The seasonal variation in the water-soluble constituents of Gracilaria lichenoides collected from Palk Bay has been studied. An economic method has been worked out for the extraction of agar from the seaweed.

The quality of an agar sample is generally determined by its ash and nitrogen contents, gel strength and setting temperature at a definite concentration. The major industrial agarophytes yield 30-50 per cent agar (dry weight basis).

The red seaweed, Gracilaria lichenoides (L) Harv., growing abundantly in many areas on the Indian coast, has been listed as one of the important agar-bearing seaweeds of India by Thivy. Efforts have been made previously in India to work out a suitable method for the efficient and economic extraction of agar from this and other species of the genus. Bose, Karimullah and Siddiqui studied Gracilaria sp. from the coastal regions of Travancore and proposed a method which requires freezing for obtaining a good quality agar. Introducing for the first time the bacterial method, Karunakar, Sanyasiraju and Varadarajan were able to prepare a standard agar from G. lichenoides. These earlier attempts have indicated that the impurities in Gracilaria agar cannot easily be removed. In view of the work on the seasonal variations in the organic constituents of seaweeds conducted in the United Kingdom, the yields from the material without seasonal classification as in previous work on agarophytes of India cannot be taken as final.

Studies on the seasonal variations in the chemical composition of some of the common seaweeds of the Indian coast were started by the author in 1952. The data obtained for Gracilaria lichenoides suggested a more economic method of agar manufacture entirely different from the methods hitherto followed elsewhere as well as in India for the extraction of the product besides having provided information on the seasonal variation in the agar content of this species.

Materials and methods

Samples of G. lichenoides were collected once a month from Palk Bay near Mandapam from April 1952 to March 1953 from the same place. These were washed and sampled as recommended by Black and used for analysis.

For the determination of the water-soluble inorganic constituents 10 g. of the fresh seaweed, immediately after collection, was ground well with acid-washed sand and extracted with a known quantity of water. The extraction was repeated three times using minimum quantity of water. The details of the final analysis of the individual components are the same as given in an earlier communication. For all other determinations the air-dried sample was used. Data were collected on the seasonal variations in nitrogen, sulphur, carbohydrates and inorganic compounds in the seaweed.

Results

Inorganic components — Table 1 gives the percentage of the various major inorganic compounds present in the water-soluble portions of the different collections of the seaweed. The results show the great variations in the percentage of these elements (sodium, potassium, calcium, magnesium and chlorine). The amount of each element present in the water extracts constitutes between 60 and 90 per cent of the total amount of the element in the seaweed (unpublished data).

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Trace elements — Only two trace elements, viz. manganese and boron, were found in the water-soluble fraction of the seaweed collected during August and December respectively. During August the plants are mature and fruiting while during December they are young and non-fruiting. The collection taken in August contained 170 mg/kg of manganese in the water-soluble form, representing 31 per cent of the total manganese in the plant, and 0·9 mg/kg of boron, representing 7·5 per cent of total boron, whereas the collection during December contained 120 mg/kg and 0·4 mg/kg respectively of manganese and boron, representing 60 and 9·5 per cent of the total of the elements in the material.

Partition of nitrogen — The total protein, water-soluble, oxidized inorganic and volatile nitrogen contents of the seaweed during the different months are given in Table 2. The results indicate that all collections contain a certain quantity of water-soluble nitrogen which varies from month to month. This variation follows the season and the growth stages of the plants. The value for the total water-soluble nitrogen is high in the young plants, i.e. during October to January, while it is low in mature plants collected between July and September. When the seaweed is macerated and leached with water, every form of soluble inorganic and organic nitrogen compounds is leached out and it results in a marked reduction in the nitrogen content of the material. It may be seen from the results in Table 2 that the amount of soluble nitrogen varies with collections made during different seasons. The minimum value is recorded for the collection made in April (0·187 per cent of the dry matter) while the maximum value (0·7 per cent) is recorded for the December collection. Fig. 1 shows that the amount of water-soluble nitrogen more or less follows the changes in the total nitrogen content of the seaweed.

![Figure 1](image-url)
Partition of sulphur — Table 3 gives the values for the total sulphur, total sulphate and ionic sulphate in seaweed collections made during different seasons. It may be seen that a good percentage of the sulphur present in the seaweed is in a water-soluble form (up to a maximum of 50 per cent). Fig. 2 shows that except in the March collection, the ionic sulphate content of the seaweed varies with the total sulphur content.

Soluble carbohydrates — The percentages of simple sugars, total cold-water-soluble carbohydrates and total hydrolysable carbohydrate in the seaweed collected during different months are given in Table 4. On the average, 20-35 per cent of the total carbohydrate exists in a cold-water-soluble form in the seaweed during all stages of growth.

Thus a good percentage of the nitrogenous matter and carbohydrates in the seaweed, the separation of which forms the major problem in the refining of agar, is present in a water-soluble form and can be leached out almost completely by macerating the material with sand and water. This observation suggests an improved method for the preparation of pure agar from the seaweed in which the removal of the impurities is effected from the seaweed itself and not from the gel as in other methods. In the methods now used for agar manufacture, refrigeration and adsorption are the chief techniques employed to remove the impurities from the agar gels. The crude agar gel is likely to contain a major portion of the water-soluble components when the extraction is made from seaweed samples without prior treatment. In these methods the seaweed was subjected to thorough cleaning, mechanized washing, prolonged soaking and bleaching in the sun which serve only to remove minor proportions of the various impurities. The principle involved in refrigeration of the agar gel in the existing methods is to expel some of the water from the gel thereby removing most of the water-solubles. As it is very difficult to handle the material at the gel stage, because of its pliability and softness, all the water-soluble impurities may not be removed from the gel. Thus the products may contain varying amounts of these impurities in different samples prepared from the same source and have to be tested every time.

Purity of agar obtained by the improved method — The improved method of agar manufacture is a modified leaching process which will cut down the cost of production compared to the processes now employed, since no chemicals or diatomaceous earth are used. In order to arrive at the optimum conditions for the extraction of agar from
G. lichenoides by this method, several trials were conducted. The properties of agar samples prepared from the seaweed after grinding and without grinding the seaweed, after leaching the weed for different periods and by allowing the gel after extraction to coagulate, have been studied. The results of some of the trials are given in Table 5. The effect of drying the gel in direct sun and over an electric oven was also studied. With leaching was more effective. Leaching for 8 hr. brings down the ash content of the material to 4-5 per cent, while in the two samples leached for 24 hr. the ash content was 3-2 per cent. The nitrogen content also is brought down to below 0-2 per cent. A sample of agar prepared by the method of Bose et al. from a sample of seaweed collected during September analysed as follows: ash, 7-79; nitrogen, 0-253; and moisture, 21 per cent. A comparison of these values with the values given in Table 5 reveals that the leaching technique is as effective as the refrigeration and coagulation method of Bose et al.

Fig. 3 represents the changes in the agar content of G. lichenoides during the different months of collection as well as the changes in the water-soluble carbohydrates. The values for agar content plotted in the graph are obtained by the conventional method of refrigeration and coagulation. The maximum agar content of the seaweed according to this is c. 34 per cent in April. But the values given in Table 5 show that a considerably higher yield can be obtained from the same seaweed by the improved technique. The yield increases to 45-50 per cent. Earlier workers have reported yield of 20 per cent refined agar for Gracilarea lichenoides obtained from Indian sources and 25 per cent from Gracilaria confervoides of Australian origin.

Description of the improved method

The following is a brief outline of the various steps involved in the manufacture of agar from G. lichenoides for adoption on a cottage industry scale:

1. The beach-dried seaweed was washed in soft water to free it from occluded particles, soaked in water for 2 hr. and spread out in the sun to dry. The material was sprinkled with water once or twice during drying to aid bleaching. The dried material was stored in baskets in a well-aerated place.

2. The dry seaweed was cleaned by gentle abrasion in a stone mortar with addition of water in the proportion 15:1 (by weight); the water was changed thrice.
3. The seaweed was allowed to soak for 24 hr. in water, the proportion of moist seaweed to water being 1:15 (by weight). The material was finally strained through cloth.

4. The soaked seaweed was crushed in a stone mortar to a pulp, with the addition of a minimum amount of water.

5. The pulp was lixiviated for 24 hr., the proportion of pulp to water being 1:10 (by weight).

6. The pulp was then churned for 10 min. in the water using an egg whisk or a household churning device, and finally strained through organdy cloth. The pulp was dried on the same cloth by spreading it on a bamboo basket-work tray and leaving it on a trellis work in the sun.

7. The dry pulp was next added to the second extract or mother liquor (the second extract is made by adding requisite amount of water to the residue from the first extraction and heating it to 90°-95°C. over a water bath) in an enamel vessel and heated at 90°-95°C. over a water bath, the proportion of dry pulp to mother liquor being 1:75 (by weight). This step is necessary to avoid the fine particles of the pulp getting suspended in the agar sol. The extraction of the pulp was continued for 15-30 min. at the same temperature. The suspension was stirred for 4 min. at the start.

8. The extraction vessel was kept in a closed chamber or box for the supernatant sol to coagulate gradually (gelation temperature of the sol prepared thus is 43°C.) and for the suspended matter to settle down.

9. The clear gel was removed with a large metal spoon, the gel cut into strips and placed on fine-meshed galvanized wire-netting frames in the sun for drying. The dried agar strips peel off easily if they are handled directly after drying in the sun.

The final product obtained by this method is clear, white and shining, analysing to: moisture, 18-0; ash, 3-0; and nitrogen, 0-19 per cent; gel strength for a 2 per cent solution, 180 g.

The possibility of extending this technique to other agarophytes is at present being investigated at the Algology Section of the Central Marine Fisheries Research Station, Mandapam.

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References