-Rios, 2012). The extracted sodium alginate was found at peak band of 3252 cm^{-1}. The band signal at 2922 cm^{-1} is comparably close to the commercial alginate which is found at 2927 cm^{-1} , which according to Mathlouti and Keoning (1986) as cited by Yudiati (2017) is interacted to C-H stratching vibrations. 1600 and 1420 cm^{-1} is assigned to COO- stretching (Sartori et al., 1997), the extracted Sodium alginate was found at 1598.3 and 1405.2 cm^{-1}. The absorbance around 1401 cm^{-1} is correlated to the deformation vibration of C-OH, which is the contribution of O-C-O symmetrically stretching vibration from carboxylate group (Mathlouti and Keoning, 1986; Silverstein and Webster, 1991). The wavelength of 1405.2 cm^{-1} was found near the reported band. The vibration measured at 1033 $^{\text{cm-1}}$ has the same value as reported by Chandia et al. (2001), which is formatted to C-C vibration. The anomeric, region (950–750 cm^{-1}) is the most discussed in carbohydrates (Mohamed et al., 2014). The spectrum band obtained by Leal et. al., (2008) of three types of alginate is at 930-940 cm^{-1} is referred to C-O stretching of uronic acid residue. The obtained band spectra is found at 939.3 cm^{-1}. In addition, the result is also comparable to the obtained FTIR results of alginates from *S. duplicatum* and *S. crassifolium* in which it has the same characteristic bands (Indrani and Budianto**,** 2013). The functional groups obtained from extracted sodium alginate were in agreement with those in commercially available alginate, in which it exhibited similarities in absorption pattern with that of the commercial alginate.

`

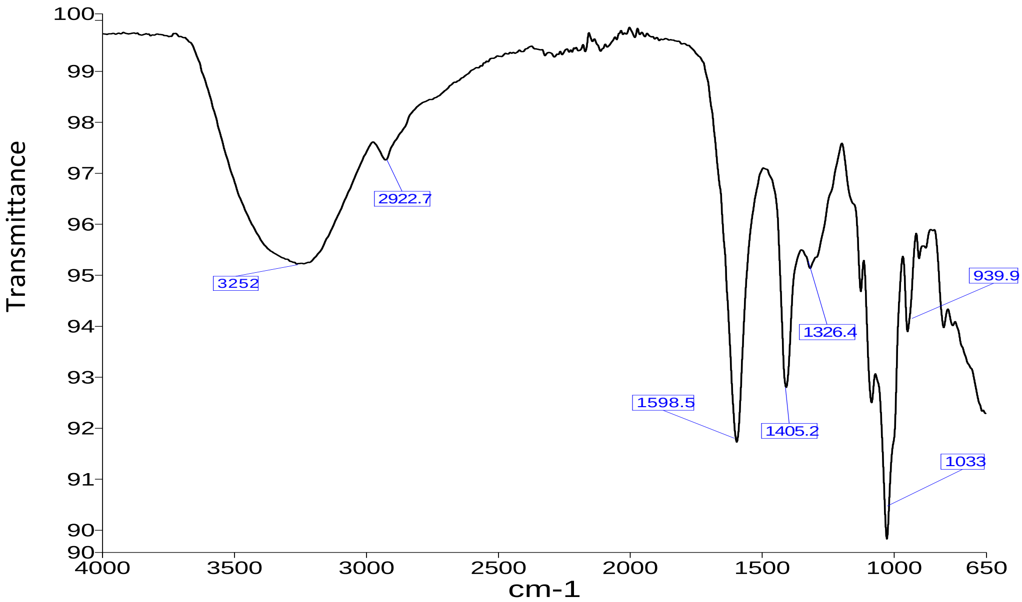


Figure 2. FTIR spectra of extracted sodium alginate at 4000 to 650 cm^{-1} absorbance

Physical Quality of Sweetpotato Beverage

Beverages with solid mixtures by natural phenomenom separates or settles at the bottom, this might be influenced by the particles density. Separation of solid particles are aided by stabilizers, which firmly fixed the solution. One of the functional property of alginates is its ability to hold in place two immiscible liquids and stabilizes aqueous solutions.

Liquid-solid separation is often observed in beverage developed from sweetpotato. Application of the extracted Sodium alginate was used to testify its effectiveness when used as stabilizers. Figure 4 show the appearrance and observation of the sweetpotato beverage applied with different levels of Sodium Alginate. It is evident that at 0% Sodium alginate a noticeable separation of the solid particles was observed and a clear liquid formation compared with the treatment having 1.5% of Sodium alginate. In addition, the samples where placed in a 100 ml gradduated cylinder and solid formation is evident at a 0% Sodium alginate which is approxiamtely about up to 10ml meniscus scale of the cylinder and no solid formation at 1.5%. It is evident that the extracted Sodium alginate has the ability to hold and make solutions at stable condition.

0% .5% 1% 1.5%

% Sodium alginate

Figure 3. Appearance of the sweetpotato beverage applied with varyieng levels of Sodium Alginates

Conclusions

Generally the study was conducted to utilize and extract Sodium alginate from *Sargassum piluleferum* and evaluate its potential as food stabilizer. Response Surface Methodology using Central Composite Design (CCD) of experiment was employed in order to achieve this. Results proved that a lower concentration of Na_2CO_3, higher extractat ratio and higher NaOCl concentration gives a better yield this is mainly because polysaccharides are more susceptible to degradation with higher amount of

Sodium Carbonate. Additionally too low extractant ratio to milled Sargassum piluleferum causes rapid vaporization that leads to the development of partially burned viscous solution. The purpose of the NaOCl is basically for bleaching which is effective in removing potential contaminants and impurities, thus also contributes to higher yield. Hence, the results of optimized extraction process demonstrated in this study, proves that alginates not only be extracted form kelps but can be extracted also from locally found tropical *Sargassum piluleferum*. These observations show a possibility of processing and utilizing the abundantly found seaweed, *Sargassum* sp into a more profitable and value added products. The extracted Sodium alginate was applied as stabilizer in sweetpotato beverage, it was observed that at only 1- 1.5% it can already stabilize the solution

Acknowledgement

The author wishes to thank the Commission on Higher Education of the Philippines for the support, Bohol Island State University-Calape Campus, Bohol and Department of Food Science and Technology of Visayas State University, Visca, Baybay City, Leyte, Philippines.

References

Arvizu-Higuera DL., Hernandez-Carmona G, Rodriguez-Montesinos YE.. 2002. "Parameters affecting the conversion of alginic acid to Sodium alginate." *Ciencias Marinas,* 28(1): 27-36. doi: https://doi.org/10.7773/cm.v28i1.208

Bixler H, Porse H. 2010. "A decade of change in the seaweed hydrocolloids industry." *Journal of Applied Phycology* 23, 321-335.doi: https://doi.org/10.1007/s10811-010-9529-3

Chandia NP, Matsuhiro B, Vasquez AE. 2001. "Alginic acids in Lessonia trabeculata: characterization by formic acid hydrolysis and FT-IR spectroscopy." *Carbohydr. Polym* 46:81–87.

Chou HN, Chiang YM. 1976. "Studies on Algin from Brown Algae of Taiwan: Estimation of Yield and quality of Algin. Taiwan" *Acta Oceanographica Taiwanica* : Science Report of the National Taiwan University. Report No. 7.

Cordero P, Caballes SM, Miranda, S. 2018. "Survey on the Seaweeds and other Fishery Products and Their Local Utilization at Higatangan Island, Naval, Biliran. Leyte, (Ph): Visayas State University"

Davis TA, Volesky B, Vieira RHS. 2000. "Sargassum seaweed as biosorbent for heavy metals." 4270-4278.doi: https://doi.org/10.1016/S0043-1354%2800%2900177-9

FCC. 1993. "Food Chemical Codex." *National Academy Press Washington* 434.

Food and Chemical CODEX. 1981. *Alginic acid, Sodium alginate and potassium alginate.* Washington DC.: National Academy Press.

Garcia-rios V, Rios-leal E, Robledo D, Frelle-Pelegrin Y. 2012. "Polysaccharides composition from tropical brown seaweeds." *Phycological Research* 2(12). doi: https://doi.org/10.1111/j.1440-1835.2012.00661.x

Gholamipor S, Nikpour-ghanavati 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.* Bandar-Abbas, Iran. 1-5.

Granato D, Calado VM. 2014. "The use and importance of design of experiments (DOE) in process modelling in food science and technology." In *Mathematical and Statistical Methods in Food Science and Technology, First Edition*, 1-18 (3). John Wiley & Sons, Ltd.

Haryanti AM, Darmanti S, Izzati M. 2008. "Absorption Capacity and Water Storage in Various Sizes of Seagrass Gracilaria verrucosa as the Base Material of Organic Fertilizer." *Bioma* 10: 1-6.

Haug A, Larsen B, Smidsred O. 1974. "Uronic acid sequence in alginate from different sources." *Carbohydrate Res.* Vol.25, 273-282. doi: https://doi.org/10.1016/S0008-6215%2800%2982100-X

Hernandez-Carmona, G., Arvizu-Higuera, D.L., and Rodriguez-Montesinos, Y.E. 1999. "Manual de tecnicas de control de calidad para el acido alginico y sus derivados." *CICIMAR-IPN.La paz.* Mexico.

Hsu JY. 1985. *Rice Pasta Composition.* Europian Patent 911030690.

Indrani D, Budianto E. 2013. "A study of extraction and characterization of alginates obtained from brown macroalgae Sargassum duplicatum and Sargassum crassifolium from Indonesia." *Dental Journal* 1-6.doi: https://doi.org/10.20473/J.DJMKG.V46.I2.P65-70

James CS. 1995. "Analytical Chemistry of Foods." *Blackle Academic and Professional London* 95-102.

Jing P, Ruan, SY, Park D, Park JM. 2011. "Optimization of purification conditions of radish (Raphanus sativus L.) anthocyanin-rich extracts using chitosan." *LWT – Food Science and Technology,* 44, 2097–2103.doi: 10.1016/j.foodchem.2013.09.107

Kasim S, Asnah M, Sumarheni 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* 1-9. ISSN: 0975-8585

Kleinubing SJ, Gai F, Bertagnolli C, Carlos Da Silva MG. 2013. "Extraction of alginate biopolymer present in marine alga sargassum filipendula and bioadsorption of metallic ions." *Materials Research* 1-9.doi: http://dx.doi.org/10.1590/S1516-14392013005000013

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

Laksomono JA, Widjaya R, Triajie F, Sastrosoeparno S. 2013. "Parameters Controlled on Indonesian Sargassum Extraction Process to obtain a Water-Soluble Alginate." *International Journal of basic and Applied Sciences* Vol. 13 No.05.

Leal D, Matsuhiro B, Rossi M, Caruso F. 2008. "FT-IR Spectra of alginic acid block in three species of brown seaweeds." *Carbohydrate Research* 343:308-316.doi: http://dx.doi.org/10.1016/j.carres.2007.10.016

Mathlouthi M, Koenig JL. 1986. "Vibrational spectra of carbohydrates." *Adv. Carbohydr. Chem. Biochem* 44:7–66.doi: https://doi.org/10.1016/s0065-2318(08)60077-3

Mazumder A, Holdt SL, Francis DE, Morales D, Mishra MA, Angelidaki I. 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.: DTU. 1-2.

Mazumder A, Holdt SL, Francisci D, Alvarado-Morales M, Mishra HN, Angelidaki I. 2016. "Extraction of alginate from Sargassum muticum: process optimization and study of its functional activities." *J. appl. Phycology* 2-11.doi: https://doi.org/10.1007/s10811-016-0872-x

Mcdowell RH. 1977. "Properties of Alginates." *4th ed. Alginates industries, Ltd., London* 67.

Moe ST, Draget KI, Skajak-Brek G, Smidsrod O. 1995. *Food Polysaccharides and their Applications.* New York.Taylor and Francis

Mohamed F, Belfikra A, Dahmane EM, Taourirte M, Brouillette F. 2014. "Extraction and characterization of Sodium algiante from Moroccan Laminaria digitata brown seaweed." *Arabian Journal of Chemistry* 2-9.doi: http://dx.doi.org/10.1016/j.arabjc.2014.05.003

Mushollaeni, W. 2011. "The physicochemical characteristics of Sodium alginate from Indonesian brown seaweeds." *African Journal of Food Science Vol. 5(6)* 349-352.doi: ISSN 1996-0794

Myers RH, Montgomery DC, Anderson-Cook CM. 2009. *Response Surface Methodology: process and product optimization using designed experiments, 3rd edn.* New York: John Wiley & Sons, Inc.,.

Ortiz AT, Trono GC. 2000. "Growth and reprodcutive 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 *Alginates and Their Biomedical Applications*, by BHA., and Moradali, MF. Rehm, 1-41. Singapore: Springer Nature.

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

Salasa, FFA. 2002. "Seaweeds and Fish Processing Technology." *Fisheries Department of Indonesia, Jakarta* 12.

Sartori C, Finch DS, Ralph B. 1997. "Determination of the cation content of alginate thin films by FTIR spectroscopy." *Polymer* 38: 43–51.doi: https://doi.org/10.1016/S0032-3861%2896%2900458-2

Silverstein R, Webster F. 1991. "Spectrometric Identification of Organic Compounds." *USA, John Wiley and Sons.*

Smidsrod, O. 1980. "Structure and properties of charged polysaccharides." *Int Congr Pure Appl Chem* 315–27.

Stephen A, Phillips G, Williams P. 2006. *Food Polysaccharides and Their Applications.* Boca Raton, FL: CRC Press.

Tiwary AK, Puri M, Sharma D,Barrow CJ. 2012. "Optimisation of novel method for the extraction of steviosides from Stevia rebaudiana leaves." *Food Chemistry,* 132, 1113–1120.doi: https://doi.org/10.1016/j.foodchem.2011.11.063

Toft K, Grasdalen H, Smidsrod O. 1986. *Synergistic gelation of alginates and pectins.* ACS Sympos Series.doi: https://doi.org/10.1021/bk-1986-0310.ch010

Torres ML, Cortizo AM, Oberti TG, Fernandez JM. 2016. "Characterization of Commercial and Algae (Undaria pinnatifida) extracted Sodium ALginate for future application in Bone Tissue Engineering." 1-13.

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.doi: https://doi.org/10.3176/chem.2001.2.04

Vauchel P, Kaas K, Arhaliass A, Baron R, Legrand J. 2008. "A new process for the extraction of alginates from Laminaria digitata: Reactive extrusion." *Food Bioprocess Technol* 1(3), pp. 297–300.doi: https://doi.org/10.1007/S11947-008-0082-X

Venkatesan J, Sukumaran A, Se-Kwon K. 2017. *Seaweed Polysaccharide.* Amesterdam, Netherlands: Elsevier Inc.

Whyte, JNC, Engler JR. 1976. "Alginate degredation in situ accompanying thermal drying of the marine algae Nereocystis luetkeana and Macrocyctis integrifolia." *Fisheries and Marine Service, Environmental Canada* 1-38.

Winarno, FG. 1996. "Teknologi Pengolahan Rumput Laut." *Pustaka Sinar Harapan.* ISSBN 9794160962

Yudiati, E, Isnansetyo, A. 2017. "Characterizing the Three Different Alginate Type of Sargassum siliquosum." *ILMU KELAUTANMaret Vol 22* 7-14.doi: https://doi.org/10.14710/ik.ijms.22.1.7-14

Zailanie, K. 2015. "Study of Alginate Sargassum Filipendula with FTIR Confirmation." *Journal of Life Sciences and Biomedicine* 5(6): 167-170. ISSN 2251-9939

Appendix Table 1. Summary of the critical and predicted value of physico-chemical properties of Sodium Alginate from *Sargassum piluleferum*

RESPONSE	VARIABLES			PREDICTED VALUE
	Na_2CO_3 Conc.	Extractant Ratio	NaOCl Conc.	
Yield	1.45017	36.50258	8.33619	17.91552
Moisture	3.20031	35.18430	1.57708	9.902621
Ash	-2.10172	28.99873	5.14351	59.6963
pH	9.52480	21.10153	2.46599	10.57535
Purity	1.87485	39.31110	6.42589	82.24642

Appendix Table 2. Analysis of Variance (ANOVA) for the different physico-chemical analysis of Sodium alginate

	F-RATIO				
REGRESSION	YIELD (%)	MOISTURE (%)	pH	ASH (%)	PURITY (%)
(1)Na2CO3 Conc.(L)	19.06284**	0.923929^{ns}	0.925122^{ns}	9.957349**	6.96449**
Na2CO3 Conc.(Q)	5.97558*	0.059672^{ns}	0.180744^{ns}	0.282958^{ns}	0.42074^{ns}
(2)Extractnt Ratio(L)	21.71178**	0.451376^{ns}	4.197251^{ns}	0.265602^{ns}	5.07526*
Extractnt Ratio(Q)	2.87622^{ns}	0.752185^{ns}	0.643417^{ns}	0.017361^{ns}	0.82112^{ns}
(3)NaOCL Conc.(L)	0.33749^{ns}	7.727138**	1.250877^{ns}	6.248347*	0.05616^{ns}
NaOCL Conc.(Q)	0.40566^{ns}	1.371373^{ns}	7.242430**	4.260257*	0.70652^{ns}
1L by 2L	0.08731^{ns}	0.385644^{ns}	1.961341^{ns}	0.081689^{ns}	0.35766^{ns}
1L by 3L	0.73141^{ns}	0.003745^{ns}	1.961341^{ns}	0.110882^{ns}	3.83214^{ns}
2L by 3L	15.66741**	0.358008^{ns}	0.379716^{ns}	2.181600^{ns}	11.86767**