

## Preliminary Studies on the Alginic Acid and Agar contents of some Marine Algae

INDRANEE ARUMUGAM, A. SIVAPALAN AND K. THEIVENDIRARAJAH

Department of Botany, University of Jaffna, Thirunelvely, Sri Lanka.

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**Abstract:** Different methods of extraction of alginic acid from brown algae were tried out and it was found that treating with  $\text{Na}_2\text{CO}_3$  followed by bleaching powder gave alginic acid of better quality. Of the several species of brown algae studied *Cystoseira triquetra* (L.) J. Agardh and *Timbinaria conoides* (Kuetzing) were found to contain the greatest amounts of alginic acid. Investigations further showed that there was a marked seasonal variation in the content of alginic acid in these algae, highest being during the months of May to July. Extraction of agar was carried out in six different species of red algae and it was found that species of *Gelidium* (Lamouroux) *Hypnea* (Lamouroux) and *Gracilaria* (Greville) could be used for extracting agar. The amount of agar in these algae varied from 16.2% to 50%. There is a marked seasonal variation in the agar content in these algae. The content of agar was found to be high in these algae during January.

### 1. Introduction

Alginic acid and agar are two important commercial polysaccharides that could be obtained from marine algae. Algin is usually obtained from some brown algae and the alginic acid producing algae are generally called alginophytes. Similarly agar can be obtained from some red algae and the agar producing algae are called agarophytes.

Both alginic acid and agar are extensively used in Sri Lanka and annually large quantities of these products are imported. Agar is mainly used in confectionaries as a substitute for gelatine. It is also used as a culture medium for the growth of micro-organisms like bacteria and fungi. Alginic acid as its sodium salt is extensively used in textile industry.

No systematic studies have been made on the content of alginic acid and agar present in the marine species found in Sri Lanka. The present investigations were undertaken to estimate quantitatively the amounts of these substances in a number of marine algae found along the coast of Mandaitivu in the Jaffna Peninsula, described by Durairatnam<sup>1</sup>. Mandaitivu, is an island situated on the South West side of the Jaffna peninsula. It is about five miles from the main land and there is a rich flora of alginophytes and agarophytes along the coast of Mandaitivu.

## 2. Methods of Study and Results

### 2. 1. Extraction of alginic acid ;

Different methods used by Rao and Modi<sup>7</sup> and Rao *et al*<sup>8</sup> were tried for the extraction of alginic acid from brown algae

2. 1. 1. - Extracted with sodium carbonate and precipitated with dilute hydrochloric acid.

2. 1. 2. - Extracted with sodium hydroxide and precipitated with dilute hydrochloric acid.

In these two methods the extract contains sodium alginate. When dilute hydrochloric acid is added precipitation of alginic acid takes place. Here the product is soft in texture and dark brown in colour. Since the colour and texture of the product obtained by these two methods were not satisfactory another method was tried.

2. 1. 3. - Extracted with  $\text{Na}_2\text{CO}_3$ , precipitated with bleaching powder, added dilute HCl, until effervescence ceases. Here calcium alginate and calcium carbonate precipitate and then the latter is removed completely. In this method the alginic acid obtained is rubbery in texture and white in colour. Therefore the ultimate method adopted for the routine estimation of alginic acid content is as follows :

20 ml of 6% sodium carbonate solution of pH around 9 is added to 0. 5g dry wt of the algal material and kept at 50°C for nearly 3 hours for the material to become a pulp. Extract is filtered through a muslin cloth, saturated solution of bleaching powder is added until complete precipitation occurs. Dilute hydrochloric acid is added until  $\text{CO}_2$  effervescence ceases, product filtered, washed in water, dried at 50°C and weighed.

This method is very economical because of the cheap reagents used.

A quantitative study on the amount of alginic acid present in the following marine algae such as *Padina* (Adanson), *Cystoseira triquetra* (L.) J. Ag., *Turbinaria conoides* (Kuetzing), *Cystophyllum* (J. Agardh), *Turbinaria ornata* (J. Agardh), *Hydroclathrus* (Bory), *Sargassum polycystum* (C. Ag), *Sargassum whightii* (Greville), *Sargassum cervicone*, (Greville), *Stoechospermum* (Kuetzing), *Dictyota* (Lamouroux) and *Sargassum tenerrimum* (L. Ag.) was carried out periodically using duplicate samples at about 1½ to 2 months interval beginning from October 1977. The results of this analysis is given in Table I.

**Table 1** – Alginic acid content (percent dryweight) of different brown algae collected from Mandaitivu during the period of survey.

Species	October 1977	December 1977	January 1978	March 1978	May 1978	July 1978	August 1978
<i>Padina</i>	10% 8%	8% 10%	4% 4%	8% 10%	14% 14%	4% 4%	—
<i>Dictyota</i>	10% 12%	—	14% 16%	—	—	—	—
<i>Stoechospermum</i>	—	—	—	—	—	14% 16%	8% 8%
<i>Cystoseira</i>	—	20% 18%	14% 16%	24% 26%	33% 32%	32% 28%	18% 16%
<i>Cystophyllum</i>	14% 16%	10% 10%	10% 12%	18% 18%	—	10% 10%	10% 10%
<i>Turbinaria conoides</i>	28% 24%	20% 20%	12% 12%	—	—	—	8% 8%
<i>Turbinaria ornata</i>	—	—	6% 6%	18% 18%	20% 20%	28% 28%	8% 10%
<i>Hydroclathrus</i>	—	—	12% 14%	—	—	—	—
<i>Sargassum polycystum</i>	20% 20%	14% 14%	12% 12%	—	22% 22%	24% 28%	—
<i>Sargassum whightrii</i>	16% 14%	12% 12%	—	—	—	—	12% 10%
<i>Sargassum cervicone</i>	—	—	—	—	—	12% 14%	—
<i>Sargassum tenerrimum</i>	—	—	14% 16%	22% 22%	28% 30%	—	—

(—) indicates that the species is absent.

Results show that there is significant difference in the alginic acid content of different species tested. Also a marked seasonal variation is present in all species. Percentage alginic acid is high during the period May to July, and is low during December to January. Of the species tested *Cystoseira triquetra* contained the greatest amount of alginic acid. However there was no difference in the texture of the alginic acid extracted from the different species.

## 2. 2. Moisture content of different alginophytes.

The percentage moisture in the thallus of the different alginophytes that have been used in this study was determined in order to find out whether there is any relationship between the alginic acid content and moisture content. Moisture

content was determined by drying 10g of freshly collected algae, after surface blotting, in an oven at 80°C until constant weight is attained. Percentage moisture of different species during the period of survey is given in Table 2.

Table 2 - Percentage moisture (percent fresh weight) of the thalli of alginophytes used in this study

Species.	October 1977	December 1977	January 1978	March 1978	May 1978	July 1978	August 1978
Padina	58%	66.6%	61.5%	64.3%	61.3%	68.7%	—
Dictyota	66%	—	64%	—	—	—	—
Stoechospermum	—	—	—	—	—	—	—
Cystoseira	—	84.4%	78.3%	80.8%	79.2%	76.2%	74.5%
Cystophyllum	75%	78.5%	73.6%	68.7%	—	81.5%	83.5%
Turbinaria conoides	75.22%	75.22%	72.22%	—	—	76.2%	73.1%
Turbinaria ornata	—	—	66%	—	—	—	71.3%
Hydroclathrus	—	—	90.3%	63.2%	61.4%	75.1%	76.2%
Sargassum polycystum	79.4%	72.2%	71.0%	—	70%	72.2%	—
Sargassum whightii	70.6%	72.5%	—	—	—	—	—
Sargassum cervicone	—	—	—	—	—	—	76%
Sargassum tenerrimum	—	—	68.4%	64.3%	62.6%	72.2%	—

(—) indicates that the species is absent.

The results indicate that there is no significant correlation between alginic acid content and moisture content. There is variation among species in the moisture content, but there is no marked seasonal variation in any of the alginophytes tested.

### 2. 3. Extraction of Agar.

Agar was extracted according to the method described by J. F. Wood.<sup>4</sup> 2 g of dry sea weed is soaked in 50ml water overnight. Depending upon the species this is either boiled or autoclaved after adding 3-5 drops of dilute hydrochloric acid. The pH of the extraction medium is around 6. When the tissue turns into a pulp it is filtered, allowed to set, sundried and weighed. Extraction of agar was tried on five different species of red algae namely *Gelidium* (Lamouroux) *Gracilaria lichenoides* (L) Harvey, *Hypnea* (Lamouroux), *Acanthophora* (Lamouroux) and *Laurencia* (Lamouroux). Routine estimation was carried out periodically using duplicate samples to see whether there is any fluctuation of agar content. Results of this analysis is given in Table 3.

Results show that of the species tested *Gelidium*, *Gracilaria lichenoides* and *Hypnea* are quite suitable for the extraction of agar while *Acanthophora* and *Laurencia* cannot be used for this purpose. Seasonal variation is present in the agar content of all species tested. Generally agar content seems to be high during January and low during May.

Table 3 - Agar content (percent dry weight) of different red algae collected from Mandaitivu during the period of study.

Species.		October 1977	December 1977	January 1978	March 1978	May 1978	July 1978	August 1978
Hypnea	1	36.18%	—	46.1%	—	16.3%	47.5%	—
	2	35.8%	—	46.28%	—	16.1%	47.45%	—
	Av	36%	—	46.2%	—	16.2%	47.5%	—
Gracilaria lichenoides	1	36.3%	—	49.9%	35%	28.5%	32.2%	26.6%
	2	36.7%	—	50.1%	35.1%	26.4%	32.75%	26.4%
	Av	36.5%	—	50%	35%	27.5%	32.5%	26.5%
Gelidium	1	40%	23.2%	39.51%	32.8%	22%	24.5%	21%
	2	40%	22.75%	39.48%	31.16%	23.1%	25.4%	22%
	Av	40%	23%	39.5%	32%	22.5%	25%	21.5%
Acanthophora	1	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—
	Av	—	—	—	—	—	—	—
Laurencia	1	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—
	Av	—	—	—	—	—	—	—

*Hypnea* and *G. lichenoides* (—) indicates absence of species.

*Acanthophora* and *Laurencia* (—) indicates that there is no extractable agar in them.

#### 2. 4. Moisture content of agarophytes.

Percentage moisture of the different agarophytes used in this study is given in Table 4. The moisture content of the agarophytes was determined as described in 2.2. There seems to be no correlation between agar content and moisture content of these algae.

Table 4 - Moisture content per cent. fresh weight of different agarophytes used in this study

Gracilaria lichenoides	..	.	—	86.5%
Gelidium	..	..	—	70%
Hypnea	..	..	—	87.1%
Acanthophora	..	..	—	88.4%
Laurencia	..	..	—	88.1%

#### 2. 5. Abundance of the alginophytes and agarophytes.

An eye estimation of the abundance of different alginophytes and agarophytes was carried out during different months from October 1977 to August 1978. Results are given in Tables 5 and 6.

Results indicate that certain alginophytes and agarophytes such as *S. tenerrimum*, *Acanthophora*, *Laurencia*, *Gelidium* are present throughout the year although the relative abundance during different months vary. Others such as *Cystoseira*, *Cystophyllum*, *S. polycystum* are present during most of the months and are completely absent during some periods of the year.

Table 5 - Relative abundance of alginophytes found at Mandaitivu during different periods of the year.

Species.	October 1977	December 1977	January 1978	March 1978	May 1978	July 1978	August 1978
<i>Pedina</i>	+	++	++++	++	+++	+	---
<i>Dictyota</i>	++	---	+	---	---	---	---
<i>Stoechospermum</i>	---	---	---	---	---	+++	+++
<i>Cyroseira</i>	---	++++	+++	+++	+++	+++	+
<i>Cystophyllum</i>	+++	+++	+++	++++	---	+++	+
<i>Turbinaria conoides</i>	+++	++++	+	---	---	---	+++
<i>Turbinaria ornata</i>	---	---	+++	---	---	+++	+
<i>Hydroclathrus</i>	---	---	+	---	---	---	---
<i>Sargassum polycystum</i>	++	++	++++	---	+++	+++	---
<i>Sargassum whightii</i>	+++	++	---	---	---	---	+++
<i>Sargassum cervicorne</i>	---	---	---	---	---	+++	---
<i>Sargassum rennerianum</i>	---	---	+	+++	+++	+++	---

(-) Indicates absence of the species.

++++ indicates the most abundant species.

+

Indicates that the species is present in trace amounts.

The population of the other species lie inbetween these two.

Table 6 - Relative abundance of red algae found at Mandaitivu.

Species	Oct. 1977	Dec. 1977	Jan. 1978	Mar. 1978	May 1978	July 1978	Aug. 1978
Hypnea	+	—	++++	—	++	+++++	—
Gracilaria lichenoides	+++++	—	++	++	+++	+++	+++++
Gelidium	++	++	++	+	+++	+	+
Acanthophora	+++++	+	+	++++	++++	+++	+++++
Laurencia	++++	++++	++++	++++	++++	+++	+++++

+++++ — The most abundant species.

— Species present in trace amounts.

The relative abundance of the other species lie inbetween these two.

(—) indicates absence of the species.

Species such as *Hydroclathrus*, *S. whightii*, *S. cervicone*, *T. ornata* appear only during certain months of the year.

### 3. Discussion.

Alginic acid and agar could be produced in small cottage industries in Sri Lanka since the natural resources necessary for this purpose are available in plenty. The crude agar obtained by the method of extraction mentioned here could be utilised in certain microbiological work. The crude agar preparation sets well at 1.5 percent. This setting property is not affected at pH ranging from 3 to 9. The alginic acid obtained in our studies is a white pulp of gelatinous nature pH being approximately 6. The technique of drying this product without any damage to the nature of it has not been perfected yet. But the alginic acid could be stored in a refrigerator in this fresh form for more than two months without any change. Further investigations have to be carried out to study the feasibility of commercialising these two products. Also experiments have to be carried out to cultivate these algae in larger quantities in order to make the production of agar and alginic acid more economical.

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