Poultry, Fisheries & Wildlife Sciences

ISSN: 2375-446X

Jayasinghe et al., Poult Fish Wildl Sci 2016, 4:1
DOI: 10.4172/2375-446X.1000150

Case Report Open Access

Effect of Extraction Methods on the Yield and Physiochemical Properties of Polysaccharides Extracted from Seaweed Available in Sri Lanka

Jayasinghe PS[1]*, Pahalawattaarachchi V[1] and Ranaweera KKDS[2]

[1]National Aquatic Resource Research and Development Agency, Crow Island, Colombo15, Mattakkuliya, Sri Lanka
[2]Faculty of Food science, University of Sri Jayewardenepura, Nuegegoda, Sri Lanka

Abstract

Agar, carrageenan and alginate are high valued seaweed polysaccharides, which are used as gelation and thickening agents in different food, pharmaceutical and biotechnological industries. The annual global production of the polysaccharides has recently reached 100,000 tons with a gross market value just above US$ 1.1 billion. In Sri Lanka although several seaweed species are available, there is no systematic study on comparison of method of extraction, yield and physicochemical properties of polysaccharides from seaweeds. Seaweed species Gracilaria edulis, Gracilaria verrucosa, Kapphaphycus alverazi and Sargussum wightti were collected from North and South coast of Sri Lanka. Gracilaria edulis and *Gracilaria verrucosa* were used for agar extraction with acid and alkaline treatments. Carrageenan was extracted from Kapphaphycus alvarezii using alkaline treatments. Three types of carrageenan (kappa, iota, lambda) were separated using freeze thawing, jelly pressing and alcohol precipitation methods respectively. For the extraction of alginates from Sargussum wightti samples were treated with acid and alkaline and hot and cold extraction methods were used. Some of the above physiochemical properties were compared with commercial available products.

In agar yield (16.6%) of acid treated Gracilaria eduliswas high when compared to Gracilaria verrucosa. The gel strength was high in alkaline treatments (429 g.cm^2) and was obtained from *G. verrucosa*. Freeze thawed method gave the highest yield (23.4%) and gel strength 715 g/cm^2 in kappa carrageenan extracts. The highest alginate yield (35%) was obtained from Sargussum wightti in hot extraction treatments. The mean viscosity of agar and carrageenan purchased from commercially and extracted polysaccharides didn't show significant difference. When considered the proximate composition mean crude protein contents of extracted and commercial products were not significantly difference on dry weight basis among agar, carrageenan and alginates. The mean value of iodine content of extracted and commercial product obtain from agar carrageenan and alginates were range from 32-58; 26-45 and 43-57 mg/l respectively. The mean sodium content of agar, carrageenan and alginates were significant difference between extracted polysaccharides and commercial product. There is also significant difference in the calcium contents of agar, carrageenan and alginates respectively. Chemical properties of agar carrageenan and alginates extracted in laboratory were more superior when compared to those properties of the products collected from the local market, but physical properties were at comparable levels. Considering the above extracted product can be promoted for commercial market.

Keywords: Polysaccharides; Agar; Carrageenan; Alginates; Extraction; Seaweeds

Introduction

Agar, carrageenan and Alginates are industrially important hydrocolloids derived from brown and red seaweeds. These hydrocolloid polysaccharides are of significant importance, both technologically and economically. They are used in the food, pharmaceutical, medicinal, and biotechnological industries due to their distinct physical-chemical properties as gelling agents, thickeners or stabilizing and emulsifying agents. Agar and carrageenan constitute of two well defined families of polysaccharide, derived from different genera in the *Rhodophyta* (red algae), *Pheophyta* (Brown algae) collectively known as Agarophytes, Carrageenophytes and Alginophytes [1]. These seaweed polysaccharides have a high nutritional property than other natural hydrocolloids such as pectin, xanthan gum [2]. These hydrocolloids have a global commercial value of approximately US$ 1.1 billion, which is prospected to increase [3]. Seaweeds have a unique source of high-value hydrocolloid polysaccharides: whereas Carrageenans currently have the highest commercial total production (60,000 ton/year) and contribute to the highest total value of US$ 626 million per year.

This paper, reports the distinctive techniques for extraction of agar carrageenan and alginates together with physical substance and wholesome properties from locally accessible species. Agar was extracted from *Gracilaria verrucos* and *Gracilaria edulis*by alkali and acid treatment and carrageenan from *Kappaphycus alvarezii* and alginate was obtained from two *Sargasum* species. The high qualities of end products were compared with each commercially available polysaccharide.

Material and Method

Sample collection and preparation

Seaweed species *Gracilaria verucosa*and *Gracilaria edulis* were collected from coastal areas of Trincomalle and Kalpitiya. *Kappaphycus alvarezii* was collected from North West coastal belt of Sri Lanka. Two

***Corresponding author:** Jayasinghe PS, Professor, National Aquatic Resource Research and Development Agency, Sri Lanka, Tel: 94 11 2521000; E-mail: pradee_jaya@yahoo.com

Received March 22, 2016; **Accepted** May 04, 2016; **Published** May 06, 2016

Citation: 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. Poult Fish Wildl Sci 4: 150. doi:10.4172/2375-446X.1000150

species of brown seaweeds, *Sargussum wightti* and *Sargussum filipendula* were collected from Southern cost of Sri Lanka. The specimens were transported to the research facility, kept in ice and washed a few times in running water to expel epiphytes and dried in an air coursed broiler. Suitable measures of the dried examples were taken, ground utilize a household processor and kept in plastic holder secured with aluminum foils. Tests were kept at 0°C for further investigation.

Extraction of agarophytes

The dried powder samples of two seaweeds species *Gracilaria edulis* and *Gracilaria verrucosa* were used with two different methods pretreated with acid [4] and alkaline (Tomas & Krishnamurthy, 1976.) to extracts dried agar powder. Then 1.5% of agar solution was prepared by boiling 9 g of agar powder in 600 ml of distilled water for 30 min. Viscosity, gel strength and gel pH were measured in prepared solution. Viscosity of the solution was determined at 80°C by using a Brookfield viscometer (BL spindle at 60 rpm, Tokyo Keiki) according to Joint FAO/WHO Expert Committee [5]. The filtrate was gelled at room temperature, kept at 20°C for 15 h and used to determine gel strength (3 replicates per sample), using a 1 cm^2 plunger (Nikkansui Shiki Gelometer, Kiya Seisakusho, Tokyo) according to Nikan S.(1988). The solidagar was cut into strips, frozen at -18°C for 24 h, thawed in tap water, soaked in ethanol and dried at 40°C in an oven for determination of agar yield.

Extraction of carragenopytes

50 g of dried material of *Kappaphycus alvarezii* was washed with tap water to remove sand and salt. The three carrageenan extraction methods, freeze thawed, gel pressing and alcohol precipitation were done according to [6]. The extracted dried powder was stored and kept at 0°C in polythene bags until further analysis. Then 1.5g carrageenan solution was prepared from 9 g of the extract, dissolved in 600 ml of distilled water which was heated at 80°C for 30 min. The viscosity of this solution was measured at 75°C using a Brookfield type viscometer (Tokyo Keiki). To measure gel strength of the solution, 0.29g of KCI was added and gel was maintained at room temperature. For kappa carrageenan analysis the gel was incubated at 20°C and for iota carrageenan incubation was done at 10°C, for a period of one hour. (3 replicates per sample)

Extraction of alginophytes

50 g of dried material of *Sargussum wightti* and *Sargussum filifendinalis* were washed with tap water to remove sand, salt, mud and dirt. Alginate was extracted according to four different methods. Alginate yield was measured by method A was performed according to [7] method B [8] and method C were done in hot and cooled methods [9]. The extracted dried powder was stored and kept in 0°C in polythene bags until further analysis. The intrinsic viscosity measurement for alginate sample were carried out at 25°C for 0.4% alginate in NaOH as solution with glass viscosity meter and Molecular weight was calculated using Mark Houwink Sakurada equation $\eta=k M^{a}_{w}$ [10].

Determination of chemical physical and nutritional parameters of polysaccharides

The method which gave highest yield was followed to extracted agar, carrageenan and alginic acid and samples from the above were subjected to compare physical and chemical parameters with market available products. The fat content, protein content, carbohydrate content, moisture content and ash content [11] were analyzed according to given procedures. Micro and macro elements were measured according to [12] method using atomic absorption spectrophotometer. The gel pH was measured using electronic pH meter. The melting point was determined according to [13] and gelling points from [14].

Results and Discussion

Effect of different extraction methods on quality of seaweed polysaccharides

Quality Index of *Gracilaria verrucosa* and *Gracilaria edulis:* The variation in quality of five different seaweed species such as *Gracilaria verrucosa*, *Gracilaria edulis*, *Sargussum wightti*, *Sargussum species* and *Kapphaphycus alvarezii* showed in Table 1. Statistical analysis of data showed that there were no significant differences among moisture percentage and purity percentage of agarophytes. The highest percentage of total impurities (TI) (26.34 ± 1.2) were recorded in 26.34*Gracilaria verrucosa* and the lowest percentage of impurities were recorded in *Sargussum species*. The highest percentage of DDMS (12.8 ± 0.23) was recorded in *Kapphaphycus alvarezii* and lowest percentage of DDMS (10.7 ± 0.77) was recorded in *Sargussum wightti* The *Gracilaria verrucosa* also recorded high percentage of insoluble matters and (20.22%) low percentage of total soluble matters (11.34%). The highest percentage of total soluble matter (12.79%) was recorded in *Gracilaria edulis.* But all the species used showed a high amount of purity. *Gracilaria verrucosa* recorded highest purity percentage (98.73%) leading to best quality carrageenan.

Effect of different extraction methods on quality of Agar

Comparison of the Physical properties of agar extracted from *G. verrucosa* and *G. edulis* pretreated with alkaline and acids is showed in Table 2. The viscosity of crude agar extracted from *G. edulis* was 150 cp which is significantly higher than that of *G. verrucosa*. In acid treatment viscosity increase to 195 cp and alkaline treatment to 120 cp where the viscosity of crude extract was 90.5 cp. This shows that there is a significant increase in viscosity when acid treatment is used. In commercial agar viscosity recorded was150 cp which was similar to value recorded in crude extract for *G. edulis*. Pretreatment of acid improve significantly the viscosity of both species leading to better thickening ability.

Species	Parameters					
	Moisture (%) (mean ± S.E)	Total soluble matter (%) (mean ± S.E)	Insoluble matter (%) (mean ± S.E)	DDMS (mean ± S.E)	Purity (mean ± S.E)	Total impurities (%) (mean ± S.E)
Gracilaria verrucosa	12.37 ± 007	11.58 ± 4.6	20.22 ± 5.9	12.70 ± 4.8	98.73 ± 6.2	26.34 ± 1.2
Gracilaria edulis	12.37 ± 0.56	12.97 ± 7.8	11.34 ± 0.02	9.67 ± 2.3	98.66 ± 6.6	23.38 ± 1.5
Sargussum wightti	13.7 ± 0.12	12. ± 6.9	15.8 ± 0.04	10.7 ± 0.77	95 ± 6.9	25 ± 2.9
Sargussum species	14.7 ± 1.78	10.9 ± 1.2	17 ± 1.3	12.3 ± 0.8	96.7 ± 4.2	22 ± 1.2
Kapphaphycus alvarezii	14.5 ± 1.98	12.7 ± 2.9	14 ± 0.9	12.8 ± 0.23	97 ± 2.8	22 ± 0.2

Table 1: Comparison of physical quality parameters in purity%, DDMS%, moisture%, total soluble matter (%) and insoluble matter (%) in five different seaweed species from Sri Lanka.

Species	Extraction methods					
	Crude extract Mean ± S.E		Acid treatment, (1.5% agar gel) Mean ± S.E		Alkaline treated agar (1.5% agar) Mean ± S.E	
	Gracilaria verrucos	*Gracilariab edulis*	*Gracilaria verrucos*	*Gracilariab edulis*	*Gracilaria verrucos*	*Gracilariab edulis*
Physical Properties Viscosity (cp)	90.5 ± 0.091	150 ± 1.37	195.27 ± 0.61	170 ± 1.03	120 ± 0.51	160 ± 0.2
Gel strength g/cm^2	450 ± 4.5	375 ± 0.76	65 ± 1.0	23 ± 0.1	429 ± 6.4	342 ± 0.76
Ph	6.27 ± 1.1	6.3 ± 0.9	4.3 ± 0.18	4.25 ± 9.4	8.3 ± 0.069	8.67 ± 0.15
Yield (%)	15.5 ± 6.4	16.6 ± 0.9	33 ± 0.069	37 ± 0.28	18.6 ± 0.154	20.5 ± 0.39
Melting Point °C	80.2°C ± 0.08	85°C ± 0.02	88°C ± 0.48	75°C ± 0.73	92.6°C ± 0.154	94.5°C ± 0.02
Gelling point °C	32°C ± 0.06	30°C ± 0.05	43°C ± 0.3	36°C ± 0.908	32°C ± 0.23	30°C ± 0.1

Table 2: Comparison of physical properties of agar extracted from *Gracilaria verrucosa* and *Gracilaria edulis*using different method.

Physical properties	Freeze thaw method	Gel pressing, 1.5% gel	Alcohol precipitation, 1.5% gel
Viscosity (cp)	80.2 ± 4.5	150 ± 0.71	130.5 ± 1.3
Gel strength g/cm^2	715.0 ± 4.54	70.50 ± 2.81	680 ± 13.5
Yield (%)	37.3 ± 0.2	29.7 ± 0.26	30.5 ± 0.26
Gelling point	50°C ± 0.6	43°C ± 0.56	40°C ± 0.3
Melting point	95°C ± 0.23	65°C ± 0.7	75°C ± 2.9

Table 3: Physical properties of extracted different carrageenan types from *Kapphaphycus alvarezii.*

The pH values of crude, acid and alkaline treated agar extracted from *Gracilaria verrucosa* and *Gracilaria edulis* species were 6.27, 6.3; 4.3, 4.25; 8.3 and 8.67 respectively.

Extracts from *Gracilaria verucosa* and *Gracilaria edulis* treated with acid gave lower gel strength when compared to alkaline treated samples. The crude agar from *G. verrucosa* and *Gracilaria edulis* showed the highest gel strength when compared acid and alkaline treated (1.5%) gel indicating 450 g/cm^2, 482 g/cm^2 respectively. Agar extracted from alkaline treated *Gracilaria verrucosa* showed an increase in the gel strength by 60% when compared to acid treated once. The commercial agar showed two fold higher gel strength than crude agar. In wild or older plants in *Gracilaria* species, agar gel strength is higher than that of younger or cultivated plants [15].

With respects to yield, agar extracted from *Gracilaria verrucosa* was recorded the lowest yield (15.6%). The yield recorded for the alkaline treated of *Gracilaria verrucosa* and *Gracilaria edulis* were 18.6% and 20.5% respectively. Acid treated *Gracilaria edulis* significantly increases agar yield from 77% to 80% when compared to alkaline treatments. These observations of present study are similar to the [16] who states that the acid treated wild samples give highest agar yield due to higher hydrolysis of the seaweed in acid than in alkaline. *Gracilaria species* which was used as a raw material for agar extraction generally give a yield of 10-25% [17].

There was increased in melting point by 15% in *Gracilaria verrucosa and* 4% *in Gracilaria edulis* of agar extracted using alkaline treatments. The melting point of agar extracted from alkaline treated *Gracilaria edulis* increased by (26%) when compared to acid treated agar. Highest gelling point (43°C) was observed for acid treated agar extracted from *G. verrucosa* where there was increased by (34%) when compared to alkaline treatments. The time taken for alkaline treatment has improved the gelling point from 32°C to 43°C in *Gracilaria verrucosa* [18]. States that gelling point change with the variation of alkaline strength and also with treatment time.

The carrageenan extracted from *Kappaphycus alvarezii* (Eucheema cottani) is referred to as kappa carrageenan while *Eucheema spinosa* iota carrageenan [3]. In the present study was used *Kapphaphycus alvarezii*. The carrageenan yield ranged from 29.70-37.3%. The gel pressing method gave the lowest yield (29.7%) whereas freeze thawed method gave the highest yield.

The viscosity of alcohol precipitation method was the highest (240 cp) while freeze thaw method showed second highest value (180.2 cp). The lowest value of 70.2 cp was obtained from gel pressing method. The commercial product showed 250 cp which is closer to the viscosity of alcohol precipitation method of carrageenan extracted in the present study (Table 3).

The gel strength of kappa carrageenan was highest in freeze thaw method and was 715 g/cm^2. The lowest gel strength (150 g/cm^2) was obtained from gel pressing method while second highest value (680 g/cm^2) was record for alcohol precipitation method.

The melting point of carrageenan was in (95°C) in freeze thaw method while it was lower than that of alcohol precipitation and gel pressing method.

Alginic acid extraction methods

The yield and apparent viscosity of alginates extracted of two different brown seaweeds using four different extraction methods showed in Table 4. The alginate from *Sargussum wightti* from hot extraction (method C) gavethe highest alginate yield (47.3%) and viscosity (120 cp) when compared to other three methods. Cold extraction method C showed its second highest yield 44.43% in *Sargussum wightti.* In the hot extraction which was soaked in 1% $CaCl_2$ at 50°C for three hours resulted in higher yield than overnight at 27°C in cold method. The similar trend was observed in *Sargussum filipendula* gave highest alginate yield (40.5%) from hot method. The lowest alginates yield (19.3%) was observed in method B where given in Table 5 in *Sargussum filipendula.*

The apparent viscosity of *Sargussum wightti* dropped significantly from 120 cp to 99 cp when it was extracted through hot method. Similar trend was observed for *Sargussum filipendula.* The apparent viscosity of alginates and for extraction method depended on species used for extraction of alginates. However it was also observed that the

Parameter/Methods	Yield (%)				Viscosity Cp			
	Method A (mean ± S.E.)	Method B (mean ± S.E.)	Method C (mean ± S.E.)	Method C (hot)	Method A (mean ± S.E.)	Method B (mean ± S.E.)	Method c (cold) (mean ± S.E.)	Method C (hot) (mean ± S.E.)
Species *Sargussum wghtti*	33 ± 0.225	22.02 ± 0.56	47.3 ± 1.2	44.43 ± 0.75	100 ± 1.85	90 ± 1.06	99 ± 3.61	120 ± 1.034
Sargussum filipendula	27 ± 0.56	19.3 ± 0.43	40.5 ± 0.67	37.3 ± 0.81	94 ± 2.85	97 ± 0.56	85 ± 1.56	112 ± 1.95

Table 4: Effect of different extraction methods on yield and viscosity of alginatesextracted from *Sargussumwightti* and *Sargussum filipendula.*

Experimental methods	Treatments and digestion	Precipitation
Method A	,0.4% formaldehyde 5 hours,2N H_2SO_4 at 27°C overnight,1% $NaCO_3$ 27°C overnight, 50ml added NaCl0.1% (m/v)	Ethanol, 50°C for 24 hours drying
Method B	Boiling water for 30 min,0.5% $CaCl_2$(100°C) 30 minutes, NaCl 10% of weed (100°C) 30 minutes, 10% of weed $NaC0_3$ (90°C) for 20 minutes	DiluteH_2SO_4, 90°C for 2 hours drying
Method C (hot)	Soaked 1% $CaCl_2$ (50°C) three hours 5% HCl (1 hour),3% $NaC0_3$ for 1 hour (27°C)	Ethanol/water (1:1,w/v),50°C for 24 hours drying
Method C (cold)	Soaked 1% $CaCl_2$ (27°C) over night 5% HCl (1 hour),3% $NaC0_3$ for 1 hour(27°C)	Ethanol/water (1:1,w/v),50°C for 24 hours drying

Table 5: Summary of experimental design of the four alginate extraction method.

Methods	Parameter			
	Intrinsic viscosity (η) (dLg^{-1})		Molecular weight M_w x 10^5	
	Hot method	Cold method	Hot method	Cold method
Species *Sargussum wightti*	8. 3 ± 0.28	14.6 ± 0.63	6.8 ± 0.26	7.3 ± 0.25
Sargussum filipendula	4.3 ± 0.0.28	12.0 ± 0.63	4.8 ± 0.25	5.9 ± 0.26

Table 6: Intrinsic viscosity and Molecular weight of alginates sample extracted from two different species.

application of concentrated H_2SO_4 for overnight in method a lead to loss of alginates viscosity.

The intrinsic viscosity of alginate from *Sargussum wightti* significantly increased by 71% in cold method when compared to hot method. The similar changes was observed in intrinsic viscosity of alginates from *S. filipendula* where significant increased by heat treatments from 4.3 to 12.0**.** *Sargussum wightti* was more heat sensitive when compared to *Sargussum filipendula.*

The calculated molecular weight (M_w) of alginates extracted from *Sargussum wightti* was given in Table 6. Alginates extracted from *Sargussum wightti* has highest molecular weight (7.3 g mol-1) in cold extraction and 6.8 g.mol-1 in hot extraction. *Sargussum wightti* was found more heat sensitive when compared to the *Sargussum filipendula*. It is apparent that due to the hot extraction of molecular weight get dropped from 7.3 to 6.8 when compared to cold extraction method. Species *Sargussum wightti* and *Sargussum filipendula* showed a similar changes.

When consider the protein content Table 7 of extracted agar (6 ± 0.35) at lower than that in extracted carrageenan (8.2 ± 0.3). The protein value of extracted polysaccharides were found higher than that of commercial products except in alginic acid. Commercial agar was reports significantly lowest (p>0.05) protein value (4.3 ± 0.08)%.

The fat content of the different polysaccharides of majority of extracted products have lower fat content. The extracted carrageenan have highest fat content (2 ± 1.2%) while commercial carrageenan gave the lowest fat contents (0.25 ± 0.23%). It was resulted that industrial polysaccharides have lowest fat content where incomparable to extracted polysaccharides.

As given in Table 7, all the polysaccharides have higher carbohydrate content, more than 40%. In the extracted alginic acid gave the highest carbohydrate value (60 ± 1.7%) while lowest value was indicated in commercial alginic acid. The carbohydrate contents of extracted polysaccharides were found statistically higher than commercial products except in carrageenan.

The ash content of the laboratory prepared polysaccharides ranged from 2.5 ± 0.031% to 4.28 ± 2.31% which is higher when compared to the commercial products. All commercially extracted polysaccharides, agar and carrageenan ranged from 2.5 ± 0.03 to 2.7 ± 0.031%. Differences were found to be statistically significant. Generally market product indicate low ash content and laboratory extracts gave high ash content. Their high mineral content may be due to different treatments and purification process adapted.

The extraction process of polysaccharides depend on specific seaweed species [19], but generally consists of laboratory agar extracted using alkali treatment from *Gracilaria verrucosa* followed by hot water extraction. The gel strength of commercial agar was two times higher than laboratory extracted agar.

Viscosity was highest (150 ± 5.4) in laboratory prepared agar than in commercial product. Alkaline treatments improved the melting points 83°C ± 1.35, but lower than that of commercial agar. The lowest gelling point was observed for laboratory prepared agar when compared to commercial agar, carrageenan extraction from alkaline (6% KOH) treatments improved gel strength two fold than in commercial carrageenan. As described above, for carrageenan the alkali treatments causes a chemical change in agar (formation of the 3,6-anhydro-galactopyranose) resulting in increased gel strength. The viscosity of laboratory prepared carrageenan was highest than commercial product Table 8.

The gelling point was higher in commercial carrageenan while melting point in both carrageenan types were lower than that of agar.

Table 9 indicates the mineral composition of three different seaweed extracts and commercial product. The commercial agar had

Parameters	Polysaccharides					
	Extracted agar	Commercial agar	Extracted Carrageenan	Commercial carrageenan	Extracted alginic acid	Commercial alginic acid
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Protein (%)	6 ± 0.35	4.3 ± 0.08	8.2 ± 0.3	5.6 ± 0.22	5.9 ± 0.09	6.2 ± 0.74
Fat (%)	1.5 ± 0.09	1.2 ± 0.11	2 ± 0.81	0.25 ± 0.35	1.2 ± 0.097	0.34 ± 0.08
Moisture (%)	17.1 ± 0.61	16 ± 2.06	14 ± 1.4.7	14 ± 1.5	5.9 ± 0.106	15 ± 0.61
Ash (%)	4.284 ± 0.13	2.7 ± 3.8	3.7 ± 0.187	2.6 ± 3.75	4.2 ± 0.3	2.5 ± 0.04
Carbohydrate (%)	53 ± 8.1	52 ± 3.4	52 ± 3.22	54 ± 3.34	60 ± 1.7	43 ± 1.01

Table 7: Proximate composition of laboratory developed and commercial purchased of agar, carrageenan and alginates.

Parameters	Products			
	Extracted Agar Mean ± SE (*Gracilaria verrucosa*)	Commercial agar Mean ± SE	Extracted Carrageenan Mean ± SE (*Kapphaphycus alverazii*)	Commercial carrageenan Mean ± SE
Parameter Solubility	Boiling water	Boiling water	Boiling Water	Boling water
Gel strength (g/cm^2)	482 ± 1.13	1000 ± 7.9	715 ± 2.4	350 ± 62.45
Viscosity (cp)	150 ± 5.4	100 ± 7.7	287 ± 5.3	250 ± 17.03
Melting point (0^0)	83°C ± 1.35	90°C ± 0.85	75°C ± 0.99	76°C ± 1.21
Gelling point (0^0)	30°C ± 1.07	40°C ± 1.19	32°C ± 0.91	50°C ± 1.06
Colour	Yellow	white	Yellow	White

Table 8: Comparison of physical properties of extracted seaweed polysaccharides with commercial purchased polysaccharides.

Minerals	Polysaccharides					
	Agar		Carrageenan		Alginic acid	
	Extracted Agar	Commercial Agar	Extracted Carrageenan	Commercial Carrageenan	Extracted Alginic acid	Commercial Alginic acid
Na	2584 ± 37.35	100 ± 5.5	9786 ± 19.6	160 ± 9.9	16944 ± 64.71	210 ± 4.8
Mg	5901 ± 3.7	750v3.9	1458 ± 5.7	400 ± 0.98	221 ± 6.6	468 ± 3.1
Ca	79018 ± 29.08	600 ± 6.1	8222v37.62	800 ± 15.1	678 ± 4.9	435 ± 10.2
K	15397 ± 57.8	1100 ± 30.5	49947 ± 8.7	1500 ± 46.4	3352 ± 566,2	2000 ± 12.7
Mn	128 ± 9.3	68 ± 4.8	15 ± 0.29	16 ± 0.49	5115 ± 8.2	1279 ± 12.6
Cr	ND	ND	8.3 ± 5.2	4.8 ± 0.98	3998 ± 0.3	435 ± 3.6
Co	ND	ND	ND	ND	ND	ND
Ni	ND	23	ND	ND	333	ND
B	ND	ND	ND	ND	ND	ND
Iodine	58 ± 1.02	32 ± 0.6	45 ± 5.2	26 ± 0.98	57 ± 1.62	0.45 ± 0.12
Sulphate	18 ± 1.4	13 ± 0.19	20 ± 5.51	14 ± 0.35	6 ± 0.34	16 ± 0.31
Phosphorus	0.07 ± 0.001	0.06 ± 0.007	0.02 ± 0.009	0.03 ± 0.087	0.09 ± 0.009	0.03 ± 0.008

Table 9: Comparison of mineral content of laboratory extracted seaweed polysaccharides and commercial purchased seaweed polysaccharides.

the lowest Na content. On the other hand alginic acid has significantly high sodium content (16944 ± 71). Carrageenan showed the second highest sodium contents (9786 ± 245 ± 26.7mg/l).

The Mg content and in the commercial products were in the range of 400 ± 0.67 to 750.21 ± 6.0 mg/kg. The level of Mg content among laboratory prepared extracts did not significantly differ ($p<0.05$). The highest Mg values were found in agar and carrageenan (5901 ± 32.1-1458 ± 42.1mg/kg). The lower Mg levels present could be attributed to the fact that elements mg have destroyed during processing.

The commercial agar, carrageenan and alginic acid tend to have a lower Ca content when compared to the laboratory prepared products. The highest Ca content was observed extracted agar 79018 ± 29.08. The second highest Ca content (8222 ± 37.62) in carrageenan indicating significantly higher amount to the commercial product. It is possible that variation in method which carried out in commercial and laboratory prepared seaweed extracts should have contributed to the differences observed.

All the polysaccharides recorded very low Mn except in alginic acid which have the highest Mn content among them (1279 ± 0.089).There was a statistically significant difference among Mn values ($p<0.05$) in commercial and laboratory prepared products. The differences in the Mn content of seaweed extracts could be traced to the possible indigestion of during processing.

When consider the potassium content carrageenan tends to have highest levels 49947 mg/kg. However the second highest value of potassium was recorded in agar (15397 ± 56.9mg/kg) which was significantly different when compared to in the value of potassium in carrageenan. When consider the agar, carrageenan and alginic acid content of commercial product is range from 1100 ± 45.0 to 2000 ± 47.4 mg/kg which exhibited low potassium content than laboratory

products. The carrageenan and agar were higher in potassium due to available considerable amount of potassium in raw seaweeds.

Iodine level was found significantly higher in laboratory prepared products when compared to the commercial ones. In the study laboratory prepared ones were rich source of iodine when compared to the others. The reason for differences in iodine content between commercial and laboratory products may be due to the differences in the processing techniques. These products can be extensively used as health food.

There were no significant differences between sulfate content of commercial extracts and laboratory prepared ones. The sulfate content ranged from 6 ±-20 ± mg/kg. The low sulfate content observed may be due to the sulfate degradation due to different bonds formation during processing.

Conclusion

Seaweed is a unique source of valuable hydrocolloids due to their functional properties and have a significant importance in the food, medicinal, and biotechnological industries. In order to improve the functional properties of these hydrocolloid polysaccharides to need identify the sustainable use of chemicals and more selective extractions techniques.

Current literature mainly focuses on hydrolysis of the hydrocolloid agar, two seaweed specific by acid and alkaline have been identified which degrade the hydrocolloid polysaccharides and thereby change the yield and gel strength of agar. Physical properties of agar extracted from *Gracilaria verrucosa* treated with alkaline was more superior to agar extracted from *Gracilaria. edulis*. The physical properties of extracted seaweed hydrocolloids are comparable to commercial hydrocolloid. In addition to extracted hydro collided has superior nutritional properties than commercial products could be demanded substitute in food industry.

Alkaline treatments increased the carrageenan properties such as gel strength, while maintaining the gelling properties and others decreased the hydrocolloid yield and viscosity interfered with the gelling abilities of the hydrocolloids. Acid treatments increased the hydrocolloid yield and viscosity and decreased the gel strength. The high viscosity hydrocolloid has demand for the food industry and low vicious hydrocolloids are demand in textile industry.

Sargussum wightti found to be very heat sensitive than *Sargussum filipendula* gave significant higher yield of alginate by the hot extraction at 50°C compare to other methods. The effect of heat on the extent of depolymerisation of the alginate molecular weight and viscosity from the two brown seaweed species was significant. The properties of these hydrocolloids could be controlled by pre heat treatments in order to full fill the requirement of industry what used application these polysaccharides. Thus the alginates extracted from Sri Lankan seaweeds species has a potential to be used in relevant industries considering to its yield, viscosity and molecular weight.

References

1. Yaphe EW (1984) Chemistry of agars and carrageenans. Hydrobiologia. 116/117, 171-1.
2. Saha D, Bhattachaya S (2010) Hydrocolloids as thickening and gelling agents in food. Journal of Food science and Technology 476: 587-589.
3. Nana RK, Marcel Tutor A, Meyer AS (2015). Seaweed Hydrocolloid Production: An update of enzyme assisted Extraction and Modification Technology Mar Drug 3340-3359.
4. Standard Operation Procedure (2003) Determination of agar properties Project Management Services Avenue.
5. Joint FAO/WHO Expert Committee on Food Additives 44th Session Rome, Italy J-4, June (1995). Preprint edition for the 52th sessions of Codex Alimentarius Committee for food additive Contaminands. Compendium of Food additive Specifications Addendum 9.
6. Ohino M, Larg'o DB, Ikumoto T (1994) Growth rate, Carrageenan yield and gel Properties of cultured kappa Carrageenan producing red algae Kapphaphycua alvarezii. Appl Phycology 6: 1-5.
7. Nishigawa K (1985) Extract method of alginic acid. In Research method of algae. pp. 624-626.
8. Dayaratnem M, Grero J (1969) Seasonal variation of Alginic acid content in Sargassum cervicone Greville from Hikkaduwa. Bul Fish Res Stn 20: 169-170.
9. Hernandez-Carmona G, McHugh DJ, Lopez-Gutierrez F (2000) Pilot plant scale extraction of alginate, From Macrocystis pyrifera 1: Effect of pre-extraction treatments on yield and quality of alginate. J Appl Phycol 10: 507-513.
10. Torres MR, Sousa APA, Piho EATS, Melo DF, Feitosa JPA, et al. (2007) Extraction and physiochemical characteristic of Sargusum vulgare from Brazil. Carbohydrate 347: 2067-2074.
11. AOAC (1984) Official methods of analysis. Determination of fat, protein, moisture, ash content.
12. AOAC (1983) Official methods of analysis. Determination of mineral content.
13. Fuse T, Goto F (1971) Studies on utilization of agar. Agri Biol Chem 35: 799-804.
14. Guiseley KB (1970) The relationship between methoxyl content and gelling temperature of agarose. Carbohydrate 13: 247-256.
15. Young EG, Langille WM (1958) The occurrence of inorganic elements in the marine algae of Atlantic Provinces of Canada. Canadian Journal of Botany 36:301-10.
16. Istin S, Ohno M, Kusunose H (1994) Methods analysis for agar carrageena and alginate in seaweeds. pp: 49-55.
17. Hoyle MD (1978) Agar studies in two *Gracilaria* species (*G. bursapastoris* (Gmelin) Silva and *G. coronopifolia* J. Ag.) from Hawii II. Seasonal aspects. Bot 21: 347-352.
18. Rath J & SP Adhikary (2004) Effect of alkali treatment on the yield and quality of agar from red alga *Gracilaria verrucosa*(Rhodophyta, Gracilariales) occurring at different salinity gradient of Chilika lake. Indian Journal Marine Sciences 33(2): 202-205.
19. Stanely N (1987) Production properties and uses of carrageenan In, Production and utilization of Products from commercial seaweed edited by D.J. McHugh, FAO Fish Tech Paper 288: 118-123.