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Methods of Analysis for Agar, Carrageenan and Alginate in Seaweed

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Abstract: The extraction of agar and carrageenan was carried out after alkali treatment, and alginate after acid treatment, of samples agarophytes and carrageenophytes were treated at high temperatures whilst the alginophytes were extracted at room temperature. Agar yield and gel strength were obtained after alkali treatment (5% NaOH, at 70 and 80°C, for 2 hours) from commercially harvested *Gracilaria chorda* (mature and young material) from Japan; *G. fisheri* (cultivated and wild material from Thailand); *G. verrucosa* from the Philippines and *G. lemaneiformis* from Chile. Agar with a high yield (18.34% of dry weight) and gel strength (692 ± 24 $g.cm^{-2}$) was obtained from *G. chorda* (mature thalli) after alkali treatment at 80°C, whilst a low yield (9.92%) and gel strength (355 ± 22 $g.cm^{-2}$) was obtained from *G. verrucosa.*

The yield and gel strength of carrageenan were obtained with alkali treatment (6% KOH), for kappa carrageenan and 6% NaOH for iota carrageenan, at 80°C for a period of 3 hours, using different extract recovery methods (e.g. freeze- thawing, gel pressing and alcohol precipitation). The largest carrageenan yield (35.47%) of kappa carrageenan was obtained by the freeze-thawing method. Alcohol precipitation provided the best extraction of iota carrageenan (34.05%). High gel strength reading were obtained from the freeze-thawing method to extract kappa carrageenan (814 ± 82 $g.cm^{-2}$) and iota car ·ageenan (191 ± 5 $g.cm^{-2}$).

The yield of alginate (1 - 2% sodium carbonate treatment) unbleached and bleached was, 32.16 and 30.65% respectively for *Laminaria japonica,* 33.95 and 33.72% respectively for *Ecklonia cava* and 27.63 and 28.68% respectively for *Sargassum duplicatum.*

Key words: Extraction, yield, physical properties, agar, carrageenan, alginate

Introduction

Agar, carrageenan and alginate are polysaccharides derived from algae. They are hydrocolloid sources, with many applications in the food, pharmaceutical, cosmetic, biotechnology industries, etc, as gelling agents, thickeners or stabilizing and emulsifying agents. Agar and carrageenan constitute two welldefined families of polysaccharide, derived from different genera in the Rhodophyta, collectively known as agarophytes and carrageenophytes (Yaphe, 1984). The basic structure of agar is a regularly alternating sequence of 3-linked-β-D-galactopyranose and 4-linked 3,6-anhydro-α-L-galactopyranose. Carrageenan is a linear polysaccharide with a repeating structure of alternating 1,3-linked β-D galactopyranose and 1,4-linked α-D galactopyranose units. The 3-linked units occur as the 2-and 4-sulfate or unsulfated, while the 4-linked units occur as the 2-sulfate, 2,6-disulfate, the 3,6 anhydrid and the 3,6 anhydrid 2-sulfate (Norman Stanley, 1987).

Alginates should be distinguished from other phycocolloids, they are extracted from brown

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seaweed (Phaeophyta). Alginic acid is a linear polymer based on two monomeric units, β-D mannuronic acid and α-L guluronic acid (Wilma, 1990).

In this paper, we report on methods for the determination of yield and physical properties of agar after alkali treatment at different temperatures from *G. chorda* from Japan, *G. fisheri* from Thailand, *G. verrucosa* from the Philippines and *G. lemaneiformis* from Chile and carrageenan from *Kappaphyous alvarezii* and *Eucheuma spinosum* from the Philippines. Sodium alginate was obtained from *Laminaria japonica, Ecklonia cava* and *Sargassum duplicatum.*

Materials and Methods

Agarophytes

Samples of young and mature *G. chorda* were collected from natural seaweed populations in the Uranouchi Inlet of Tosa Bay, Japan in 1990. Cultivated and wild *G. fisheri* were collected from Songkhla, Thailand in 1991. *G. verrucosa* was collected from Bohol, Philippines in 1989 and *G. lemaneiformis* was collected from Chile in 1990.

Agar extractions were performed as described by Orosco *et al.*, 1992. Samples of *Gracilaria* were washed in tap water to remove excess salt, dried at room temperature and cut in small pieces. 50g of the samples were incubated in 1 litre of 5% NaOH solution at 70 and 80°C in a water bath for 2 h and washed in tap water for 30 min. The algae were then stirred lightly in 1 *l* of 1.5% sulphuric acid solution at room temperature for 2 h and further washed in tap water overnight to completely eliminate the acid. Samples were boiled for 90 min in 1 *l* of distilled water in 2 *l* Erlenmeyer flasks fitted with a reflux condenser. The agar extract was filtered through muslin cloth. Viscosity of the solution was determined at 80°C by a Brookfield viscometer (BL spindle at 60 rpm, Tokyo Keiki). The filtrate was gelled at room temperature, kept at 20°C for at least 15 h and used to determinate gel strength (3 replicates per sample), using a 1 cm^2 plunger (Nikkansui Shiki Gelometer, Kiya Seisakusho, Tokyo). The solidified agar was cut into strips, frozen at -35°C for 24 h, thawed in tap water, soaked in acetone and dried at room temperature for determination of agar yield, after which, gel pH was measured using electronic pH meter. A 1.5% agar solution was prepared from by boiling 9 g of agar powder in 600 ml of distilled water for 30 min. Viscosity, gel strength and gel pH were measured .

Carrageenophytes

Kappaphyous alvarezii and *Eucheuma spinosum* were collected from the Philippines in 1993. Carrageenan extraction was performed as described by Ohno *et al.*, 1994. 50g of dried material was washed with tap water to remove sand and salt, and then incubated in 2 *l* of 6% KOH for *Kappaphycus alvarezii* and 6 % NaOH for *Eucheuma spinosum* solution in an 80°C water bath for 3 hours. The samples were washed overnight in slowly tap water. The samples were then stirred lightly in 1 *l* of distilled water and boiled for at least 1 h until the algae disintegrated. The carrageenan extract was filtered with pressure pump.

In order recovery of the carrageenan extract was undertaken after the methodology of Stanley (1990). Carrageenan was precipitated in alcohol (ethanol). 50 *ml* of 10% NaCl solution was added to the filtrate. The filtrate was then added to double its volume of ethanol by continuous application of smaller volumes together with stirring using a glass rod. The stringy precipitate usually attaches to the glass rod. Precipitated carrageenan was then soaked in alcohol and dried at room temperature for 2 days or dried at 50°C in a drying oven.

To prepare carrageenan by the freeze-thaw method, 0.2% KCl solution was added to the filtrate, the resulting gel was kept at room temperature. Carrageenan gel was frozen at -35°C for 24 h, and then thawed in tap water. Following this the carrageenan extract was soaked in etha-

nol and dried at 50°C in a drying oven.

In the gel pressing method, the gel was sliced and put between two cloths, which was pressed between absorbent newspaper and cardboards for 2 days (the newspapers was changed regularly until the water was removed completely), the gel was then dried at room temperature.

1.5% 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). In order to measure gel strength of the solution, 0.2% KCl (m/v) was added, the gel was maintained at room temperature. For kappa carrageenan analysis the gel was incubated at 20°C and for iota carrageenan analysis incubation was at 10°C, for a period of 1 h following which the gel strength (3 replicates per sample) was measured.

Alginophytes

Material of wild population *Laminaria Japonica* and *Sargassum duplicatum* and *Ecklonia cava* was collected from Tosa Bay in 1994.

Sodium alginate extraction was performed according to Nishigawa (1985). 10 g samples of dried samples were chopped in to small pieces, and treated with 500 ml of 0.2 N sulphuric acid in a slow shaker overnight, at room temperature, in order to remove the acid soluble salt. The mixture was filtered through nylon and washed with 50-100 ml distilled water and filtered again. The residue was extracted with 500 ml of 1% sodium carbonate solution and shaker at room temperature overnight. Before filtration the sample was dilluted with distilled water to 1 litre and, filtered through nylon. In order to recover the alginate extract, 50 ml of 0.1-0.2% (m/v) NaCl was added to the filtrate and the solution was stirred. The filtrate was then added to ethanol (2 times filtrate volume) by addition of small volume continuously stirred with a glass rod. The stringy precipitate usually attaches itself to the glass rod. The precipitate was washed with ethanol and dried in an oven at 50°C for 24 hours.

In order to bleach the alginate , the samples were treated with formaldehyde solution (0.1-0.4%) for 3-5 hours at room temperature and then washed with water before acid pretreatment (McHugh, D.J, 1987).

Table 1. Yield and physical properties of agar from *Gracilaria* sp. (alkali treatment temperature 70℃).

Properties	*G. chorda*		*G. fisheri*		*G. verrucosa*	*G. lemaneiformis*
	Young	Mature	Cultivated	Wild		
Agar crude extracted						
viscosity (cp)	8.2	28.1	2.6	3.6	4.0	5.1
Gel strength* ($g.\ cm^{-2}$)	347±20	479±25	171±56	199±3	178±12	90±5
pH	6.82	6.81	6.78	6.80	6.79	6.69
Yield (%)	10.25	11.20	13.0	14.20	9.22	13.20
1.5% agar gel						
Viscosity (cp)	53	69.9	37.67	66.8	9.6	377
Gel strength* ($g.\ cm^{-2}$)	467±65	486±41	270±4	382±12	237±9	289±1
pH	6.65	6.58	6.53	6.52	6.52	6.57

* mean ± SD.

Results

Agar gel strength was found to increase after alkali treatment with 5% NaOH, for 2 hours at 70 and 80°C. The crude agar extracted from all species, after alkali treatment had a viscosity range from 2.6 to 71.5 Cp. The viscocity of extracted crude agar from *G. chorda* and *G. lemaneiformis* were higher than that of *G. fisheri* and *G. verrucosa.* The pH of crude extracted agar of all species , ranged from 6.67 to 6.89 and the gel strength ranged from 90 ± 5 g. cm^{-2} to 502 ± 71 g. cm^{-2}. Crude extracted agar from G.chorda (mature) had the highest gel strength value. (Table 1)

High agar yields (18.34%) were obtained from samples (9.92%) of *G. chorda* (mature) incubated at 80°C; Low agar yields were obtained from samples of *G. verrucosa.*

The viscosity of the agar extracted from *G. lemaneiformis* was higher than that of the other species. The pH value of the 1.5% the agar from all samples did not differ, values varied from 6.52 to 6.75. In general, samples treated at 80°C gave a higher gel strength than samples treated at 70°C (Table 2). Highest gel strength (692 ± 24 g.cm^{-2}) was obtained from 80°C incubated samples from *G. chorda* (mature). The agar yield from *G. fisheri* (cultivated) and *G. fisheri* (wild stock), treated at 70° and 80°C did not differ greatly, but the gel strength of *G. fisheri* increased from 270 ± 4 to 393 ± 32 g.cm^{-2} for (cultivated) and from 382 ± 12 to 548 ± 57 g.cm^{-2} for (wild stock). Agar yield from *G. verrucosa* and *G. lemaneiformis* did not differ, but gel strength increased from 237 ± 9 to 355 ± 22 g. cm^{-2} for *G. verrucosa* and 289 ± 1 to 360 ± 22 g.cm^{-2} for *G. lemaneiformis.*

Table 2. Yield and physical properties of agar from *Gracilaria* sp. (alkali treatment temperature 80℃).

Properties	*G. chorda*		*G. fisheri*		*G. verrucosa*	*G. lemaneiformis*
	Young	Mature	Cultivated	Wild		
Agar crude extracted						
Viscosity (cp)	30.2	71.5	4.7	6.6	4.6	38.6
Gel strength* (g. cm^{-2})	365 ± 67	502 ± 71	291 ± 34	385 ± 16	235 ± 29	158 ± 8
pH	6.82	6.89	6.63	6.74	6.67	6.69
Yield (%)	15.9	18.34	14.0	15.63	9.92	12.01
1.5% agar gel						
Viscosity (cp)	87.6	91.4	71.6	79.1	25.8	499
Gel strength* (g. cm^{-2})	565 ± 8	692 ± 24	393 ± 32	548 ± 57	355 ± 22	360 ± 22
pH	6.75	6.58	6.86	6.76	6.75	6.52

* mean ± SD.

The carrageenan extracted from *Kappaphycus alvarezii* is a kappa carrageenan and carrageenan extracted from *Eucheuma spinosum* is of the iota form (Stanley, 1987). The yield for both types of carrageenan examined was found not to differ. Carrageenan yield, ranged from 21.80 to 35.47% for kappa and ranged from 20.60 to 34.05% for iota carrageenan, (Table 3). The lowest yield for both carrageenan types was obtained from the gel pressing method. The highest carrageenan yields were obtained from the freeze-thawing method for kappa carrageenan and alcohol precipitation method for iota carrageenan.

The viscosity of both carrageenan types ranged from 68.17 to 246 Cp (kappa carrageenan) and 147.7 to 790 Cp (iota carrageenan). The highest viscosity recorded for iota carrageenan

Table 3. Yield and physical properties of carrageenan from *Kappaphycus aluarezi* and *Eucheuma spinosum.*

Properties	*Kappaphycus alvarezii*	*Eucheuma spinosum*
Freeze-thawing:		
Yield (%)	35.47	31.45
Viscosity of 1.5% sol (Cp)	93.10	790
Gel strength (g. cm^{-2})*	814±82	191±5
Gel Pressing:		
Yield (%)	21.80	20.60
Viscosity of 1.5% sol (Cp)	68.17	146.7
Gel strength (g. cm^{-2})*	155±3	131±8
Alcohol precipitation:		
Yield (%)	32.80	34.05
Viscosity of 1.5% sol (Cp)	246	700
Gel strength (g. cm^{-2})*	668±57	158±1

* mean ± SD.

was 790 Cp (freeze-thawing method) and 246 Cp for kappa carrageenan (alcohol precipitation method).

The gel strength of kappa carrageenan was higher than that of iota carrageenan, gel strength ranged from 155±3 to 814±82 $g.cm^{-2}$ (kappa carrageenan) and 131±8 to 191±5 $g.cm^{-2}$ (iota carrageenan), respectively. The highest gel strength of extracted kappa carrageenan (814±82 $g.cm^{-2}$) was obtained by the freeze-thawing method. The highest gel strength values of both kappa carrageenan (814±82 $g.cm^{-2}$) and iota carrageenan (191±5 $g.cm^{-2}$) were obtained from the freeze-thawing method.

The alginate yield, after extraction with 1-2% alkali solution from *L. japonica* and *E. cava* did not differ greatly. The yield of sodium alginate from *L. japonica* ranged from 31.62 to 32.16% (unbleached treatment) and ranged from 27.36 to 30.65% (bleached treatment, Table 4). The yield of sodium alginate from *E. cava* ranged from 31.56 to 32.95% (unbleached treament) and ranged from 28.92 to 33.72% (bleached treatment). Sodium alginate from *S. duplicatum* was lower than the other specimen examined and ranged from 20.71 to 27.63% (unbleached treatment) and from 20.49 to 28.68% (bleached treatment).

Table 4. Yield of sodium alginate from *Laminaria japonica, Ecklonia cava* and *Sargassum duplicatum.*

Alginophytes	*Sodium carbonate (%)*	*Yield (%)*	
		Unbleached	*Bleached*
Laminaria japonica	1	31.62	27.36
	1.5	31.92	29.52
	2	32.16	30.65
Ecklonia cava	1	31.56	28.92
	1.5	33.12	32.4
	2	33.95	33.72
Sargassum duplicatum	1	20.71	20.49
	1.5	27.63	24.05
	2	27.35	28.68

Discussion

In this study, analysis of methods for the extraction of agar indicated that the yield and physical properties (viscosity and gel strength) of the agarophytes tested had a potential commercial value. Agar extracted using alkali treatment was converted from the biological percursor of agar, L-galactose-6-sulphate into 3,6-anhydro-L-galactose which resulted in a higher gel strength.

Commercially the material of *Gracilaria* were used as a raw material for agar produced, that usually give an agar yield of 10-25% (Hiroshi Tsukakoshi, personal comunication). The agar produced in Japan showed that agar extracted from *Gracilaria sp.* mixed with *Gelidium* sp had gel strength 300-400 $g.cm^{-2}$. (Shimitsu Co) .

Agar extraction at a temperature 80°C was more effective and the yield and physical properties were improved. Yield and gel strength of agar from *G. chorda* (mature) were higher than *G. chorda* (young), yield and gel strength of agar from *G. fisheri* (wild) were higher than *G. fisheri* (cultivated). In wild or older plants of *Gracilaria* agar formation is complete, thus the yield and gel strength is higher than younger or cultivated plants (Orosco *et al.*, 1992).

There was a low yield of agar from *G. verrucosa* (9.92%). The gel strength was almost the same as that *G. lemaneiformis*. The agar yield of *G. lemaneiformis* treated at 80°C, slightly decreased from 13.2 to 12.01% when the viscosity was very high and difficult to filter.

Using methodology similar to the extraction of agar, carrageenan extracted with alkali converted the precursor of carrageenan, D-galactose 6-sulphate into 3, 6-anhydro galactose. In kappa and iota carrageenan types the gel solution is gelled by potasium ions made available by alkali treatment. These are characterized by having their 1,3-linked residues either unsulphated or sulphated only at C-4, giving a high gel strength after alkali treatment (Stanley, 1990).

The yield of kappa carrageenan and iota carrageenan did not differ. Gel strength of kappa carrageenan was higher than that of because the sulphate content iota carrageenan is greater than that of kappa carrageenan. Carrageenan extracted by the freezethaw method gave gel strength value which were higher than gel extracted by the pressing method. In recovering carrageenan by the freeze-thaw method, the greatest part of the water content is eliminated due to the insolubility of the gel under cold conditions which inturn gives rise a stronger gel. In the case of the gel pressing method the water is eliminated by applying a force (Armisen and Galatas, 1987). The gel strength will be greater if more of the water content can be eliminated.

In this methodology the carrageenan precipitation method is followed by alcohol washes to dehydrate the coagulum. Carrageenan precipitation in alcohol media is easier than that of agar because the precipitate of agar is more flocculated, with lower cohesion, thus being difficult to recover quantitatively. In the alcohol precipitation method the gel strength of the agar did not differ from that extracted by the freeze-thawing method.

In general, the alcohol precipitation method is used for extraction by the carrageenan industry. In this method the spent alcohol is recovered by distillation, but it is not economically viable.

Iota and kappa are carrageenan have different gel properties and texture, and there are used for different purposes. Iota carrageenan gel is softer than that of kappa carrageenan, Which has a harder gel. In the food industry iota carrageenan is used in combination with kappa carrageenan to control the gel structure (Stanley, 1990).

The properties of alginate was different amongst the species tested. Alginate was extracted with an alkali solution, the purpose is to convert the alginate to a soluble form so that extracted alginate can be removed from the rest of the seaweed. The yield of sodium alginate

from *L. japonica* and from *E. cava* did not differ greatly, but the yield from *S. duplicatum* was lower. In general, yield was improved by increasing the alkali concentration. The yield of unbleached sodium alginate was higher than the bleached sodium alginate, since material was lost during the bleaching process.

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