EXTRACTION OF ALGINIC ACID FROM LOCAL BROWN ALGAE

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ALGIN EXTRACTION

HAGOPIAN

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A B S T R A C T

This study deals with the determination of the alginic acid content of three genera of brown algae, Sargassum, Padina and Fucus, that grow along the seashore of the American University of Beirut. Three different procedures of extraction were employed. The procedures differed from one another as to the thermal conditions to which the weeds were subjected. Several additional steps, such as treatment with alcohol, ether, acetone and NaOCl, were also tried on Procedure 3 with the purpose of improving the purity of the alginic acid yield. All the products obtained were analyzed as to their chemical and physical properties, viscosity and purity. After evaluation of the procedures and the purification treatments, Procedure 3 was employed with three additional steps of treating the weeds and wet alginic acid with alcohol and bleaching the sodium alginate solution with NaOCl.

Of the three brown algae studied, the most promising source of alginic acid was found to be Sargassum linifolium because of its high yield (16-19%) and purity (92.2%-). Lower percentage yields (14%), but high purity (92.6%) were obtained from Padina while Fucus yielded the least pure (86.2%) products.

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PART I

INTRODUCTION

In recent years interest has been directed towards the use of seaweeds as a source of raw materials. The list of products that can be obtained from marine algae is long but several years ago its use was limited to only a few. Even though the method of preparation of several of these products has been known since the beginning of this century, their full exploitation was achieved only recently. Algin, a phycocolloid prepared from brown algae, has proved to be of major importance in the utilization of seaweeds, especially in the United States of America, where it is manufactured in greater quantities than any other algal product. What has proved to be of such major economic importance for certain countries could be the same for Lebanon. The brown algae of Lebanon, unlike those of other countries, are mainly rockweeds which are smaller in size and difficult to harvest

The purpose of this study is to examine the possibilities of the Lebanese rockweeds, in particular of Sargassum linifolium (Turn.) J.Ag. as a raw material for algin production. The study also includes an evaluation of certain procedures of extraction as to yield and purity when Sargassum is used because it is more difficult to obtain pure algin from rockweeds than from the large bottomweeds generally used.

PART II

HISTORICAL REVIEW

Algae in General

In taxonomic study algae are placed at the bottom of the evolutionary tree. They differ from higher plants in having no special organs such as, true roots, stems and leaves. Even though they are primitive and simple plants, they perform "well-defined functions"; the most significant of which is photosynthesis. As a group they bind inorganic matter into organic form ten times more than the higher plants.[67]

The algae are divided into seven major divisions based upon their cell structure, reproductive processes and pigmentation. The divisions are Chlorophyta, Euglenophyta, Pyrrophyta, Chrysophyta, Phaeophyta, Cyanophyta and Rhodophyta.[46] As to habitat, algae are found in water, and on land, where they appear in soil, on rocks, and on tree trunks. Symbiotically, they live with fungi, within certain Protozoa and in the intestinal tracts of higher animals.[67] But they occur more abundantly in fresh and salt waters. The marine habitat of the algae can be divided into pelagic and benthic regions. The algae of the pelagic region are the phytoplankton that are microscopic in size and occur in free floating masses. Those of the benthic region are usually attached to the sea bottom and are commonly referred to as seaweeds. Chlorophyta, Rhodophyta and Phaeophyta comprise the majority of the seaweeds. Even though algae do not have special organs, the seaweeds have root-like structures called holdfasts by which they are firmly attached to the sea bottom.

They also develop a stem-like structure, the stipe, and leaf-like flattened parts, the laminae.[12] Since sand and mud are unfavourable for firm attachment, seaweeds are usually absent from such substrates except in quiet bays where there is very slight wave action. In regions of high waves, they are firmly attached to rocky bottoms of either smooth or rough surfaces.[15]

According to their place of occurrence, seaweeds can be divided into rockweeds, bottomweeds and castweeds. The rockweeds are found on rocks between high and low tide lines in the temperate regions. Consequently for long periods of time, they are exposed to the air. Most of the rockweeds are members of the order Fucales, such as Ascophyllum nodosum, Fucus serratus and Fucus vesiculosus. The bottomweeds are beyond the tide line, in deep waters. Sometimes they grow to such giant sizes that their laminae reach the surface of the water while they are still attached at the base. Examples of bottomweeds are the several species of Laminaria. Besides these two, there are the castweeds which are thrown to the seashore by strong currents. Commercially, castweeds are used as a major source for several products.[11] Examples of castweeds are Laminaria digitate and clausti.

The structure of seaweeds depends upon the following: the material which composes the cell wall. The cell wall contains little cellulose. Instead mucilaginous and gelli-fying substances cement the cells together. These are the commercially important substances. The unit of construction which is the branched thread or the filament, not a tissue.

And lastly, the design according to which the threads are interwoven to produce different structures.[16]

The brown algae are of direct concern to this study. The members of Phaeophyta are the largest in size, having no unicellular forms. They are also well-organized and have well differentiated parts. They occur in cool and temperate seas with the majority of their members growing in between the tide-marks. The pigments that play a role in photosynthesis are chlorophyll a and another green pigment which Strain and Manning[5] call chlorofucine. They consider it to be different from chlorophyll c. Besides the green pigments there are the carotenes and the xanthophylls.

The organic composition of brown algae varies according to the species, the geographic location, the depth of water and the part of the plant. A very general analysis shows the following ranges of concentration of organic and inorganic substances:[61]

SUBSTANCES	PERCENTAGE
Carbohydrates	
Manitol	3-27
Alginic acid	11-33
Laminarin	0-36
Fucoidin	2-11
Cellulose	1-10
Proteins	3-16
Oils, fats, waxes	1.8-3.6
Ash	14-46

On the basis of the life cycles, the Phaeophyta is subdivided into three classes:

1. Class Isogeneratae shows similar alternating gametophyte and sporophyte generations. It includes the following five orders: Ectocarpales, Sphacelariales, Tilopteridales, Cutleriales and Dictyotales.

2. Class Heterogeneratae members have a large sporophyte generation alternating with a microscopic gametophyte generation. It includes the following six orders: Chordariales, Sporochnales, Desmarestiales, Punctariales, Dictyosiphonales and Laminariales.

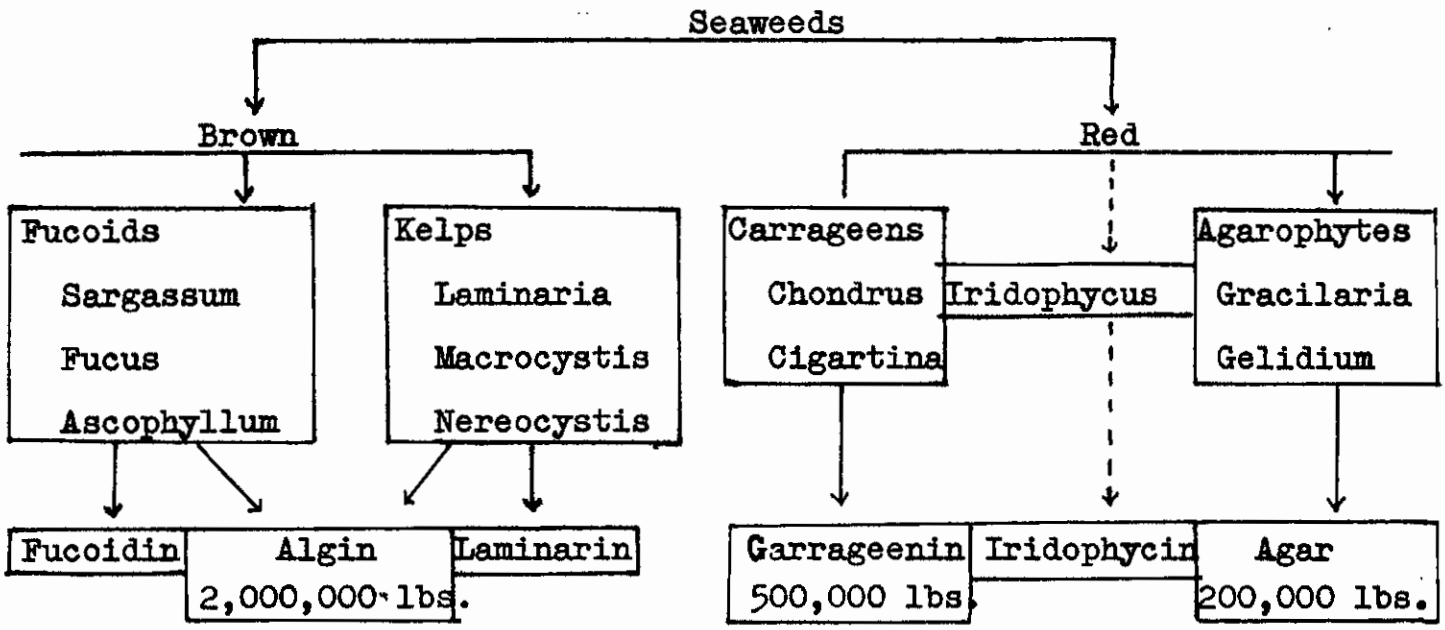
3. Class Cyclosporeae members have only the macroscopic sporophyte generation. It includes only the order Fucales.[46]

Since early times, seaweeds have been utilized by man. Their uses can be divided into the following:

1. Use as fodder and as fertilizer by some farmers, because of its high mineral content.

2. Use as food in the Far East where delicacies are prepared from some seaweeds. Their nutritional value is not well known. However, they provide minerals and act as roughage.

3. Use in the preparation of certain commercial products like potash, agar agar, alginic acid and glue.[16] The following scheme prepared by Tseng [55] shows clearly the great use made of seaweeds commercially.



Note. The figures in lbs. of agar, algin and carrageenin refer to the yearly production of each in the U.S.A.

The above-mentioned scheme clearly brings forth the commercial importance of seaweeds. The great amount of algin (3 tons world output in 1955)[53] extracted from brown algae indicates its industrial significance. In the following pages a short review of the discovery, methods of extraction, occurrence, chemistry and industrial potentiality of algin will be presented.

E.C.C. Stanford, an English chemist, discovered that some seaweeds contained a gelatinous material which was responsible for their firm structure.[34] He came across this by-product in 1883 when he was trying to improve the yield of iodine from seaweeds near Scotland. He extracted this subs-

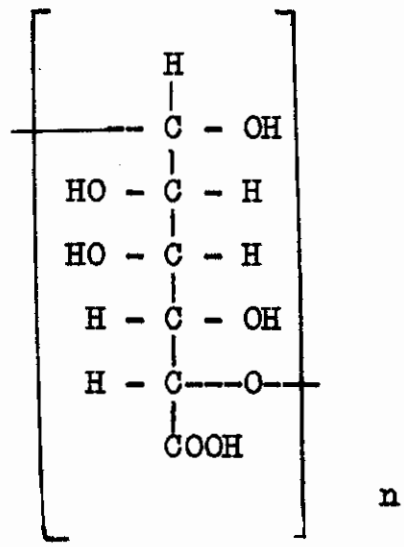
tance with sodium carbonate digestion, particularly from Phaeophyta. He named it algin, after the word alga which means seaweed in Latin.[30] In the following years (1883-1887), a great deal of experimental work was carried on for better methods of extraction, for its physical and chemical properties and possible uses. 34 But it was first sold on the market in 1910 [53] and for its full exploitation it had to wait another twenty years.[61] Today, by the suggestion of Tseng, the word algin refers only to the soluble sodium alginate, the first product in the process of manufacture. Alginic acid is obtained when algin is treated with a mineral acid and all the other alginates are derived from it with treatment of their respective salts.[56]

Chemistry

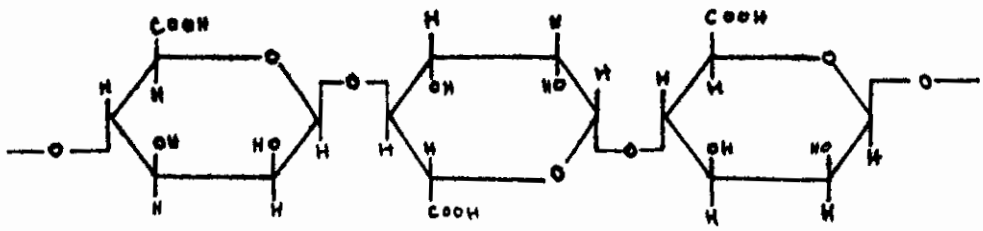
Since the discovery of algin, many workers have studied its chemical nature. The investigations were pioneered by its discoverer Stanford (1883-6) who proposed $C_{76}H_{76}O_2\cdot(NH_2)_2$ as the empirical formula. He was mistaken in placing nitrogen as one of the chemical constituents of the molecule. His erroneous conclusion was due to the presence of impurities. Unlike Stanford, Krefting's (1896-1898) analysis of a similar product extracted from seaweeds in Norway showed no nitrogen constituent. Alginic acid was first prepared in pure form by Hoagland and Lieb [26](1915). Its study led them to the correct conclusion that alginic acid was nitrogen free. The empirical formula given by them as $C_{21}H_{27}O_{20}$ with two re-

placeable hydrogens, and the molecular weight of 599 proved later to be incorrect. Nevertheless, they were the first to attempt hydrolysis with HCl. Their claim to have obtained pentoses, xylose and arabinose, was erroneous. After them, other workers used hydrolysis to identify residue units of alginic acid. Schmidt and Vocke (1926) used formic acid and H_2SO_4 for hydrolysis and obtained glucuronic acid.[8] While Nelson and Cretcher (1930) obtained D-mannuronic acid. They concluded that alginic acid was a polymer of D-mannuronic acids with a free carboxyl group and a bound aldehyde group. By methylation experiments they also established linkage as 1-5 (pyranose).[36]

After a critical analysis of the results of the former workers, Bird and Haas [8] (1931) agreed with Nelson and Cretcher that on hydrolysis a mannuronic acid was obtained, and disproved the presence of pentose constituents claimed by Hoagland and Lieb. In 1947 Spoehr,[49] after developing a reliable and easy method of hydrolysis with formic acid, conclusively established the presence of D-mannuronic lactone. Dillon (1938) stated that this mannuronic acid existed in the hydrated form, $(C_6H_8O_6.H_2O)n$ where n=80-83. The structural formula put forth by him is the following:[12]



In opposition to Dillon, Lunde et al [12] (1938), Hirst[24] (1939), March [12] and Speakman [12] (1944) claimed that the molecular structure was in the anhydrous ring form $(C_6H_8O_6)_n$ Hirst et al,[24 25] also proved that C_2 and C_3 were free and hence the glycosidic ring is either 1:4 (furanose) or 1:5 (pyranose). The pyranose ring seemed highly probable because of the stability and large levorotatory value of alginic acid. The findings of the above mentioned workers were confirmed by Astbury[2] (1945) with the help of x-ray diagrams of regenerated alginic acid fibers. Barry, (1954), on the other hand, used chemical methods to confirm the proposed ring structure.[53] Chanda et al[10] (1952) think that alginic acid is made of 100β-D-mannuronic acid residues arranged in a straight chain. The following formula represents the results of the later workers.[12]



1. C_2 and C_3 have hydroxyl groups attached to them[24].
2. The glycosidic linkage is 1-5 (pyranose). [24]
3. The carboxyl group is free to react while the aldehyde is not.
4. The chain is linear or slightly branched.
5. Alginic acid is very similar to cellulose in configuration.

Properties and Reactions

The industrial importance of alginic acid and its derivatives depends on their physical and chemical properties. Several of these properties will be mentioned below.

Alginic acid is usually obtained from sodium alginate solutions by precipitation with strong acids. It is insoluble in cold water, most organic solvents (alcohol, ether and glycerol) and is slightly soluble in dilute alkali and boiling water.[26] When moist, it can absorb 200-300 times its weight of water. While when dry it becomes a horny and fibrous substance. It is a weak acid but can liberate CO_2 from carbonates like other acids.[52] Alginic acid cannot reduce Fehling's solution but after hydrolysis it gives a positive test.[63] The hydrolysis is brought about by mineral acids and heat.[26]

Chemically it is very reactive. It forms water soluble salts when treated with compounds of alkali metals and those of Mg and NH_3. While its alkali-earth salts are insoluble in water.[35]

The salts of alginic acid can be divided into two ca - tagories: water soluble (Na, K, Mg, NH_3) and water insoluble (Ca, Co, Cu, Be, Zn, Al).[42] The water soluble alkali salts are odorless, tasteless and white or slightly yellow in color.[12] Industrially their most widely used member is sodium alginate which usually is the first product obtained during extraction. It can be obtained in powder form by addition of excess alcohol as it is insoluble in organic solvents. Sodium alginate is used as an emulsifier, stabilizer and suspending agent because it is compatible with glycerine, gums, sugar, starches, proteins, soaps, wetting agents, some acids and alcohol up to 25% concentration.[30] Like alginic acid, sodium alginate is very reactive. It reacts with NH_3, Mg and ferrous ions to form water soluble salts and with heavy metals to form water insoluble compounds. The water insoluble salts when moist are plastic and can be moulded. After drying they set hard. Also their property of water resistance is used by production of thin flexible films.[42]

Two other important derivatives of alginic acid are alginic acid acetate [66] and alginic acid proplene glycol ester.[3] The first has great adsorbing properties while the second has emulsifying, thickening, stabilizing and suspending capabilities.

The water soluble salts are hydrophilic colloids and form viscous solutions. The degree of viscosity depends upon

method of preparation, temperature, presence of foreign materials and concentration.[20 30] A knowledge of the factors that affect viscosity is essential as a change may "impair its usefullness" in several industrial products.[44] Solation and gelation of these viscous solutions do not depend on temperature. Change from sol to gel occurs by addition of heavy salts like $CaCl_2$.[63] The degree of thickening can be regulated by the quantity of calcium ion added. Intermediary degrees of consistencies can be obtained. The gels are stable and do not bleed.[22] All these solutions must be protected against bacterial attack by preservatives such as formalin, sodium benzoate, parahydroxybenzoate esters etc.[4] As shown by several workers, [64,65] bacteria decompose algin both in the sea and in purified preparations. The action of bacteria is hydrolyzing rather than oxidizing.[1]

Sources and Production

The countries that produce most of the algin in the world are the United States of America and England. Lesser quantities are produced by Norway, France and Japan. The United States make use of the brown seaweeds growing along its two coasts. On the Atlantic coast, Laminaria digitata (horsetail kelp) and Laminaria saccharina (broadleaf kelp) are the main raw materials used;[58] while on the Pacific side, Macrocystis pyrifera (giant kelp) is the main source. The major kelp sources used by the Europeans are Laminaria saccharina and L. digitata.[30] The British also make use of the

casteweed. Even though India and Australia do not produce algin in great quantities, surveys of their resources have been conducted. Valsan[59] has measured the percentage concentration of the five commonest genera: Sargassum, Tubinaria, Homophysa, Cystophyllum and Padina, of the Gulf of Mannar area in India. The yields of Sargassum and Tubinaria were found to be equivalent to those of Macrocystis of the United States of America. According to him, it is wise to exploit all of them except Padina. Womersly[68] claims that Australia has extensive resources, although there is no algin production yet. According to him the best potential sources are Macrocystis pyrifera (21%) and Durvillea potatorum. Besides these large bottomweeds, rockweeds have been investigated and used. The commonest ones are the different species of Fucus, Ascophyllum and Sargassum.[63]

People have developed several ingenious methods of survey for seaweed resources as well as for their collection. For rockweeds growing at the tide-line, calculation of amount can be done by simple observation. For weeds that are always covered with water, aerial photography and the echo-sounder indicate dense growth. Another method is collection of samples by grappel from a boat. For accurate results, it is advisable to use a combination of methods.[11] Methods of collection depend upon the habits of the plant. The giant kelps with large floating fronds are collected by a mechanical harvester which cuts the weeds and collects them with an elevator. In Europe, where the weeds are not of the floating type,

they are hauled up with a grapple from 12-15 ft.[54] The British also use trawls to cut the weeds and then collect them in large nets.[12] In France, for harvesting, fishermen use long poles to which knives are attached.[54] While the Japanese tear and pull them up by hooks and prongs.[27] Of course there is always the method of collecting by hand.

Yields and Variation

Much work has been done to determine the percentage concentration of algin in different species of brown algae. In general the percentage of alginic acid content is high. The following are a few examples. The first series were prepared by Lunde[12] (1937-1938).

Laminaria digitata	15-40%
Laminaria saccharina	15-35%
Ascophyllum	20-30%
Fucus serratus	18-28%
Fucus vesiculosus	18-28%
Macrocystis	14-19%

Nereocystis leutkeana concentrations were found to be 15-21% by Sager and co-workers[41] (1946). Davies[14] (1950) has found a range of 7-17% in different species of Sargassum. The following are the mean values determined by Valsan[51] in India.

Sargassum	17.5%
Turbinaria	16 %
Homophysa	16.8%
Crystophyllum	13.5%

Padina 8.4%

All the determinations were made on dry basis. The above mentioned are enough to indicate the high percentage composition of alginic acid of the brown seaweeds.

The studies of yields confronted the workers with the problem of variation. They noticed that changes in percentage concentration occured according to habitat, locality in the world, latitude, depth of water, parts of the plant and seasons of the year. A great deal of data is available for the last two but not much work has been done for the rest. Sager et al[41] determined yields of stipes and of fronds separately of Nereocystis leutakeana. They found that stipes had 15.33%, while the fronds had 20.96% concentration of alginic acid. Moss[37](1950) has made extractions from three different parts of the Fucus plant. According to her, the highest concentration occurs in the sterile tips, next in receptacles and the lowest occurs in parts below the receptacle. She[38] concludes that alginic acid increases in concentration from basal to distal regions of the thallus. The high percentage of alginic acid at the tips seems to be protective against the slashing of violent waves. Same kinds of studies were carried on Himanthalia elongata by Jones.[28,29]

Seasonal variations were first observed by Ricard (1931) and Lunde (1937). They found that the content reached a maximum between September and November.[12]

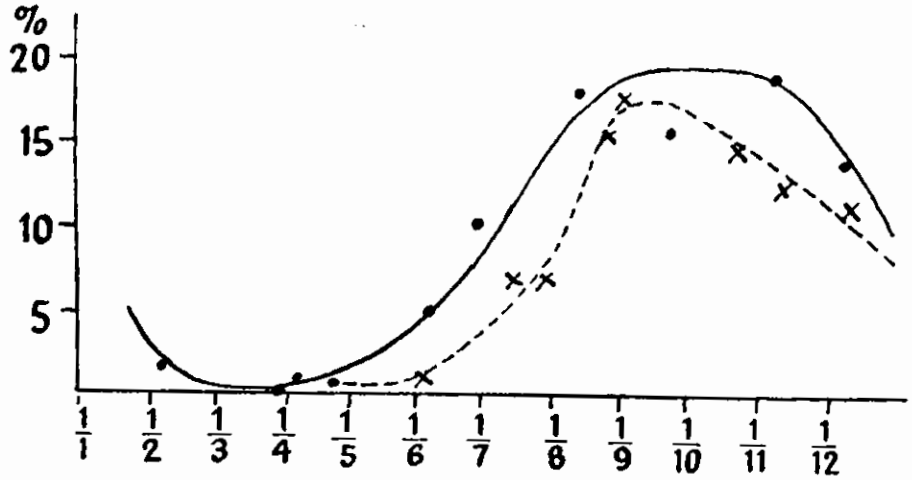


FIG. 40. Seasonal variation of Algin in *Laminaria digitata* (After Lunde)

Black[9] (1948) made percentage determinations in different seasons for stipes and fronds separately. He found that the perennial stipe had almost a constant alginic acid content, while the annual frond showed a lot of variation. Fucus species and Ascophyllum nodosum yields were measured by Macpherson et al.[32] (1952) between July 1949 and July 1951. In all maximum concentrations occurred between November and March, after which a sharp fall occurred to a minimum in May.

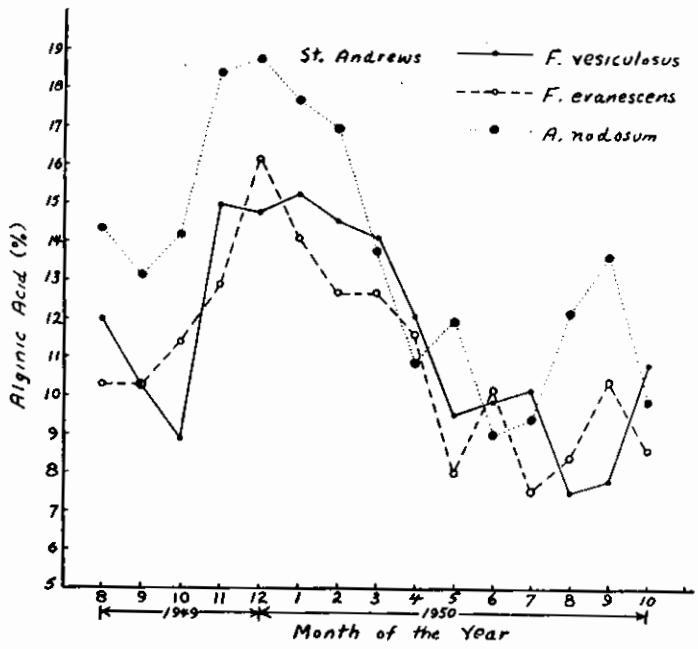


FIG. 5. Seasonal variation in composition of alginic acid at St. Andrews, N.B., 1949-50, as percentage of total solids.

Seasonal fluctuations are ascribed to metabolic activities. Alginic acid synthesis is supposed to be related to mannitol synthesis via mannose, the presence of either depending upon the temperature. In warm weather mannitol accumulates while in cold weather alginic acid synthesis is favored.

Mannitol ⟶ mannose ⟶ mannuronic acid ⟶ alginic acid

Mannitol is the main product of photosynthesis in brown algae. Hence anything that affects photosynthesis such as light, CO_2 concentration, O_2 concentration, pH, salinity, nutrient amount, will produce a change in mannitol and alginic acid concentrations.[69]

Occurrence in the Cell

The specific location of alginic acid is regarded to be the cell wall. Kylin (1915) was the first to suggest that algin was a major constituent of the cell wall.[26] The cell wall of brown algae is of two portions; an inner firm region of cellulose and an outer gelatinous part of algin.[46] Moss[38] in 1948 stained Fucus vesiculosus sections before and after algin extraction. She found that the cell walls of cortex of stipe were mainly of cellulose. Apparently, the frond cell walls contain most of the algin.

The exact chemical state of algin in the plant is still uncertain.[12] Kylin assumed that it occurred as an insoluble calcium salt. When extracted with hot water, an alkali-metal salt was obtained; while when extracted with Na_2CO_3, the yield was soluble sodium alginate. According

to him, sodium alginate is the result of double decomposition of Na_2CO_3 with the insoluble calcium salt. Unfortunately, he failed to support his assumptions with experiments.[26] In 1931 Bird and Hass[8] by experiments proved Kylin wrong. They stated that algin occurred in two forms, one, as a soluble alkali salt, about 1%; and two, as the free acid which is the one extracted by Na_2CO_3. In the same year, Dillon and McGuiness suggested that the major part of algin occurred in connection with iron and calcium colloidal compounds.[12] Even though the exact state of occurrence is still uncertain, many workers feel sure that it occurs in more than one form. They state that it is a mixture of the free acid and its Ca, Mg and Na salts.[35]

Manufacture

Historically, the very first method of extraction was the one employed by Stanford in 1883.[30] His method is rather simple. He macerated the kelps, mainly Laminaria, in cold sodium carbonate solution for 24 hours. The resulting viscous liquor was filtered through coarse linen to get rid of the cellulose tissue mass. Upon addition of a mineral acid, he obtained a jelly-like product which was the alginic acid. Later, several other methods with modifications and improvements were developed, but almost all made use of the basic steps of Stanford's original method. The basic step in all the methods is to transfer the "algin" material in the plant into its water soluble sodium salt. Any of the known salts of alginic acid can be ob-

tained upon addition of their proper ion.[54] In 1932 Gloess introduced the improvement of washing the weeds with HCl before Na_2CO_3 digestion to remove the mineral salts. Dillon (1938) has used a completely different approach where he omits the step of maceration with Na_2CO_3. He first boiled the weeds to remove fucoidin and then left them in dilute HCl for one day. Instead of obtaining the sodium salt, he extracted with ammonia. In the above-mentioned methods, wet seaweeds were used. Gloess claims that better extraction is achieved if dry weeds are used, but it is not always practical economically.[12]

Investigators have also devised steps for hastening the process, some of which are mentioned below. Several methods crush or mill the dry weeds beforehand to increase the surface area for Na_2CO_3 action. Others reduce the 24 hours period in Na_2CO_3 by boiling the mixture twice for 30 minutes each.[16] Still some manufacturers add $CaCl_2$ before the mineral acid and first obtain calcium alginate, which renders the purification easier. Neither the cited methods nor the shortcuts yield pure products.

The preparation of the pure material is rather difficult and was first accomplished on a laboratory scale. Several patents have been issued by different countries for the development of methods that yield purer products. In the United States two patented principal commercial processes are used which yield highly pure alginates. They are Green's Cold Process and Le Gloahec-Herter Process.

These processes are presented in scheme form by Tseng.[55] Besides these complicated commercial processes several other simpler procedures have been employed to secure pure algin specially on a laboratory scale. One such way is to redissolve the obtained alginic acid in Na_2CO_3, to filter and to reprecipitate it several times.[63] Another way was employed by Waksman and coworkers [65] who achieved some degree of decolorization by washing the product with water followed by alcohol and ether. Lucas and associate[31] (1940) obtained a fine white powder when they washed extensively the wet alginic acid with 50% alcohol. Vincent[60] (1956) has made a detailed study of purification and decolorization of algin products obtained from rockweeds, specially Fucus vesiculosus, which yield extremely colored substances upon extraction with Na_2CO_3. His criteria of quality were color, viscosity, percentage yield and the percentage purity of the yield. The following are the methods of decolorization and purification used by him.

He first tried several adsorbents. Darco G-60 carbon adsorbed most of the color from sodium alginate solutions. His results upon precipitation with ethanol, HCl and 20% $CaCl_2$ are listed below.

Method	Yield	Viscosity	% alginate
Ethanol	2.7%	13.9	76.6
HCl	3.5%	4	92.6
20% $CaCl_2$	4.0%	3.6	95.4

As already seen, the yield is very poor. Another adsorbent used was Celite Magnesol where a pale ivory yield of 16.4% was obtained. Aliminum and silica gels were also tried but were found to be ineffective. Slight decolorization was obtained when the seaweeds were placed in 40% formaldehyde before extraction. Another approach used by Vincent was washing the wet alginic acid with several organic solvents. He obtained the following results.

Solvent	Color	Yield (%)	Viscosity
Ethanol	white	8.8	5.6
Methanol	white	15.8	3.0
Benzene	light brown	16,5	2.8
Ether	light brown	16.7	3.7
2:1 Benzene ethanol	pale ivory	16.5	5.6
water	light brown	2.4	1.2

Lastly, he tried bleaching with sodium hypochlorite. The yield was 10.4%, while the viscosity was 1.34 c.s. which indicates a great loss. Therefore, according to Vincent adsorbents besides carbon are not very effective. Most of the color can be removed by washing with ethanol either the weeds or the wet alginic acid.

Uses

It has been possible to use algin and its derivaties in numerous ways because of its various chemical and physical properties. Programs of research are still carried on to tap all of its potentials. The well established

uses are so extensive that it would be impossible to discuss all of them adequately. It is best to divide them into several catagories.[43]

Uses of Algin in Food

The most important and extensive use of algin is in ice-cream industry where it has replaced gelatin. It is used as a stabilizing agent that gives a smooth body and texture and prevents the formation of ice-crystals when the ice-cream is stored. Algin being tasteless does not interfere with the flavor of the ice-cream[58] but gives it bulk. Its caloric value is 1.4 cal/gr. and since most foods use less than 1% it becomes valuable when low calory diets are desired.[19]

Now instead of gums, algin is also used to stabilize ices (lemon and orange), sherbets, whipped cream, cream cheese, jellies, jams, icings, candies, fruit juice powder[58] etc. as it can absorb water and hold it close to the food product preventing crystal formation upon freezing.[5] Propylene glycol alginate is used in salad dressings and sauces as an emulsifier.[19]

Nilson and Wagner[39] have conducted feeding tests on rats, mice, guinea pigs, cats and chicks to determine the possibilities of algin itself as food. Its physical sticky state when mixed with water presented a problem in chewing and swallowing when high concentrations of algin were used. Algin, as such, cannot be used as food, but in the amounts used in the food products, and ten times more, no

harm against health was observed.

Pharmaceutical and Cosmetic Preparations.

The controllable viscosity of sodium-alginate solutions by addition of different concentrations of Ca-ions, its emulsifying and stabilizing properties and its being odorless, tasteless and colorless[57] make it a very convenient ingredient in pharmaceutical and cosmetic preparations. It is a useful constituent of many tablets and pills (aureomycin, aspirine, triple sulfa) as it swells in aquous media and helps in their disintegration.[30] As an emulsifying agent it is employed in sulfanilamide ointment preparations that are used in treating skin wounds and burns.[58] It has been found to be an excellent base for toothpastes, hand lotions, hair pomades,[22] wave sets etc. where it acts as a stabilizer and is not sticky or waxy. It is also an advantageous addition to brushless shaving creams. The algin present helps in easier spread, stabilizes the lather, delays its drying on the skin and is easily washed off the razor.[5]

Industrial applications:

1. Rubber industry: In manufacture of natural rubber, algin (generally ammonium alginate) acts as a creaming agent i.e. separates the rubber from the serum. It is also used in synthetic rubber manufacture.[57]

2. Textiles: algin is employed in the preparation of water proof cloth. The material is first placed in sodium alginate which is then made insoluble by dilute acids or metals.[54]

The highly viscous solutions of alginic acid and its salts have suggested to several workers that fibers which will be woven into textiles can be prepared from it. In 1912 Sarason described a spinning method and in 1934 Tadashi obtained patents for spinning cuprammonium algin solution which yielded fibers easy to dye and of great tensile strength.[34] Unfortunately alginic acid, Na-alginate and Ca-alginate fibers are easily dissolved in alkali solutions of soap and soda. The fibers must be made alkali resistant. Speakman[48] (1942 - 1945) replaced the Ca-ion with beryllium and chromium ions which made the fiber alkali resistant. These artificial yarns have great strength,[45] are non-inflammable, have great affinity to dyes and some of them are naturally colored, like chromium alginate which is blue, while cobalt alginate is red.[12] Its use as textile has not been perfected yet.

Alginate rayon is also used as "disappearing fiber". The normal twist of cotton is removed by weaving it with Ca-alginate fibers which are later removed by alkali.[12]

3. Paints: sodium and ammonium alginates are added to paints as emulsifiers. After use, a dilute acid or $CaCl_2$ is added which makes an insoluble water-proof film over the paint. Algin is also used as stabilizer in camouflage paints.[58]

4. Agriculture: small amounts of algin activate and reduce the quantity of the insecticide added to sprays.[57] Brown seaweeds are also used as manure. Their algin en-

ters into physico-chemical reactions with the soil particles, increasing the water holding capacity and available O_2 content.[61]

5. Boiler Treatment: for boiler treatment, crude algin is used. Algin reacts with the calcium of hard water forming a Ca-alginate mass, which can be removed easily from the boiler.[57]

6. Liquor clarification: alginic acid adsorbs the impurities when added to the solutions of sugar extracted from beet. Alginic acid, being a precipitate, can be removed with the adherent impurities.[57]

Uses in the Medical Sciences.

1. Dentistry: in dentistry, algin is used instead of tinfoil to coat dentures made of resins. The coatings are first made with sodium alginate which are later changed to the insoluble calcium alginate form. Its advantages over tinfoil are that it is cheaper and easier to apply.[33]

Since 1939 algin is sometimes used instead of agar for dental moulds. It is easier to use than agar, but it gives less accurate results, specially in inexperienced hands.[58] Powdered alginic acid, sometimes mixed with penicillin, is placed on bleeding gums after tooth extraction. Bleeding soon stops as alginic acid acts with Ca to form a clot.[30]

2. Internal medicine: recently, research for the use of alginic acid has been more directed towards its application in medicine. Patients with high blood

pressure were fed alginic acid as cation exchange resin by Gill and coworkers.[21] They found that it was less efficient as cation exchanger than the usual resins. Feldman and associates[17] tried it as sodium ion adsorber in the intestine and found it to be adequate. Its advantage is that it causes no nausea or vomiting, while other resins do. Before it can be widely used for such a purpose, more work needs to be done. Richards[40] has used alginate wool to trap mold spores found in the air which cause allergies. He could them culture and study them.

3. Surgery: since alginates can form fibers that can be woven, their use as gauze and swab in surgery has been studied by Blain.[6] The advantages of alginate gauze over gelatin and fiber cellulose are that it can be autoclaved, is cheaper and does not inhibit penicillin action. Chenowitz[13] and Franz,[18] on the other hand, found that absorbable alginate gauze was toxic, as it produced clots. It is possible that different forms of algin could be prepared which would be non-toxic. Blain and coworker[17] also used it in eye surgery on rabbits. They found that healing was accelerated and no irritation was caused, but it penetrated into the vitreous humon, affected the focusing power of the eye.

Miscellaneous Uses: there are several other uses some of which can only be mentioned. When CS_2 or CCl_4 is added to algin a rubbery mass results which is used in making typewriter rollers.[16] A horny mass produced in another way is used to make wall or ceiling boards. Gloess, on the

other hand, has suggested weatherproofing cement buildings by coating them with soluble alginates. It is also used in making waterproof cloth for tents and wagon covers.[12] Algin can produce a transparent film like cellophane which is cheaper, non-inflammable and less brittle, and can be used as wrappings.[16] Other uses are as thickener in printing ink, separator of plates in storage batteries,[58] and adsorbent for decolorizing solutions.[12]

PART III

MATERIALS AND METHODS

Materials

The materials used in this work were several species of brown algae growing on the rocky seashore of the American University of Beirut. The weeds were generally found at the intertidal zone. The specimens used were collected by hand at low tide in October of 1960 and April of 1961. The experiments were mainly performed on the most abundant and largest rockweed, Sargassum linifolium (Turn) J.Ag. This brown alga has a holdfast which is usually attached to rocks, and a short upright stipe bearing many entire and narrow laminae. The lamina has a distinct midrib and bears receptacles. The plant also bears spherical air-bladders which aid in floating. Sargassum linifolium (Turn) J.Ag., having such a structure is easily collected by hand. It is a member of the Order Fucales, and of the Class Cyclosporeae.

Another brown alga that grows very abundantly during spring is Padina species. Padina was mainly obtained from a rocky shelf which is alternately exposed to air and water. As to size, Padina is smaller than Sargassum linifolium (Turn) J.Ag. Its fan-shaped thallus is either entire or irregularly split in several regions along the margin. It has concentric hair lines and bears the sporangia. Padina belongs to the Order Dictyotales and the Class Isogeneratae.

A third specimen collected was Fucus serratus L. which belongs to the Order Fucales and to the Class Cyclosporeae. This brown alga is still smaller in size and occurs less abundantly than the first two. The thallus is flat and has the characteristic dichotomous branching. No vesicles appear on it.

Methods

There are many methods for extracting algin from seaweeds most of which are patented. All of them are based on the same principle, i.e. converting the "algin" material in the plant to its soluble sodium alginate form by Na_2CO_3 maceration. In the first part of this study, three different procedures were tried with the purpose of standardizing a method that would yield the highest algin percentage and purest product within the shortest possible time. For all these experiments dried Sargassum linifolium (Turn) J.Ag. was used. The three procedures differ mainly from one another as to the thermal conditions to which the weeds were subjected: boiling, 60° C. and cold.

Procedure 1

This procedure is based on the method outlined in Textbook of Pharmacognosy by T.E. Wallis, 2nd edition, 1951.[63]

The algae were washed twice with tap water and left to dry in the sun. Before use, they were put in the oven (120°C) for about one hour to insure complete drying. Ten

grams of the oven dried algae were placed in 400 ml. of 0.33% HCl to wash off the mineral salts. After decanting they were cut into small pieces by scissors. In later experiments, the oven-dried specimens were milled by a waring blendor before washing. The crushed particles were then placed in 200 ml. of 2% Na_2CO_3, boiled for 30 minutes, filtered, and the residue reboiled another 30 minutes, in 100 ml. of 2% Na_2CO_3. To the combined filtrates, 100 ml. of 10% $CaCl_2$ was added. The floating brown calcium alginate was then collected. 100 ml. of 5% HCl were used to change the Ca-alginate to fibrous alginic acid. For the sake of purification, the alginic acid was redissolved in 200 ml. of 2% Na_2CO_3 which was then filtered. The extracted material now exists in the soluble Na-alginate state. It is possible to obtain the acid or any of the salts by addition of the proper reagent. 95% ethyl alcohol will precipitate it as Na-alginate, 10% $CaCl_2$ as Ca-alginate, and 5% HCl as alginic acid.

Procedure 2

This procedure is a modification of the commercial Green's Cold Process.[12] Ten grams of the oven-dried Sargassum linifolium (Turn) J. Ag. were ground in a waring blendor, washed with 400 ml. of 0.33% HCl and left overnight in 200 ml. of 2% Na_2CO_3 at PH 10. After decantation, the weeds were again digested in 200 ml. of 2% Na_2CO_3 and diluted with six volumes of distilled water. The combined liquors were added to 75 ml. of 10% $CaCl_2$ with constant shaking (100 lbs.

of $CaCl_2$ for 8 tons alginate liquor). The resulting calcium alginate product was placed in 5% HCl (1:42) to obtain the alginic acid which was then successively washed with 1% HCl, 0.33% HCl, 0.1% HCl and distilled water.

Procedure 3

This procedure is taken from Vincent, D.L.,[60] "The Preparation of Sodium Alginate from Rockweeds", Can. J. Tech. 34: 220-221, 1956. Ten grams of the milled Sargassum were stirred at 60°C. for 30 minutes with 200 ml. of distilled water containing 0.5 grams $Ca(OH)_2$. After cooling the algae were washed three times with distilled water. Maceration was carried on for 2 hours with 100 ml. of 3% Na_2CO_3 at 50°C. The mixture was then diluted with distilled water to 600 ml. and left overnight. On the next day, the liquor was filtered and the residue washed with distilled water. The combined solutions were poured into 100 ml. of 25% $CaCl_2$ and stirred for 30 minutes. The floating calcium alginate was removed and washed with water containing 5% alcohol. The fibrous acid was then obtained by the addition of 0.5 N HCl and was washed successively with distilled water, 50% alcohol, 95% alcohol and ether.

Several variations were also tried on Procedure 3 to obtain purer products.

A. Treatment of the algae before extracting to remove the pigments.

1. With hot and cold ethanol.
2. With ether for twenty days.

3. With acetone for twenty days.

4. With sodium hypochlorite for 4 hours just before extraction.

B. Treatment of the sodium alginate solution for the sake of decolorization.

1. Decolorized by activated carbon in the ratio of 1 gram carbon to 50 ml. solution.

2. Bleached with 10 ml. of NaOCl.

C. Treatment of the wet alginic acid product.

1. Extracted in 95% alcohol for several days.

2. Bleached with NaOCl.

All the yields were analyzed as to the following:

1. Color of the final product.

2. Viscosity of the 1% sodium alginate solution as determined with the Ostwald viscosimeter.

3. Percentage purity of alginic acid determined by titration with 0.0425 N NaOH. As the acid is insoluble, a suspension was used.

4. Percentage yield by weight.

5. Checked against the following table to determine whether it is the right product.

	Na-alginate	Ca-alginate	Alginic acid
Microscopic view	powder	fibrous	fibrous
Solubility			
cold water	soluble	insoluble	insoluble
hot water	soluble	insoluble	slightly soluble
alcohol	insoluble	insoluble	insoluble
Ether	insoluble	insoluble	insoluble
Glycerol	insoluble	insoluble	insoluble
$CaCO_3$ sol.	---	---	Liberates CO_2
Fehling's A & B	---	---	No reduction
Hydrolysis then Fehling's A & B	---	---	Reduction
5% Na-citrate	---	rapidly dissolves	

After evaluating the three above-mentioned methods and the modifications, it was decided to employ,

Procedure 3 with the following additions:

1. Extraction of pigments from the dried weed with alcohol.

2. Bleaching of the alginate solution with NaOCl for about five minutes.

3. Treating extensively the wet alginic acid with 95% ethanol.

This standard procedure was the one employed to determine the percentage yield of *Padina* species and *Fucus serratus* (L.).

PART IV

RESULTS

Percentage Yield of Alginic Acid

Table 1 represents the percentage yields of alginic acid obtained by the different procedures that were described previously. The range of yields of procedures 1, 2, and 3 were almost the same i.e. 16-19%. Treatment of the oven-dried and milled Sargassum, by the solvents and NaOCl bleacher to remove the pigments, did not affect the amount of the yield except in extraction with hot alcohol where no alginic acid could be obtained. Decolorization of the sodium alginate solution by both activated carbon and NaOCl was successful. The yield of the carbon treated looked to be poor, but could not be measured as it was impossible to clear the solution of all the carbon particles. Different methods employed to try to remove the carbon, such as extensive centrifugation, filtering through paper pulp and glass wool, were not completely successful and resulted in the loss of most of the product. The addition of NaOCl caused immediate bleaching and did not adversely affect the yield. Extensive treatment of the wet alginic acid with 95% ethyl alcohol had no affect on the amount of acid obtained, but bleaching it with NaOCl caused some loss in the final product. Table 1 also includes the percentage yields of alginic acid which were obtained from Padina and Fucus serratus by the standardized procedure.

TABLE 1. PERCENTAGE YIELD OF ALGINIC ACID

Treatment on			Weight of oven-dried algae (in grams)	Weight of alginic acid obtained (in grams)	Percentage Yield (%)
1. Sargassum					
Procedure	Trial				
1	1		10	1.64	16.4
1	2		10	1.81	18.1
1	3		10	1.58	15.8
2	1		5	0.94	18.8
2	2		10	1.92	19.2
2	3		10	1.67	16.7
3	1		10	1.70	17.0
3	2		10	1.68	16.8
3	3		10	1.81	18.1
Treatment as in A (pp. 31-32)					
Hot Alcohol			5	No Result	0
Cold Alcohol			5	0.74	14.8
Ether			5	0.64	12.8
Acetone			5	0.73	14.6
NaOCl			5	0.94	18.8
Treatment as in B (p. 32)					
Carbon			12	No Result	0
NaOCl			10	1.96	19.6
Treatment in C (p.32)					
95% Alcohol			5	0.93	18.6
NaOCl			10	0.76	7.6
2. Padina			6	0.83	14.0
3. Fucus			5	1.35	27.0

Viscosities of 1% Sodium Alginate Solutions

Table 2 shows the viscosities of 5 ml. samples of 1% sodium alginate solutions that were measured by the Ostwald viscometer. The average results of the two readings are represented in seconds. The relative viscosity of each sample was calculated as the ratio of the viscosity of the 1% solution to that of the standard liquid, distilled water.

$$RV. = \frac{ts}{tw}$$

RV. = relative viscosity.

ts = time in seconds for the sodium alginate samples to flow.

tw = time in seconds for the distilled water to flow: 126 seconds.

The products of Procedure 1 had the lowest viscosities which most probably is due to boiling the weeds during the process of extraction. High viscosity results were obtained from the yields of Procedures 2 (no heating) and 3 (50°C). Any additional treatment, especially bleaching of the weeds and wet alginic acid, caused a lowering of the viscosity. Surprisingly, little viscosity loss was observed when the sodium alginate solution was bleached with 10 ml. of 1% NaOCl. This could be due to the very short exposure of the alginate to the NaOCl.

TABLE 2. VISCOSITY OF 1% SODIUM ALGINATE SOLUTIONS MEASURED AT 22°C BY THE OSTWALD VISCOMETER

Treatment on		Time for flow of Samples (in sec.) (average of 2 readings)	$RV = \frac{t\ s}{t\ w}$
1. Sargassum			
Procedure	Trial		
1	1	202.2	1.62
1	2	212.5	1.68
1	3	230.4	1.82
2	1	1242.2	9.85
2	2	1357.6	10.71
2	3	1232.3	9.78
3	1	1232.05	9.78
3	2	1300.35	10.32
3	3	1399.6	11.10
Treatment as in A (pp 31-32)			
Cold alcohol		518.5	4.11
Ether		462.7	3.67
Acetone		362.9	2.88
NaOCl		371.7	2.95
Treatment as in B (p. 32)			
NaOCl		1217.9	9.67
Treatment as in C (p.32)			
95% alcohol		965.2	7.66
NaOCl		375.1	2.97
2. Padina		571.1	4.53
3. Fucus		553.6	4.38

Percentage Purity of Alginic Acid Products

The percentage purity of each alginic acid product, as determined by titration, is tabulated in Table 3. Being insoluble in water and organic solvents, a given weight of alginic acid was suspended in 50 ml. of distilled water (Table 3). Each suspension was titrated either by 0.0421 N NaOH or by 0.0942 N NaOH, using phenolphthalein as the indicator. The % purity, in each case, was calculated as follows:

1. Neutralization Equivalents (N.Eq.) were determined by the following formula:

$$\text{N.Eq.} = \frac{\text{Weight of Acid in grams} \times 1000 \text{ milliequivalents}}{\text{Normality of Base} \times \text{ml. of Base}}$$

2. The obtained Neutralization Equivalents were compared with the theoretical value of the Neutralization equivalent of pure alginic acid which is 176 and calculated on a percentage basis as follows:

$$\text{Percentage Purity of Alginic Acid} = \frac{176 \times 100}{\text{N.Eq. of Sample}}\%$$

As clearly set forth by Table 3, it was difficult to obtain very pure products by means of the first three procedures. The additional treatments with alcohol etc. improved the purity of the yield. The purity of alginic acid obtained by the standardized procedure from Sargassum and Padina was rather high, while that from Fucus serratus was somewhat lower.

TABLE 3. PERCENTAGE PURITY OF ALGINIC ACID PRODUCTS DETERMINED BY TITRATION WITH NaOH

Treatment on		Weight of Alginic Acid Titrated (in grams)	Amount of NaOH used (ml.)	% Purity
1. Sargassum			.0421 N NaOH	
Procedure	Trial			
1	1	0.1837	20.2	83.8
1	2	0.2669	29.2	81.1
2	1	1.1858	21.1	84.2
2	2	0.2687	30.5	83.8
2	3	0.2673	30.1	83.4
3	1	0.1580	18.3	85.8
3	2	0.1212	14.1	86.3
3	3	0.4149	47	85
Treatment as in A (pp.31-32)			.0942 N NaOH	
Alcohol		0.110	6	90.9
Ether		0.1227	6.55	88.4
Acetone		0.1362	7.1	86.2
NaOCl		0.1492	7.7	86.2
Treatment as in B (p.32)				
NaOCl		0.1195	6.63	92.2
Treatment as in C (p.32)				
95% alcohol		0.1695	8.9	87.1
2. Padina		0.1575	8.8	92.6
3. Fucus		0.158	8.2	86.2

Properties of Alginic Acid, Sodium and Calcium Alginates

Table 4 shows the physical and chemical properties of alginic acid and the alginates of Na and Ca as obtained in this work. The sign (+) indicates a positive result, and (--) a negative one. All the yields had the characteristic properties expected, except for the alginic acid that was bleached with NaOCl and did not show the fibrous constitution. The color of the products is represented by Plate, No., and Letter taken from Maerz, A. and Paul,R.M., A Dictionary of Color, New York, 2nd ed., 1950. The three procedures, without the special treatments, yielded colored alginic acids. The least colored (ivory) results were obtained when carbon and NaOCl were used, and the next best were the ones treated with 95% ethyl alcohol.

TABLE 4. PROPERTIES OF ALGINIC ACID, SODIUM AND CALCIUM ALGINATES

Treatment on	Alginic Acid				Ca and Na Alginates		
	Color			Fibrous	Solubility		
	Plate No.		Let.	View	Solvents	Water	
						Na-alg.	Ca-alg.
1. Sargassum							
Procedure							
1	13	7	D	+	-	+	-
2	13	9	C	+	-	+	-
3	13	7	C	+	-	+	-
Treatment A (pp. 31-32)							
Alcohol	12	7	B	+	-	+	-
Ether	12	7	C	+	-	+	-
Acetone	12	7	F	+	-	+	-
NaOCl	12	8	D	+	-	+	-
Treatment B (p. 32)							
Carbon	10	1	A	+	-	+	-
NaOCl	10	2	B	+	-	+	-
Treatment C (p.32)							
95% Alc.	12	8	F	+	-	+	-
NaOCl	10	2	B	-	-	+	-
2. Padina	10	2	D	+	-	+	-
3. Fucus	10	2	F	+	-	+	-

TABLE 5. A SUMMARY OF THE FOUR TABLES

Treatment on	Color Plate No.	Color Letter		%Yield	Viscosity	%Purity
L. Sargassum						
Procedure						
1	13	7	D	16.8	1.71	81.5
2	13	9	C	18.2	10.11	83.8
3	13	7	C	17.3	10.40	86.7
Treatment as in A (pp.31-32)						
Alcohol	12	7	B	14.8	4.11	90.9
Ether	12	7	C	12.8	3.67	88.4
Acetone	12	7	F	14.6	2.88	86.2
NaOCl	12	8	D	18.8	2.95	86.2
Treatment as in B (p.32)						
Carbon	10	1	A	0	0	0
NaOCl	10	2	B	19.6	9.67	92.2
Treatment as in C (p. 32)						
95% alcohol	12	8	F	18.6	7.66	87.1
NaOCl	10	2	E	7.6	2.97	92.2
2. Padina	10	2	D	14.0	4.53	92.6
3. Fucus	10	2	F	27.0	4.83	86.2

PART V

DISCUSSION

Vincent [60] has stated that it was more difficult to extract colorless pure alginic acid from rockweeds than from the larger generally used bottomweeds. Three procedures of extraction that generally employ bottomweeds were applied to the rockweed, Sargassum linifolium. These three methods gave rather high percentage of yields. Unfortunately, the products were colored and somewhat impure, even though most of the plant pigments remained in solution when calcium alginate was precipitated out. Additional treatment to secure whiter products was considered essential, and it was performed, for the sake of consistency, on one of the procedures. Procedure 1 which would extract in a shorter time could not be applied because it lowered the very high viscosity of the product. The loss of the product is most probably due to the great heat employed in extraction which will depolymerize the molecule. It was difficult to make a choice between Procedures 2 and 3 as both yielded alginic acid of similar properties in about the same amounts. Procedure 3 was preferred as it could be completed in a shorter time.

As mentioned previously, the modified treatments were divided into the following three groups:

A. The oven-dried Sargassum plants were treated with organic solvents and NaOCl to remove the pigments before

extraction. The organic solvents, alcohol, ether and acetone separately removed the chlorophylls. Consequently, the products were purer and less colored but of lower viscosity. The bleacher, NaOCl, used only for four hours just before extraction, had about the same effect as the organic solvents, except that the viscosity of the yield was decreased more. The pigments were also extracted with hot alcohol, but no alginic acid could then be obtained. The weeds were extracted with cold alcohol, as an additional step, as it was found to be slightly better than the other solvents.

B. In the second group, decolorization of the sodium alginate solution was achieved by the addition of either activated carbon or of NaOCl. Vincent [60] suggested the use of 70 grams of carbon for 10 grams of dried weeds, but it was found that all the color could be adsorbed with carbon added in the ratio of 1 gram to 50 ml. solution (total about 5-6 grams). A small amount of very white alginic acid was obtained. This carbon treatment could not be made use of, because not all the carbon particles could be removed from the solution. Few carbon particles remained in spite of extensive centifugation, and filtration through filter paper, glass wool, paper pulp and cotton successively. These clearing methods caused excessive loss in yield. On the other hand, addition of 10 ml. NaOCl caused immediate bleaching after which a very pale yellow product was obtained. NaOCl generally affects the viscosity but contrary to ex-

pectation the loss, in this case, was slight. The reason could be the very short exposure (about 5 minutes) of the alginate solution to the NaOCl.

C. Color was also removed by washing the wet alginic acid either with 95% alcohol or bleaching it with NaOCl. In this case NaOCl caused a great loss in viscosity. Extensive washing with 95% alcohol removed a lot of the color and had no adverse effects.

One step from each one of the above-mentioned three groups of purification procedures was added to Procedure 3 and all together insured whiter and purer products with fairly high viscosities. The major additional steps were the following:

1. Extraction of pigments from the weeds by 95% alcohol at room temperature.

2. Bleaching of the alginate solution with NaOCl for about five minutes.

3. Treating the wet alginic acid with an excess of 95% alcohol and making two changes in a month.

This standardized procedure of extraction was employed in the case of Padina and Fucus serratus. The Padina itself, being lighter in color than Sargassum, made the extraction of whiter products easier. The percentage of the alginate obtained from Padina was lower than that of Sargassum. Fucus serratus yielded the least pure and the most colored alginate product of all the three brown algae used, even though the

amount of alginate obtained was high. The high percentage yield is probably due to the impurities present in it.

The rockweeds employed in this study seem to have fairly high percentage concentrations of alginic acid. With some difficulty, it is also possible to obtain pure results. Of the three brown algae studied the most promising source of alginic acid seems to be Sargassum as to yield and purity. Other advantages of Sargassum are its abundance and its large sizes which render its collection easy. Pure extraction from Padina is easier than from Sargassum, but the former here is less abundant, smaller in size and more difficult to collect. Apparently Fucus serratus is the most inconvenient raw material to make use of as compared to the other two. Fucus is very small in size, less abundant hence difficult to collect in large quantities. Extraction of pure alginic acid from Fucus was also found to be more difficult. In an enterprise it would be wisest to employ Sargassum as the raw material because it seems to be the best potential source of algin. Padina can be considered as a less important source, while the choice of Fucus would not be very wise here.

BIBLIOGRAPHY

1. Allen, M.C., "The Decomposition of Alginic Acid by Micro-organisms," J. Bact., v. 27,159, 1934.
2. Astubury. W.T., "Structure of Alginic Acid", Nature, v. 155,667, 1945.
3. Becker, C.H., "Thickening Agents Used in Pharmacy," Am. Prof. Pharm., v. 20, 939, 1954.
4. Bergy, G.A., "Algin Finds New Uses in Pharmacy," Am. Prof. Pharm., v. 5, 367, 1939.
5. _____. "Sodium Alginates" Am. Prof. Pharm., v.5, 430, 1939.
6. Blain, M.G., "Experimental Observations on Absorbable Alginate Products in Surgery," Ann. Surg., v. 125, 102, 1947.
7. _____. et al. "The Use of Plastic Materials for Scleral Wounds," Trans. Ophthal. Soc., v. 64, 188, 1944.
8. Bird G.M. and Haas, P., "On The Nature of The Cell Wall Constituents of Laminaria spp. Manuronnic Acid," Biochem. J., v. 25, 403, 1931.
9. Black, W.A.P., "Seasonal Variation in Chemical Constitution of some British Laminariales," Nature, v.161, 174, 1948.
10. Chanda, S.K. et al. "The Structure of Alginic Acid," J. Chem. Soc., 1883, 1952.
11. Chapman, V.J., "Seaweed Resources Along the Shores of Great Britain," Econ. Bot., v. 2, 363, 1948.

12. _____., Seaweeds and Their Uses. London, Methuen and Co. Ltd., 1950.

13. Chenowith, M.B., "The Toxicity of Sodium Alginate in Cats," Ann. Surg., v. 127, 1173, 1948.

14. Davis, F.W., "Algin from Sargassum," Science, v. 111, 150, 1950.

15. Dawson, Y.E., How to know the Seaweeds. Dubuque, W.M.C. Brown Co., 1956.

16. Delf, M.E., "Nature and Uses of Seaweeds," Nature, v. 152, 149, 1943.

17. Feldman, H.S. et al. "Cation Adsorption by Alginic Acid in Humans," Proc. Soc. Exptl. Biol. Med., v. 79, 439, 1952.

18. Franz, V. K., "Experimental Studies of Alginates as Humostatics," Ann. Surg., v. 127, 1165, 1948.

19. Gibson, K.F. and Rothe, L.B., "Algin Versatile Food Improver," Food Eng., v. 27, 87, 1955.

20. Gilbert, D.L. et al. "The Influence of High Oxygen Pressures on the Viscosity of Solutions of Sodium DNA and Sodium Alginate, " J. Am. Chem. Soc., v. 79, 5677, 1957.

21. Gill, R.J. et al. "The Clinical Use of Alginic Acid as a Cation Exchanger," Am. J. Med. Sci., v. 224, 572, 1952.

22. Green, M.W., "Some Pharmaceutical Necessities," J. Am. Pharm. Assoc. Prac. Pharm. Ed., v. 7, 297, 1946.

23. Hariot, P., Atlas des Algues Marines Paris, Librairie des Science Naturelles, 1892.

24. Hirst, E.L. et al. "Structure of Alginic Acid," Nature, v. 143, 857, 1939.

25. _____. "The Structure of Alginic Acid," J. Chem. Soc., 1880, 1939.

26. Hoagland, D.R. and Lieb, L.L., "The Complex Carbohydrates and Forms of Sulfur in Marine Algae of the Pacific Coast," J. Biol. Chem., v. 23, 287, 1915.

27. Holmes, E.M., "The Japanese Seaweed Industry," Pharm. J. v. 77, 346, 1906.

28. Jones, R.F., "On the Chemical Composition of the Brown Alga Himanthalia elongata (L.) S.F. Gray," Biol. Bull., v. 110, 169, 1956.

29. _____. "Variation of Nitrogen and Carbohydrate Constituents During the Development of Himanthalia elongata (L.) S.F. Gray," Biol. Bull., v. 112, 81, 1957.

30. Lesser, M.A., "Alginates in Drugs and Cosmetics," Econ. Bot., v. 4, 317, 1950.

31. Lucas, H.J. et al. "Oxidation of Alginic Acid by Periodic Acid," J. Am. Chem. Soc., v. 62, 1793, 1940.

32. Macpherson, M.G. and Young, G.E., "Seasonal Variation in the Chemical Composition of the Fucaceae in the Maritime Provinces," Can. J. Bot., v. 30, 67, 1952.

33. Matthews, E., "The Alginates in Dentistry," Brit. Dent. J., v. 76, 8, 1944.

34. Mauersberger, H.R., Matthew's Textile Fibers, New York, John Wiley and Sons, Inc., 1947.

35. Mautner, H.g., "The Chemistry of Brown Algae," Econ. Bot., v. 8, 174, 1954.

36. Mori. T. "Seaweed Polysaccharides," Advances in Carbohydrate Chemistry, v. 8., 349, 1953.

37. Moss, B.L., "Studies in the Genus Fucus,I," Ann. Bot., v. 12, 275, 1948.

38._____. "Studies in the Genus Fucus II," Ann. Bot., v. 14, 406, 1950.

39. Nilson, H.W. et al. "Feeding Tests with Some Algin Products," Proc. Soc. Exptl. Biol. Med., v. 76, 630, 1951.

40. Richards, M. "A Water-Soluble Filter for Trapping Airborne Micro-organisms," Nature, v. 176, 559,1955.

41. Sager, R.W. et al. "A Phytochemical Study of Nereocystis luetkeana, Am. J. Pharm., v. 118, 157, 1946.

42. Sanford, B.F., "Seaweeds and Their Uses," Fishery Leaflet, v. 469, 11, 1958.

43. Scheffer, V.B., "The Commercial Importance of Seaweed Gums in the United States," Fish. Mark. News, v. 5, 1, 1943.

44. Schwarz, T.W. et al. "Viscosity Changes of Sodium Alginate Solutions After Freezing and Thawing," J. Am. Pharm. Assoc., v. 46, 562, 1957.

45. Sherman, J.V. and Sherman, S.L., The New Fibers. New York, D. Van Nostrand Co., Inc., 1946.

46. Smith, G.M., Cryptogamic Botany. Algae and Fungi. Vol I. New York, McGraw-Hill Book Co., Second ed., 1955.

47. "Sodium Alginate," Am. Prof. Pharm., v. 18, 125, 1952.

48. Speakman, J.B., "Seaweed Rayon," Nature, v. 155, 655, 1945.

49. Spoehr, H.A., "The Hydrolysis of Alginic Acid with Formic Acid," Arch. Biochem., v. 14, 153, 1947.

50. Strain, H.H. and Manningm W.M., "Chlorofucine, A Green Pigment of Diatoms and Brown Algae," J.Biol. Chem., v. 144, 625, 1942.

51. "Substitute for Tinfoil," J. Am. Dent. Assoc., v. 29, 1349, 1942.

52. Taylor, E.W. et al. "Investigation of the Properties of Cellulose Oxidized by Nitrogen Dioxide. II. The Evolution of CO_2 from Uronic Acids and Polyuronides," J.Am. Chem. Soc., v. 69, 342, 1947.

53. Trease, G.E., A Textbook of Pharmacognosy. London, Bailliere, Tindall and Cox, 1957.

54. Tseng, C.K., "Algin," Contributions from the Scripps Institution of Oceanography, New Series No. 351.

55. _____."Colloids from Kelp," Chem. Met. Eng., June 1945.

56. _____."The Terminology of Seaweed Colloids," Science, v. 101, 597, 1945.

57. _____. "Seaweed Products and Their Uses in America," J. New York Bot. Garden, v.47, 553, 1946.

58. _____. "Seaweed Resources of North America and Their Utilization," Econ. Bot., v. 1, 69, 1947.

59. Valsan, A.P., "Alginic Acid Content of Some of the common Seaweeds of the Gulf of Mannar Area," Cur. Sci. v. 24, 343, 1955.

60. Vincent, D.L., "The Preparation of Sodium Alginate from Rockweed," Can. J. Tech., v. 34, 220, 1956.

61. Walford, L.A., Living Resources of the Sea. New York, The Roland Press Co., 1958.

62. Wallis, T.E., Practical Pharmacognosy. London, J.A. Churchill Ltd., 1953.

63. _____. Textbook of Pharmacognosy.London, J.A. Churchill Ltd., 2nd ed., 1951.

64. Waksman, S.A. et al. "Bacteria Decomposing Alginic Acid," J.Bact., v. 28, 1934.

65. _____ and Allen, M.C., "Decomposition of Polyuronides by Fungi and Bacteria," J. Am. Chem. Soc., v.56, 2701, 1934.

66. Wassermann, A., "Alginic Acid-Acetate," Nature, v. 158, 271, 1946.

67. Weiss, F.J., "The Useful Algae," Sci. v. 187, 15, 1952.

68. Womersley, H.B.S. "The Marine Algae of Australia," Bot. Rev., v. 25, 546, 1959.

69. Wort, D.J., " The Seasonal Variation in the Chemical Composition of Macrocystis integrifolia and Nereocystis luetkeana in British Coastal Waters, "Can. J. Bot., v. 33, 331, 1955.

7o. Zaneveld, J.S., "The Utilization of Marine Algae in Tropical South and East Asia," Econ. Bot., v.13, 89, 1959.