JNSF9-1-7

J. Natn. Sci. Coun. Sri Lanka 1981 9 (1)

Carbohydrate Constituents of the Marine Algae of Sri Lanka Part I. Some Physico-chemical properties of Phycocolloids from Eight Species of Red Algae

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(Date of receipt : 11 March 1980)

(Date of acceptance : 03 November 1980)

Abstract: Phycocolloids from eight species of red algae were extracted, and the yields estimated. *Gracilaria edulis (lichenoides)* and *Gracilaria salicornia* were found to be the most suitable for commercial exploitation. The gel strength properties and their relationship to chemical constituents of the phycocolloids are discussed in relation to similar properties of a commercially available sample of agar.

1. Introduction

Agar and the alginates are two phycocolloids of commercial importance. Agar is extracted from red algae, whilst alginates are obtained from brown algae.

The polysaccharides, agarose and agaropectin are the main constituents of Agar. Agarose is a linear chain of alternating neutral sugar residues - mainly 1, 3 linked D-galactose and 1, 4 linked 3, 6- anhydrous L-galactose residues. Agaropectin consists mainly of D-galactose, 3,6 anhydro-L-galactose, some ester sulphate and D-glucuronic acid. It has many of the structural features of agarose.

Agar is insoluble in cold water but dissolves readily in boiling water, and sets to a firm gel at concentrations as low as 0.5%. It is a valuable colloidal substance because of its hydrophilic nature and its high gel strength. These two important properties are responsible for its wide use in the food, pharmaceutical and textile industries as a thickening, emulsifying, stabilizing and gelling agent. It is also used in medical and bacteriological laboratories as a culture medium for microorganisms.

More than 50 species of red algae have been used as raw materials in the manufacture of agar. Principal sources are species of *Gelidium*, *Gracilaria*, *Ahnfeltia*, *Pterocladia* and *Phyllophora*. In Japan, the largest amount of agar, is extracted mainly from plants of the genus *Gelidium*.

Durairatnam and Medcof[1] made a preliminary survey of the Sri Lankan sources of algae for the extraction of agar on an industrial scale. Durairatnam[2] after a systematic survey reported that *Gracilaria confervoides* and *Gracilaria edulis (lichenoides)* were found in large quantities, had a high gel content and could be used for the production of agar. We now report a preliminary study made of the feasibility of extracting phycocolloids from eight species of red algae collected in Sri Lanka and some physico-chemical properties of these phycocolloids.

2. Experimental Methods and Materials

The locality, habitat and distinguishing morphological features of the eight species of red algae studied are given in Table 1. Of the eight species studied, two species showed some biological variation. Brown and red thalli of *Gracilaria edulis*, and also orange and green thalli of *Hypnea musciformis* were collected. But for the extraction of phycocolloids these samples were bulked and replicate subsamples (50 g) were used. *Gracilaria corticata* is a polymorphic species but data reported in this paper refer to one form collected from Hikkaduwa.

The seaweed samples were washed and thoroughly sun dried (2-3 days) close to the site of collection. The problem of sample deterioration due to microbial activity was not observed. The dried samples were ground in a mill and extracted according to the procedure used by Durairatnam *et al.*[3] The sun dried product was stirred successively with ethanol and acetone.

The moisture content of the sun dried samples was determined by heating the samples at 110°C for 4 hours and the ash content obtained by incinerating the samples at 550°C for 5 hours. The water soluble Na^+, K^+, and Ca^{++} salts present in the ash of the algae and the agar, respectively, were recorded by using an EEL flame photometer.[6] The sulphate content was determined as $BaSO_4$ after a preliminary treatment with 0.5 N HCl. The gel strength, temperature of gel formation and the melting temperature of the gel were determined as described by Ramarao and Krishnamoorthy.[6]

3. Results and Discussion

Table 2 gives the percentage yield and colour of the phycocolloids isolated. The best yield of phycocolloid was obtained from *Gracilaria salicornia*. All other species of *Gracilaria* studied gave reasonable yields of phycocolloids. Of these only *G. salicornia* and *G. edulis (lichenoides)* can be gathered or collected in large quantities and hence be considered worthwhile for commercial exploitation.

Table 1 – Locality, habitat and morphological features of red algae

	Species	Locality	Habitat	Form and size of thallus
1.	*Gracilaria corticata*	Hikkaduwa	Protected calcareous reef	10 cm, flat, dichotomous, fastigiate cartilaginous thallus
2.	*Gracilaria fergusonii*	Hambantota	Exposed rocky boulders	ca. 7 cm, cylindrical, pseudodichotomous, cartilaginous
3.	*Gracilaria crassa*	Koggala	Calcareous reef	ca. 8 cm, cylindrical, dichotomous, succulent, arched, decumbent
4.	*Gracilaria salicornia*	Jaffna	Muddy lagoons	Grows in clumps, 20–30 cm wide, 10–15 cm high, greenish yellow, cylindrical with constrictions
5.	*Gracilaria edulis (lichenoides)*	Jaffna	Protected inshore lagoons	10–30 cm, high, cylindrical, tapers to a point, irregularly dichotomous, distichous brown/green
6.	*Acanthophora delile*	Jaffna	Lagoon	10–15 cm. cylindrical, covered with spiny outgrowths, drying black.
7.	*Gelidiella acerosa*	Jaffna	Intertidal reef	5–9 cm, cylindrical, alternate, pinnate, wiry and cartilaginous
8.	*Hypnea musciformis*	Jaffna	Protected inshore waters	ca. 8 cm, cylindrical, irregularly branched, tips of branches curled, membranous.

Table 2 – Moisture, ash and sulphate contents

	Species	Yield % of crude phycocolloid	colour of phycocolloid	Moisture (%)		Ash (%)		Sulphate (%)	
				Algal material	Phyco-colloid	Algal material	Phyco-colloid	Algal material	Phyco-colloid
1.	Gracilaria corticata ..	51·3	pale brown	14·86	18·40	15·61	6·77	2·71	2·93
2.	Gracilaria fergusonii ..	53·0	pale brown	15·25	13·70	8·76	8·37	2·63	5·15
3.	Gracilaria crassa ..	46·0	white	15·53	14·10	14·27	3·33	2·43	3·11
4.	Gracilaria salicornia (cast ashore) ..	51·0	white	14·86	12·50	12·27	3·37	1·88	2·25
5.	G. salicornia ..	62·0	white	13·43	16·80	14·54	4·04	1·92	2·43
6.	Gracilaria edulis (lichenoides) ..	47·0	off white	13·73	18·50	15·90	5·05	4·23	2·27
7.	Acanthophora delile ..	29·6	dark brown	16·38	21·50	12·97	14·00	5·55	10·25
8.	Gelidiella acerosa ..	48·0	white	15·91	13·90	12·78	11·29	0·62	0·79
9.	Hypnea musciformis ..	30·0	white	17·30	13·50	13·07	11·27	4·19	5·09
10.	Spanish agar ..	—		—	7·50	—	1·69	—	0·64

The moisture, ash and sulphate contents of the algae and the phycocolloids isolated are recorded in Table 2. The sulphate content of the phycocolloids from *Gracilaria fergusonii*, *Acanthophora delile* and *Hypnea musciformis* was noticeably higher than that of the other species investigated, while that from *Gelidiella acerosa* was significantly low and comparable to that of Spanish agar.

Table 3 gives the percentage of the water soluble sodium, potassium and calcium ions present in the ash from the algal and phycocolloidal material respectively. It is interesting to note that the ash of the phycocolloid from *G. fergusonii* and *G. crassa* has a very high concentration of potassium while those of *G. corticata* and *G. edulis (lichenoides)* is relatively low.

Table 3 – Water soluble Na, K and Ca contents of the ash

Species	Algal material			Phycocolloid		
	% Na	% K	% Ca	% Na	% K	% Ca
1. Gracilaria corticata ..	7·50	26·50	3·20	3·20	0·72	4·32
2. Gracilaria fergusonii	8·50	11·00	2·65	3·57	8·50	2·50
3. Gracilaria crassa .	9·00	11·00	2·80	3·60	8·50	1·85
4. Gracilaria salicornia (cast ashore) ..	12·50	27·00	3·10	1·80	2·00	1·80
5. G. salicornia ..	9·75	20·00	4·90	5·35	3·00	2·60
6. Gracilaria edulis (lichenoides) ..	7·80	19·25	2·55	2·70	1·50	0·92
7. Acanthophora delile ..	8·50	12·50	4·32	5·00	5·00	3·50
8. Gelidiela acerosa ..	15·00	26·00	4·10	3·10	2·60	4·60
9. Hypnea musciformis ..	17·00	4·50	5·85	4·50	2·50	1·95
10. Spanish agar ..	—	—	—	6·50	0·35	2·90

The gel strength, temperature of gel formation and the melting temperature of the gels formed by 1.0%, 1.5%, 2.0% and 2.5% solutions respectively, are given in Table 4. In general, species with high ash content also have a high sulphate content and correspondingly low gel strength, eg. *Acanthophora delile* and *Hypnea musciformis*. Further phycocolloids with a high content of water soluble potassium salts appear to form gels with better properties. Thus the phycocolloids from *G. fergusonii*, *G. crassa* and *G. salicornia* yield gels with a wider setting and melting temperature range. The low gel strength of the sample from *G. fergusonii* is probably due to its high sulphate content. The low potassium content of the phycocolloid from *G. corticata* may be responsible for its low gel strength. It may be possible to improve the setting properties of such a phycocolloid by the addition of a soluble potassium salt.[6]

Table 4 – Gelling properties of the phycocolloids

Species		1·0% solution			1·5 % solution			2·0% solution			2·5% solution		
		T_1 (°C)	T_2 (°C)	gel strength (l/cm2)	T_1 (°C)	T_2 (°C)	gel strength (g/cm)2	T_1 (°C)	T_2 (°C)	gel strength (g/cm2)	T_1 (°C)	T_2 (°C)	gel strength (g/cm2)
1.	Gracilaria corticata ..	27	38	* * *	31	43	3·72	36	46	7·69	39	48	13·52
2.	Gracilaria fergusonii ..	37	63	5·70	41	65	10·85	43	68	22·35	45	69	36·20
3.	Gracilaria crassa ..	37	55	16·21	43	60	64·14	44	63	139·85	46	65	281·77
4.	Gracilaria salicornia (cast ashore) ..	36	47	3·10	40	52	12·86	44	56	43·64	46	60	59·07
5.	G. salicornia (fresh) ..	35	46	6·10	43	55	53·57	45	60	76·24	47	67	148·87
6.	Gracilaria edulis (lichenoides) ..	40	49	13·18	42	55	88·39	45	58	122·36	46	63	262·21
7.	Acanthophora delile ..		No gel			No gel		30	53	6·27	36	55	12·53
8.	Gelidiella acerosa ..	33	41	3·83	38	52	10·54	41	58	43·64	43	62	34·55
9.	Hypnea musciformis ..	66	34	* * *	28	36	* * *	33	37	5·69	33	39	9·23
10.	Spanish agar ..	40	60	132·00	41	64	290·51	43	69	378·66	45	73	596·29

T_1 — setting temperature of gel
T_2 — melting temperature of gel
* Could not be determined.

The gel strength of a gel is determined largely by the amount of polymeric material which has precipitated in the form of a network. It has been reported that 3, 6–anhydrogalactose is less hydrophilic than galactose.[4] Therefore galactans having a high 3, 6-anhydrogalactose content yield very strong gels.[4] Furthermore, the presence of sulphate groups makes the galactans more soluble and thus lowers the gel strength and the melting temperature of the gel, while the presence of K^+ will lead to an increase in gel strength. This has been attributed to the fact that potassium salts of D-galactose sulphates are less hydrophilic than the sodium and barium salts of these compounds.[5]

A combination of these three factors together with the presence of inorganic impurities may be responsible for the variation in gelling properties observed during this investigation.

The potassium content of the Spanish agar sample is very low compared to that of the phycocolloid samples prepared in our laboratory. Hence the potassium content alone cannot markedly affect the gelling properties of these substances. The superior gelling properties of this sample is probably attributable to its 3, 6-anhydrogalactose content of the agarose fraction and low sulphate content. The absence of inorganic impurities which is reflected in the very low value for its ash content, is another factor which must be taken into account. Improvement of the gel forming ability and gel strength properties of the phycocolloids isolated is part of our future programme of research

4. Conclusion

Of the red algae studied *Gracilaria edulis (lichenoides)* and *G. salicornia* appear to be the most suitable for commercial exploitation.

Acknowledgements

We thank the National Science Council of Sri Lanka and the International Foundation for Science for financial assistance.

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