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STUDIES ON THE MANUFACTURE OF ALGIN FROM BROWN ALGAE

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INTRODUCTION

Algin was first extracted by STANFORD¹⁾ from the bladder wrack (*Fucus vesiculosus*) with sodium carbonate. His process may be described as follows: drift-weed is macerated, with or without heating, for twenty-four hours with one-tenth of its weight of sodium carbonate. At the end of this period, the plants are completely disintegrated and a very viscous, semi-gelatinous mass is obtained. The alginic acid is precipitated from the filtrate by addition of either sulfuric or hydrochloric acid.

There are available only a few theoretical studies regarding the process for

manufacturing algin, although some improvements of manufacturing method have been reported.

One of the simpler ways for obtaining algin is by pretreating algae first with dilute hydrochloric acid in order to remove any soluble mineral salts, mannit and impurities ; then the algin is extracted by using a solution of sodium carbonate resulting in a viscous extract ; the extract is filtered, and finally the algin is precipitated by treating it with hydrochloric acid.

DILLON²⁾ has described another easy method of preparation as follows ; the weed is first extracted for short time with boiling water in order to remove fucoidin. It is then soaked in dilute hydrochloric acid for a day, after which the alginic acid is removed with ammonia. Wet weed was commonly used for these extractions, but GLOESS³⁾ considered that the maximum extraction was achieved when the weed was dry rather than wet. Fresh wet weed has been used preferably by the factories in Europe and America in order to obviate the drying cost which may outweigh the advantage of using dried weed, whilst dried algae have been used in Japan to manufacture algin as they are produced abundantly for preservation as food.

There have been reported several procedures of pretreating algae as mentioned below. These pretreatments have been worked out without any consideration as for the mechanism of algin manufacture, only for the purpose of extracting mineral matter and pigment or other impurities and of making maceration with sodium carbonate easy. Pretreatments have been reported with lime water and then with water, with acid and hydrocarbon,⁴⁾ with hydrogen peroxide or chlorine in neutral or acidic solution,⁵⁾ with sea water containing aluminium sulfate in weak acid⁶⁾ or with saturated solution of sodium bisulfate for 2-10 hours at 80-90°C.⁷⁾

As for the details of commercial process in Europe and Japan, no published report is available ; on the other hand, a report concerning the commercially used processes in America was published by CHAPMAN.⁸⁾ He described two processes, of which one was so-called "cold process" and the other was "Le Gloahec-Heyter process." His description is summarized here briefly as follows.

In the first process, or cold process, fresh kelp is first leached for several hours with 0.33 per cent hydrochloric acid. After being chopped and shredded, the leached kelp is digested with soda ash solution (40-50 lb per ton of fresh kelp) at a pH of about 10 ; this first digestion occupies about 30 minutes and it is then repeated. The crude pulp is shredded again and six volumes of treated water are added at pH of 9.6-11. At this point the crude fibrous material can be dried and sold as crude sodium alginate.

To obtain a purer product the liquor is filtered, using filter aid and presses, and the temperature may be raised to 120°F to assist the process. The filtered liquor is added to a 10-11 per cent calcium chloride solution under constant agitation, and when the agitation is stopped calcium alginate slowly rises to the top. The remaining liquor is drained off and more water and bleacher (sodium hypochlorite) is added to the

precipitate. The bleached precipitate is separated off and added to a 5 per cent hydrochloric acid solution (1 part alginate solution to 42 parts acid). This converts the calcium alginate into fibrous alginic acid, which is purified by the use of dilute acid between each screening. The alginic acid so produced can be filtered and stored or else converted into salt by treatment with carbonate, oxide or hydroxide.

In the Le Gloahec-Heyter process the raw kelp can be either fresh or dried. To one part of kelp three parts of 0.8-1 per cent calcium chloride solution are added, the solution being either hot or cold. The function of the calcium chloride solution is to remove laminarin, mannit and other salts. These salts and the calcium are then removed by washing with softened water, after which the remaining material may receive an additional treatment with 5 per cent hydrochloric acid in order to dissolve any residual alkaline earth salts. It is washed once more with softened water, and is then digested with 4 per cent soda ash solution in the proportions of two volumes solution to one volume of kelp. Lixiviation is continued for about two hours at 104°F and the kelp is macerated at the same time until it is reduced to a paste.

The resulting paste is diluted with water in the ratio 3 : 7, and after being beaten into a homogenous suspension it is vigorously aerated, but if ozone or hydrogen peroxide is used it is merely agitated mechanically. The liquor is then passed continuously at high speed through a centrifuge, where it is charged with air bubbles, and then led into a clarifying tank. Here the cellulose particles agglomerate to form a floating cake and the liquor is removed.

The coloured liquor is decolourised by addition of an absorbent jelly, usually made of hydrated alumina, gelatinous silica and aluminium alginate. The jelly is removed by centrifuging and can be reclaimed by various methods.

The alginic acid is now precipitated by running it into mixing baffle where it meets a stream of strong hydrochloric acid, arranged in such a way that the precipitate runs at once into another tank. All through this process the pH of the solution is maintained at 2.8-3.2. The precipitated alginic acid is placed in baskets and drained, after which it is purified and dried.

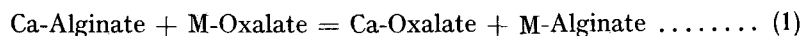
TAKAHASHI⁹⁾ described pretreatment of brown algae as follows. Raw weeds should be washed with water in order to remove dirt on the weeds, and the soluble materials of the weeds, such as iodine, mannit, potassium chloride, sodium chloride, laminarin or fucoidin were extracted from the weeds by this treatment. Extractive power would be enhanced by the addition of some chemicals in pretreating water; hydrochloric acid was the most excellent one.

Pretreating of algae with certain solution should be done to prevent the plant material from swelling in order to enhance the efficiency for extraction of soluble mineral salts, mannit or impurities.

Algin has been manufactured by macerating algae with alkaline solution, such as sodium carbonate, sodium hydroxide or ammonium hydroxide solution. TAKAHASHI¹⁰⁾

attempted to digest algae with neutral salt, ammonium oxalate, as follows. He attempted to extract algin with oxalate in the same way as in the case of extracting pectic constituents¹¹⁾ from higher plants because alginic acid fairly resembles pectic acid in respect to chemical configuration.

It was explained that insoluble alginate, such as calcium or aluminium alginate, in brown algae was dissolved into soluble alginate by the following double decomposition (1):



where, "M" represents alkali metals or ammonium radical. He did not carry out any further theoretical investigation.

It is usual to pretreat algae with water, hydrochloric acid or other solutions in order to extract potassium salts, iodine, mannit and other impurities and to treat them with sodium carbonate to make them easily macerated, but little consideration has been given to the matter of converting algin into a certain definite form. In previous paper,¹²⁾ the present worker reported on the significance of converting algin in brown algae into certain definite forms e.g., free alginic acid or calcium alginate, by pretreating algae with some solution.

In the present paper are reported the process of manufacture of algin from brown algae and the theory thereof under the condition of converting algin in brown algae into free alginic acid and calcium alginate by pretreating the plants with hydrochloric acid or calcium chloride solution. The manufacturing conditions and by-products in algin industry are also reported.

ACKNOWLEDGEMENT

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I. CHEMICAL STUDIES ON RAW MATERIALS OF ALGIN MANUFACTURE

1. Occurrence of Algin in Brown Algae

The term algin was first applied by STANFORD¹⁾ to a mucilageous polyacid occurring in *Laminaria* fronds which was soluble in dilute soda solution and precipitated by mineral acids. KYLIN¹³⁾ applied the term to a supposed insoluble calcium salt which he assumed to occur in the cell wall; he stated that it was insoluble or very slightly soluble

in water and suggested that hot water would extract from the weed an alkali salt of alginic acid produced from calcium salt, but gave no experimental evidence. Moreover, he explained that soluble sodium alginate was produced by double decomposition between insoluble calcium alginate and sodium carbonate. BIRD & HAAS¹⁴⁾ stated that hot water did not remove all the alginic acid from the cell wall, even though they could obtain 2 g of an algin-like substance from 180 g of *Laminaria* fronds. Analysis of this substance showed that it contained 3.46 per cent of Ca and 0.45 per cent of Mg, together with undetermined quantities of alkali metals. From these facts they stated that the above-mentioned substance was to be considered as a water soluble calcium, magnesium, sodium and potassium salt of alginic acid, since it yielded on acidification a gelatinous precipitate resembling alginic acid in every respect.

BIRD & HAAS concluded that the cell wall of *Laminaria* contained alginic acid in two forms, one in the form of soluble calcium, magnesium, alkali metal salt being present only in such an amount as about 1 per cent, and the other in the free state being met with in the material extracted by cold diluted sodium carbonate.

The state of the algin in brown algae can not be decided from the above mentioned facts, because both alginic acid and insoluble alginates undergo cation exchange partially or perfectly with electrolytes as mentioned later.

MIWA¹⁵⁾ investigated the calcium and magnesium contents of six species of brown algae, and found in all of the species but one that such contents did not exist in sufficient quantities to combine with alginic acid in the fronds. He suggested that some amounts of algin existed in some other states of combination than calcium or magnesium alginate, whilst he detected calcium in the cell wall of the fronds through a microchemical examination.

Figs. 1 and 2 show the results of the author's microchemical test for the presence of calcium in the cell wall of *Laminaria angustata* KJELLM. Crystals of calcium oxalate were found in the intercellular spaces in the sections that had been treated with 0.5 per cent oxalic acid.

Since algin is considered to exist as an intercellular substance in the cell wall of brown algae, the above-mentioned examination suggests the existence of calcium alginate in the cell wall.

The existence of algin in the cell wall of *Laminaria angustata* was detected by the following two methods: 1. Frozen microtome sections of the fronds were put into 1 per cent H_2SO_4 solution and then into 2 cc of I_2KI solution (1 g of iodine was dissolved in 100 cc of 5 per cent KI solution) and the blue colouration of the wall was examined under the microscope. A preliminary examination¹⁶⁾ has proved that the algin, especially calcium alginate, was coloured blue by the above reagents. Fig. 3 shows the result of this test which suggests the existence of calcium alginate in the cell wall of *L. angustata*. 2. Frozen microtome sections were steeped in 0.5 per cent calcium chloride solution and boiled for 15 minutes, whereupon almost all of the algin was

converted into calcium alginate. Thus treated sections were microchemically examined by the above described method. The results are shown in Fig. 4. The blue colouration of the walls is more marked than that shown in Fig. 3.

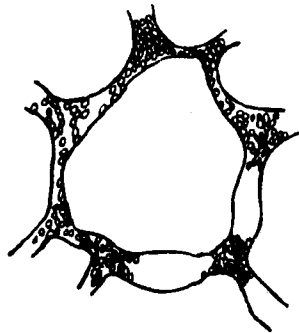


Fig. 1

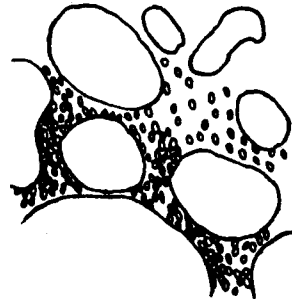


Fig. 2



Fig. 3

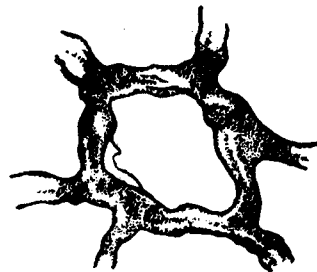


Fig. 4

- Fig. 1. Calcium oxalate crystals in the intercellular spaces of the parenchymatous tissue
- Fig. 2. Calcium oxalate crystals in the intercellular spaces of the filamentous tissue
- Fig. 3. Ca-alginate in the walls of parenchymatous cell is detected by blue colouration (dotted points) due to $I_2KI-H_2SO_4$
- Fig. 4. Ca-alginate in the walls of parenchymatous cells previously treated with 0.5% $CaCl_2$ solution (dotted points) due to $I_2KI-H_2SO_4$

It may therefore be said that the algin occurs partly or mostly as calcium alginate in the cell wall of *L. angustata*, and that the algin which would be converted into calcium alginate through the treatment with calcium chloride solution is also apparently present there. The algin may be combined to form complex compounds with mannitol, proteins or amino acids and other components of algae. It is then considered that the algin occurs in not so simple state but very complicated state, and that the

mechanisms of algin manufacture can not be studied without first causing the algin in algae to convert into certain definite states of combination.

2. Chemical Components of Brown Algae

Chemical analyses of brown algae have been repeatedly reported by many authors, but there are only a few works in which the preparation of materials has been made under well-thought-out management and the seasonal variation of the components has been reported. Even the specific differences could not be discussed without considering the season or locality of collection, because the seasonal and local differences of components of brown algae are fairly remarkable.

The present author analysed a number of brown algae especially those which are commonly used as the raw materials of algin manufacture and he studied the seasonal, local and specific differences of the components.

(i) *Experimental*

Preparation of samples :

The brown algae for the present study were collected by the author at various localities along the coast of southern Hokkaido and identified by the late Professor Chiyoichi KANDA in 1943. Collected algae were washed with sea water and dried on rocks or straw mats. Dried materials were shattered and analysed.

Analytical methods :

Moisture and ash contents were analysed by ordinary methods.

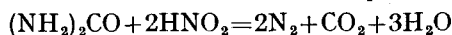
Potassium contents were determined by the author's own method¹⁷⁾ as follows : The ash obtained from 10 g of the material was extracted by hot water and the extract was filled up to 200 cc with water. Ten cc of 1 n tartaric acid solution and 5 cc of n/2 sodium acetate solution were added to 10 cc of the extract which had been pipetted into a conical beaker. After mixing the solution, 12 cc of 95 per cent ethanol was added in the course of 3 minutes, with gentle shaking. Produced $\text{KH}(\text{CHOHCOO})_2$ crystals were collected on a glass filter under reduced pressure and washed several times with 10–15 cc of 67 per cent ethanol. The separated crystals were dissolved in hot water and titrated with 0.1 n NaOH solution. Potassium content was calculated as K_2O by the following equation.

$$\text{K}_2\text{O} \% = a \times 0.47/m$$

where, a is volume (cc) of titrated 0.1 n NaOH solution and m is weight (g) of the sample.

Iodine contents were determined by the author's method¹⁸⁾ as follows : Each 5 cc of 10 per cent H_2SO_4 and 0.5 per cent NaNO_2 solution was added to 25 cc of sample solution, which contained 1–10 mg iodine, in an Erlenmeyer flask with stopper and the mixture was allowed to stand for 5 minutes. Ten cc of 10 per cent urea solution was then added, the flask was stoppered and the solution was shaken gently to mix well and

was allowed to stand for 30 minutes. After that time, the NO_2 reaction with Griess reagent has become negative. Liberated iodine was determined through iodometry with 0.01 n $\text{Na}_2\text{S}_2\text{O}_3$ solution. Principle of above mentioned process is based on the following reaction, where excess of NO_2^- is decomposed by urea.



Chlorine contents were determined by titration with silver nitrate solution as ordinarily, and total halogen contents were calculated as chlorine.

Crude fat and crude protein contents were also determined by ordinary methods.

Mannit contents were determined by the author's improvement of Smit's method¹⁹⁾ to prevent interference by ferric ion. The method was as follows: Each 25 cc of 4 n NaOH solution and 12.5 per cent CuSO_4 solution was added to 50 cc of sample solution and the mixture was shaken for several minutes, and allowed to stand for 15 hours. After that period, 5 cc of 6 n CH_3COOH and 5 cc of 5 per cent KI solution were added to 25 cc of the filtrate. The liberated iodine was determined with 0.01 n $\text{Na}_2\text{S}_2\text{O}_3$ solution, and the amount of mannit was calculated from Smit's table as usual.

Alginic acid was determined as follows: Ten grams of the materials were macerated with 3 g of sodium carbonate anhydride and 250 cc of water on the boiling water bath for 2-5 hours until the weeds were completely disintegrated. Dilute hydrochloric acid solution, (1 : 2), was added to the filtrate after diluting the disintegrated mass with water to 600-700 cc until the alginic acid gel was completely precipitated. The precipitated gel was washed with hot water several times, with 95 per cent ethanol, and weighed after drying to constant weight at 95°C.

(ii) *Results and discussion*

Analytical results are shown in Table 1. From the results the following discussion is suggested.

It is shown that a high content of ash, especially potassium salts, and the existence of alginic acid, mannit and iodine are characteristic of brown algae.

Potassium contents of Laminariaceae are generally higher than that of Fucoaceae. Since the seasonal variation of iodine contents is very remarkable as is described later, the difference in iodine contents among various species can not be here discussed. However, iodine contents are characteristically high in Laminariaceae, amounting to 0.1 per cent in their maximum season.

Since the seasonal variations of crude fat, crude protein and mannit are remarkable as is later described, the difference in their contents among various species can not be here discussed, although the contents of both fat and protein may here be stated to be poor in their maximum period in spring. Mannit contents in brown algae amount to 20-30 per cent in the maximum, while they amount to only several per cent in their minimum season as is described later.

Alginic acid contents in brown algae do not show obvious seasonal variation as is

described later. Alginic acid contents in Laminariaceae are generally larger than those in Fucaceae, that is to say, alginic acid contents amount to about 20–30 per cent in the former and about 15–20 per cent in the latter.

3. Seasonal Variation of Chemical Components

The seasonal variations of the chemical components of *Laminaria japonica*, *L. angustata*, *Alaria crassifolia* and *Costaria costata* were investigated. The materials were collected at a definite station in Muroran Bay.

Results and discussion

The results of the investigation are shown in Figs. 5–11.

Seasonal variations of laminarin and mannit contents of *Eisenia bicyclis* were reported by NISHIZAWA.^{20–22)} He stated that mannit and laminarin of *Eisenia bicyclis* were considered not to be direct photosynthetic products but reserve substances. From this viewpoint, he investigated the variations of mannit and laminarin contents in relation with season and growth stage. LUNDE²³⁾ reported on the seasonal variations of the contents of ash, laminarin, mannit and alginic acid of *Laminaria digitata*. His obtained results are summarized as follows. Laminarin contented to 20 per cent in autumn, but it decreased to almost nothing in spring; mannit content amounted to 20

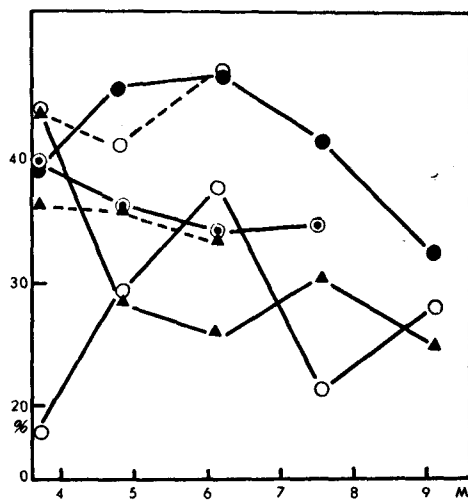


Fig. 5. Seasonal variation of ash content

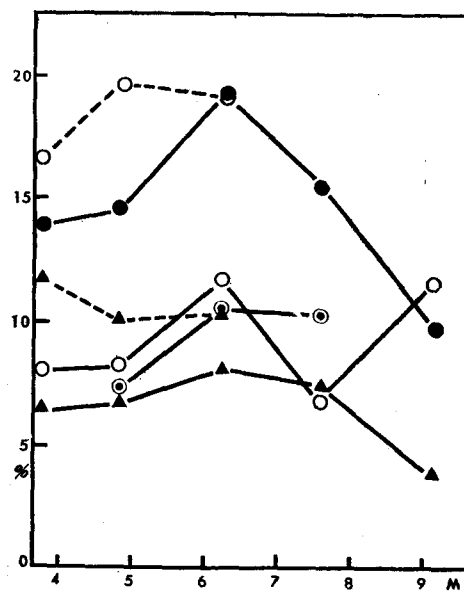


Fig. 6. Seasonal variation of K₂O content

●----- *Laminaria japonica* ARESCH.
▲----- *Alaria crassifolia* KJELLM.

○----- *Laminaria angustata* KJELLM.
⊙----- *Costaria costata* (TURN.) SAUND.

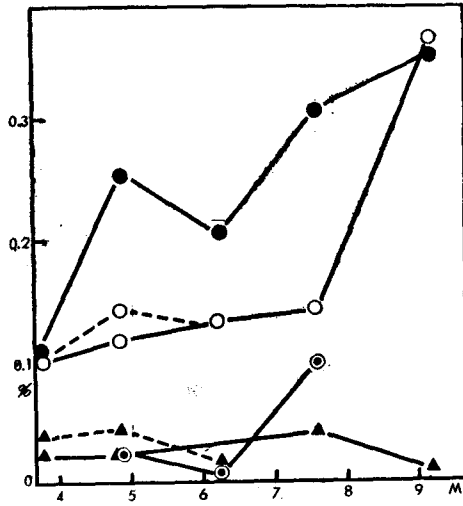


Fig. 7. Seasonal variation of iodine content

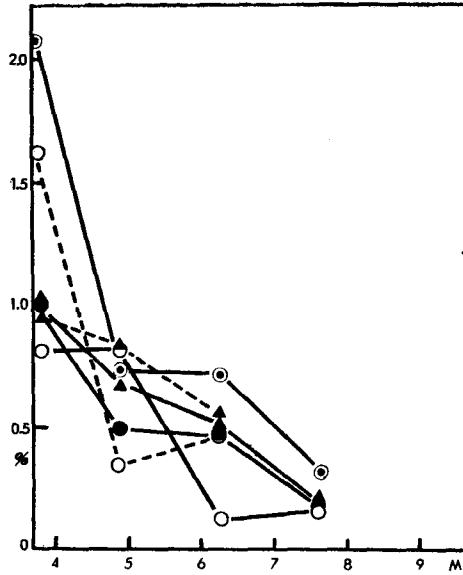


Fig. 8. Seasonal variation of fat content

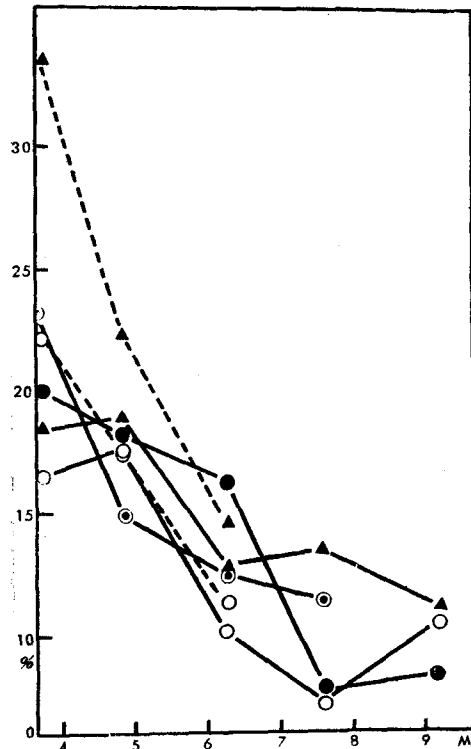


Fig. 9. Seasonal variation of protein content

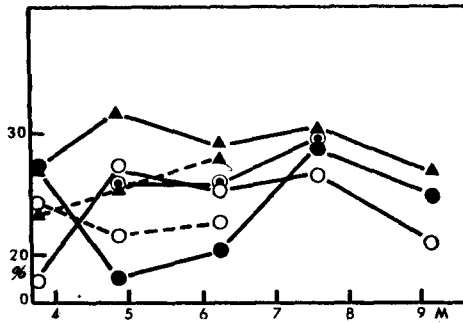


Fig. 10. Seasonal variation of alginic acid content

- *Laminaria japonica* ARESCH.
- ▲----- *Alaria crassifolia* KJELLM.
- *Laminaria angustata* KJELLM.
- ◉----- *Costaria costata* (TURN.) SAUND.

per cent in summer and it decreased to 4-6 per cent in winter. The ash content showed maximum in spring and minimum in autumn. Alginic acid content attained to over 30 per cent in spring and decreased to under 20 per cent in autumn.

From the author's results, the following points are noteworthy. Because the seasonal variations of the chemical components interact upon each other regarding their contents, the absolute amount of each component in the materials can not be discussed but their relative contents can be. Very obvious seasonal variations are observed in the amounts of iodine, mannit, protein and fat. The former two components show minimum amounts in spring and evidently increase toward autumn. On the contrary, the latter two show their maxima in spring and minima in autumn. The above mentioned relation is considered to be significant in the life of brown algae, because the photosynthesis of plants is at its maximum in summer and the metabolic processes are at their minima in winter. The contents of ash, potassium and alginic acid vary seasonally to some degree, but the variations can not be distinguished from the variations which may be caused by alternative relations among other obviously varying components.

Alginic acid, from the above noted results, would be considered a standing component of brown algae because its content does not alter very obviously with season or species. Alginic acid is considered to exist as an intercellular substance in the cell wall of brown algae, and so to play some important role in thier skeleton. As for the role of algin in brown algae, further studies should be made.

II. MECHANISM OF ALGIN MANUFACTURE

Because algin in brown algae exists in various types of combination as described above, it is desirable to convert algin in brown algae into a certain definite form in order to study the mechanism of algin manufacture as well as manufacture algin in practice. It is practically a useful method to pretreat algae with hydrochloric acid or

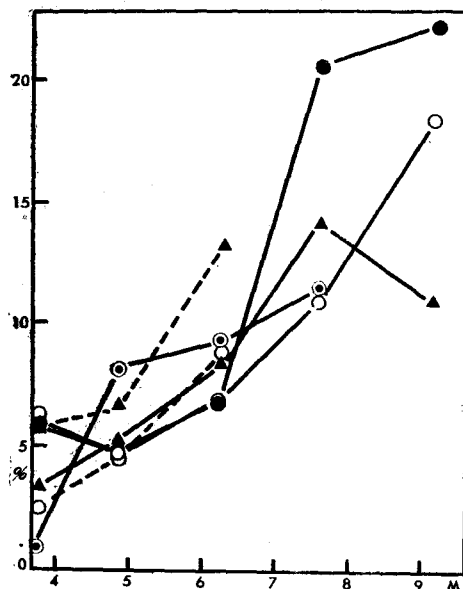


Fig. 11. Seasonal variation of mannit content

- *Laminaria japonica* ARESCH.
- *Laminaria angustata* KJELLM.
- ▲----- *Alaria crassifolia* KJELLM.
- ⊙----- *Costaria costata* (TURN.) SAUND.

calcium chloride solution. Theoretical and practical studies on algin manufacture reported in this paper were carried out by pretreating the material with the above-mentioned reagent solutions and converting the algin in brown algae into free alginic acid and calcium alginate.

1. Dissolution of Free Alginic Acid

It is customary to pretreat algae with 0.1 n hydrochloric acid solution, but that is done as a general practical step without any consideration of converting various types of algin in brown algae into free alginic acid. The pretreatment has usually been made to extract potassium salts, iodine, mannit and pigment or other impurities, but the author proposes to pretreat algae with some certain solution in order to convert algin into a definite type of combination. Having this point in mind, fundamental investigations on the pretreatment of algae with hydrochloric acid were carried out with purified alginic acid.

(i) *Preparation of alginic acid*

Alginic acid was prepared by the author's own method²⁴⁾ as described later, and it was purified well through repetition of dissolution by NaOH solution and of precipitation by HCl solution following the ordinary process, and then dialysed in distilled water till Cl ion reaction was eliminated.

(ii) *Dissociation of saturated alginic acid solution*

The dissociation degree of alginic acid as determined for the first time by PAULI & STENRBACH²⁵⁾ was 1.69~0.270 in the concentrations of alginic acid 59.7~1.6×10⁻³ mol. SEIYAMA²⁶⁾ determined the pK values and dissociation degrees of alginic acid solutions, as 2.87~4.60 and 0.285~0.316 respectively in the concentrations of alginic acid 11.88~0.19×10⁻³ mol. He calculated the above mentioned values from the values of pH and from electric conductivities; the values obtained by both methods agreed fairly well with each other. The author determined the dissociation degree, pH, ionization constant and pK of the saturated solution of alginic acid.²⁷⁾

The concentration of the solution was determined through the titration curves with 0.01 n NaOH solution. The dissociation degrees were calculated from the values of hydrogen ion concentration obtained from pH values and the concentration of alginic acid. The pH values were measured by glass electrode pH meter made by MITAMURA Shoten. The ionization constants were calculated from pH values granting alginic acid to consist of mono-mono-valent mannuronic acid, and then the values of pK were obtained from ionization constants.

The above mentioned values obtained from saturated alginic acid solutions are shown in Table 2. Each solution is prepared by saturating 0.5 g of alginic acid in 100 cc of redistilled water, "A" represents the solution allowed to stand for 20 hours at 25°C after boiling for 30 minutes, "B" represents the solution allowed to stand for an hour at 25°C after boiling for three hours.

Table 2

Solution	concentration 10^{-3} mol	α	pH	K	pK
A	2.776	0.102	3.55	3.55×10^{-5}	4.45
B	16.790	0.095	2.80	1.64×10^{-4}	3.78

The titration curve of the "A" solution is shown in Fig. 12. As the figure shows the titration curve of alginic acid solution is very characteristic. This fact would be due to the colloidal effect of high molecular electrolyte or due to the existence of carboxyl radicals having various dissociations, although that point needs further investigation. For that very reason, the ionization constant described in Table 2 means only the average ionization constant of saturated alginic acid solution. At all events, it may be said that the alginic acid is a fairly weak acid. Alginic acid is rather dissoluble in water, that is to say, about 0.3 g is dissolved in 100 cc of water when boiled for three hours, and thus obtained concentrated solution is not transparent but a protein-solution-like opalesque material.

(iii) *Dissolution of alginic acid by cation exchange reaction*

Since the phenomenon of ion exchange may take place under certain conditions in all ionic solids, an investigation with regard to dissolution of solid alginic acid was attempted because the alginic acid would be dissolved into soluble alginate if cation exchange takes place between the acid and certain electrolytes whose cations are alkali metals, magnesium or ammonium radical.

Alginic acid is a rather weak acid as noted above, but sometimes it behaves like a strong acid as follows: Alginic acid dissolves into soluble alginate when it reacts with many sorts of salts of rather strong acid through double decomposition as described in a previous paper²⁸⁾; also it liberates iodine from the mixed solution of potassium iodide and sodium nitrite as a strong acid does and it liberates sulfur from the solution of sodium thiosulfate too. The above facts may be explained as due to the cation exchange which takes place between hydrogen ion of carboxylic radical of alginic acid

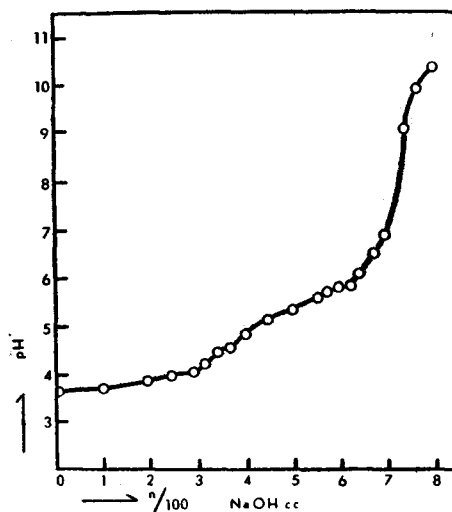
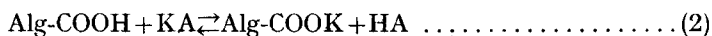


Fig. 12. Titration curve of saturated alginic acid solution "A", with n/100 NaOH

and the cation of the salts.

Corresponding acid of the salt should be produced if the cation exchange reaction would take place between alginic acid and a salt following the reaction (2):



where, "K" represents a cation, and "A" an anion.

A preliminary examination of the above mentioned theory was made as follows: Alginic acid 0.2 g and the equivalent amount of various salts were put into 100 cc of distilled water and the change of pH values were measured after the passage of one hour; pH value without salt was 4.5. The several results are shown as Table 3.

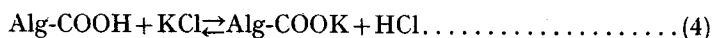
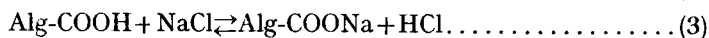
Table 3

Salt	pH	Remarks
CH ₃ COONa	4.2	Alginic acid is dissolved entirely within half an hour.
Na ₂ HPO ₄ *	4.4	Alginic acid is dispersed into small particles and partly dissolved.
C ₄ H ₄ O ₄ Na ₂ *	4.1	Same as above
NaF	4.0	Almost insoluble
HCOONa*	4.1	Alginic acid is dispersed into small particles and partly dissolved.
C ₄ H ₄ O ₆ KNa	4.0	Alginic acid is dissolved entirely within half an hour.
K ₂ CrO ₄ *	4.4	Alginic acid is dispersed into very small particles and slightly dissolved.
C ₂ O ₄ Na ₂ *	3.8	Alginic acid is dispersed into very small particles and partly dissolved.
NaAsO ₂	4.4	Almost dissolved
Na ₂ B ₄ O ₇	4.8	Alginic acid is dissolved entirely within half an hour.
NaCl	3.3	Apparently insoluble
KCl	3.4	Same as above

* See text

The mixed solutions asterisked in the table do not form true transparent colloidal solutions because they retain small particles of algin. However, they precipitate fibrous alginic acid gel by the addition of hydrochloric acid as usual soluble alginate solutions do. If the cation exchange proceeds as an almost or completely irreversible reaction, the dissolution of algin would take place, and if the reaction is almost or partly reversible, such dissolution would not take place, though alginic acid or algin with its cation replaced would be dispersed into small particles. That is to say, the ion exchange reaction between hydrogen ion of carboxylic radical of alginic acid and the cation of salt proceeds until equilibrium is reached.

The cation exchange equilibrium between alginic acid and NaCl or KCl was studied as follows: The cation exchange equilibria may be shown respectively by the following formulas, (3) and (4).



Since hydrochloric acid ought to be produced if the above-mentioned reactions take place, hydrochloric acid was determined by neutralization with sodium hydroxide or by titration of Cl ion. Two-tenth gram carefully purified alginic acid powder prepared after the author's method, was put into conical flask together with the equivalent amount of NaCl or KCl, and 20–80 cc of redistilled water was added. After standing for 20 hours, the cation exchange equilibrium was determined by the following two methods: A) The equilibrium mixtures themselves were distilled by steam until the distillate amounted to 400 cc, and the distillate were titrated with $n/40$ NaOH solution to determine acidity; on the other hand Cl ion of the distillate was determined by titrating with $n/100$ AgNO₃ solution after Mohr's usual method. B) After removal of matter left filtration, acidity and Cl ion of this filtrate were determined in the same way as above.

The purposes of these two experiments were as follows: Experiment A aimed to learn whether the titratable acid was truly hydrochloric acid or not. Experiment B was done to calculate the amount of absorbed electrolyte. The absorbed electrolyte was then calculated by subtracting titrated Cl ion of filtrate for added total Cl ion. The results are illustrated in Figs. 13, 14 and 15.

It may be said that the produced acid is hydrochloric acid on the ground that the titrated acidity agrees with Cl ion content of the distillate as shown in Fig. 13, though the amount of distilled acid is less than that obtained directly from the filtrate as is shown in Fig. 14. Under the experimental conditions, H ion of alginic acid exchanged with Na or K ion of NaCl or KCl though in small amount. That is to say, in NaCl solution about 0.045 to 0.08 mol of H ion of alginic acid is replaced by Na ion for 1 mol of alginic acid by cation exchange, and in KCl solution about 0.03 to 0.04 mol is replaced. The absorbed amount of electrolyte differs in the two cases, NaCl and KCl, as shown in Figs. 14 and 15. In NaCl solution, alginic acid scarcely absorbs the salt, whilst it does in KCl solution to as much as 0.48 to 0.55 mol for 1 mol of the acid.

2. Dissolution of Calcium Alginate

(i) Preparation of calcium alginate

MIWA¹⁵⁾ investigated some properties of calcium alginate prepared from *Undaria pinnatifida*. The calcium content in the calcium alginate prepared by him was 9.5 per cent although the theoretical calcium content in calcium alginate calculated from the formula $C_6H_7O_6 \cdot 1/2Ca$ was 10.25 per cent. He also prepared calcium alginate adding excess amount of calcium chloride solution to sodium alginate and dialyzing the calcium alginate gel until the reaction of Cl ion had disappeared. Calcium alginate thus obtained contained 21 per cent Ca, but Cl ion reaction was not found in this calcium alginate. From the above mentioned fact, he suggested that calcium would be combined not only with carboxyl radical of alginic acid but with its OH groups too. He did not recognize any remarkable difference concerning the solubilities of both

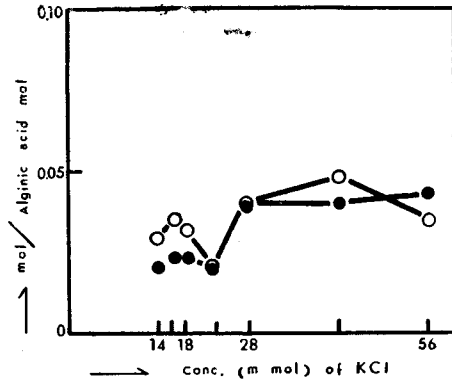


Fig. 13. Titrated acidity and chloride ion content of the distillate after method "A", where NaCl coexists with free alginic acid

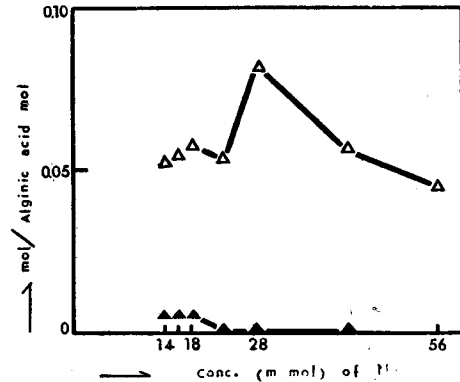


Fig. 14. Amounts of exchanged H ion and of the absorbed NaCl

○----- Titrated acidity
●----- Chloride ion content

△----- Amount of exchanged H ion
▲----- Amount of absorbed NaCl

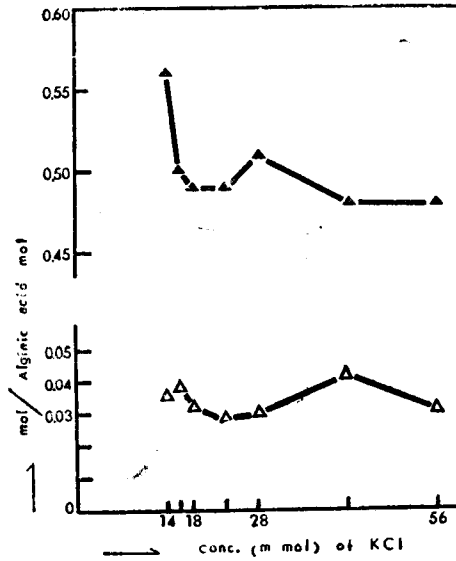


Fig. 15. Amounts of exchanged H ion and of the absorbed KCl
△----- Amount of exchanged H ion ▲----- Amount of absorbed KCl

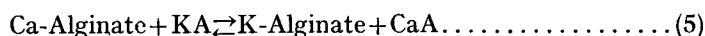
preparates against sodium carbonate, sodium hydroxide and ammonium hydroxide solutions.

The author also obtained several preparates of calcium alginate containing calcium to as much as 7 to 20 per cent. They were prepared by adding various

amounts of 5 per cent calcium acetate solution to sodium alginate solution and dialyzing in distilled water until Ca ion reaction in dialyzing water had disappeared. The preparates thus obtained were dehydrated by ethanol and washed with ether and dried at 95°C. Since no remarkable difference was recognized concerning their solubilities as MIWA described in the range of calcium content 9.7 to 10.5 per cent, the preparates containing calcium in the range of 9.8 to 10.5 per cent were used for ascertaining the mechanism of manufacturing algin from calcium alginate.

(ii) *Dissolution of calcium alginate by cation exchange reaction*

The cation exchange reaction between calcium alginate and an electrolyte should take place following the formula (5).



where, "K" represents a cation, and "A" an anion.

Calcium alginate would be dissolved into soluble alginate, if the above described reaction (5) is almost or perfectly irreversible and "K" belongs to a certain alkali metal or to ammonium radical or to magnesium. Those KA were previously reported¹⁹⁾ as shown in Table 4.

Table 4

Na ₂ CO ₃ ;	Na ₂ HPO ₄ ;	Na ₃ PO ₄ ;	(COONa) ₂ ;	Na ₂ SO ₃ ;	NaF;	Na ₂ S ₂ O ₃ ;
KIO ₃ ;	KSiO ₃ ;	Rochelle salt;	K ₄ Fe(CN) ₆ ;	etc.		

It is considered that the reaction (5) would be made irreversible if produced CaA is removed from the system of the reaction. There are two procedures by which that removal may be effected. One is as follows: Produced CaA is removed by precipitation because its solubility product is scanty. The other is as follows: Produced CaA is removed by the formation of a complex salt between CaA and KA; examples of this type are Na₂S₂O₃ and rochelle salt in the table. In the former case a turbid solution is obtained because of the precipitation of CaA and in the latter, on the contrary, a transparent solution is obtained because of the formation of a soluble complex salt. The dissolution would not take place if the reaction is almost or perfectly reversible even though "K" belongs one of alkali metals, ammonium radical or magnesium. The cation exchange equilibrium between calcium alginate and sodium chloride solution was investigated as follows.²⁴⁾

Portion of calcium alginate, each 0.7667 g as anhydride, were put into 100 cc of water, various amounts of sodium chloride were added and the mixtures were boiled for 30 minutes. After boiling, the mixtures were filtered and washed. The dissolved algin, calculated as sodium alginate, and Ca content were determined with the results shown in Table 5.

Because Ca content of the investigated calcium alginate was 79.74 mg, if the

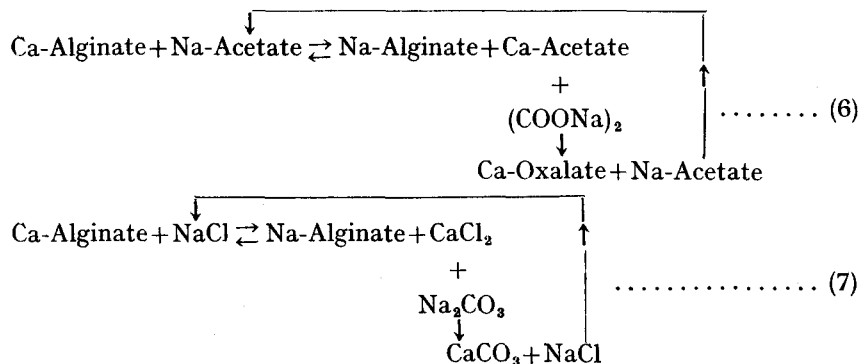
Table 5

NaCl added (g)	0.10	0.20	0.30	0.50
Dissolved Ca (mg)	7.36	12.27	14.89	15.81
Dissolved algin (mg)	25.6	44.3	45.2	73.7

calcium alginate is converted into sodium alginate entirely by cation exchange, 774.5 mg of sodium alginate would be produced. When 0.50 g of NaCl was added, 19.8 per cent of Ca was dissolved and it was calculated that 9.5 per cent of calcium alginate was converted into sodium alginate in this condition. The reason why the dissolved Ca content is larger than the dissolved algin content which is calculated as sodium alginate, is considered to be that the dissolved Ca content involves both what is produced by cation exchange and what is combined with soluble algin.

It is well known that the inverse reaction takes place easily, that is to say, calcium alginate is ordinarily gelled out by adding excess amount of calcium chloride solution to sodium alginate solution. This fact is usually applied to obtain calcium alginate both in laboratory and in commercial practice. Calcium alginate gel thus obtained is very contracted, but the gel is remarkably swollen when it is treated with sodium chloride solution by the cation exchange reaction.

The cation exchange equilibrium (5) would proceed to produce sodium alginate as described above, if the produced calcium salt is removed from the system of the reaction by any procedure. The examples of those attempts are illustrated in formulas, (6) and (7), as follows :



Algin may be manufactured with smaller amount of sodium oxalate or sodium carbonate by employing methods which follow the formulas above stated. The new recommendable process is described later applying a principle based upon the formula (7).

3. General Theory Concerning the Process of Algin Manufacture

(i) *General theory concerning the process of algin manufacture*

It has been mentioned above that alginic acid or insoluble alginates are dissolved into soluble alginate by cation exchange reaction. The general theory of the process of algin manufacture may be described as follows.

The cation exchange equilibrium between insoluble algin, such as alginic acid or insoluble alginate, and an electrolyte concerned with the dissolution of algin is presented in the following formula (8) :



where "X" represents a cation which forms an insoluble algin combining with Alg-COO^- , such as H or Ca, and "M" a cation which forms a soluble algin combining with Alg-COO^- , such as an alkali metal, ammonium radical or magnesium, and "A" an anion.

An insoluble algin is dissolved into soluble algin when the above reaction (8) is almost or perfectly irreversible. That is to say, the dissolution of algin takes place in the special case of cation exchange reaction. Practical application of this phenomenon to algin manufacture is described later.

(ii) *Dissolution by cation exchange reaction of insoluble algin*

Only a few data have been published regarding the cation exchange reaction of insoluble algin. SEIYAMA²⁰⁾ reported the effect of neutral salts on the dissociation of alginic acid. He concluded that the effect of neutral salts on that dissociation was dependent upon the ion exchange reaction. He also calculated the ion exchange equilibrium constant, and from the results of the calculation he reported that the ion exchange reaction did not occur quantitatively. He used sodium chloride solution and free alginic acid in his research and obtained the ion exchange equilibrium exponent pK_2 in the range of 1.5~3.0 for the concentration of alginic acid, as $-\log C$, 2.0~4.5.

The author also obtained pK_2 in company with solubility, pH, α and pK values. The experiments were undertaken as described in this chapter keeping equilibrium of the reaction for 20 hours at 25°C, adding various amounts of NaCl or KCl. Results are shown in Table 6.

Table 6

Added electrolyte $-\log C$	Solubility of alginic acid $-\log C$	pH	α	pK	pK_2
without electrolyte*	2.93	4.45	0.029	6.00	—
NaCl 1.85	3.07	3.85	0.162	4.56	2.76
1.55	2.95	3.80	0.140	4.59	3.15
1.37	2.96	3.50	0.289	3.90	2.57
KCl 1.85	3.10	3.75	0.222	4.30	2.53
1.55	3.09	3.75	0.212	4.31	2.85
1.37*	2.96	3.15	0.648	2.89	1.40

The titration curves of the equilibrium solutions denoted with asterisks in the table are shown in Figs. 16 and 17. The curves were obtained by titrating with $n/100$ NaOH after removal of the matter left after filtration. It is discussed through above mentioned results as follows.

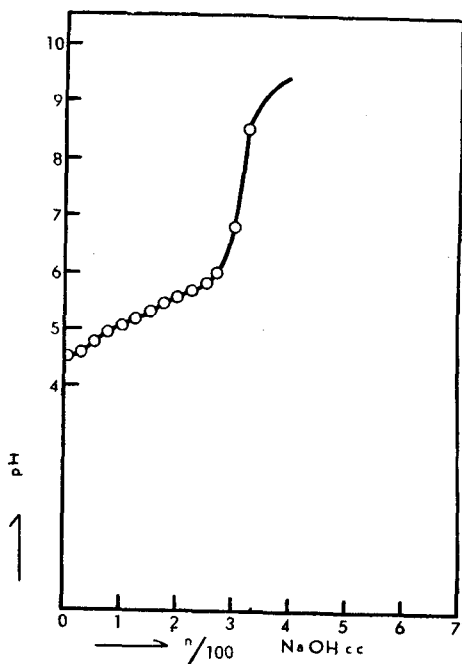


Fig. 16. Titration curve of saturated free alginic acid solution with $n/100$ NaOH allowed to stand for 20 hours at 25°C

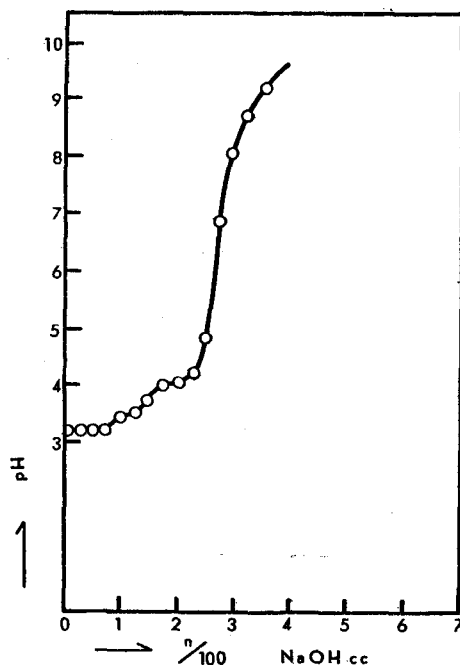


Fig. 17. Titration curve of saturated free alginic acid solution, where KCl coexists with the acid, allowed to stand for 20 hours at 25°C

The dissociation degree of the equilibrium solution is remarkably increased by addition of electrolyte, especially of KCl. This fact shows that the hydrogen ion of alginic acid was released to the solution as is shown by the decrease of pH. The author has calculated the cation exchange equilibrium exponent after SEIYAMA's equation, although the mechanism of ion exchange of alginic acid is considered very complicated and it should be further studied how to present the exponent. At all events, it may be said that the cation exchange reaction between alginic acid and electrolyte, NaCl or KCl, has taken place because pH, pK and pK_2 decrease and the dissociation degree of the solution is increased by addition of the electrolyte. It is then considered that the cation exchange reaction between free alginic acid and the salt of a weaker acid easily takes place and the alginic acid is dissolved into soluble alginate, though the reaction between free alginic acid and the salt of strong acid such as sodium chloride or

potassium chloride proceeds very slightly.

It is already noted above that the titration curve of saturated alginic acid solution was very characteristic and the reason for this fact was considered to be the existence of carboxyl radicals having various dissociation constants or the certain colloidal effects. The characteristic of the titration curve did not disappear by adding electrolyte to such a dilute alginic acid solution as shown in Fig. 16 or 17 and, therefore, the existence of carboxyl radicals having different dissociations is considered to be fairly possible although further investigations are needed because the colloidal effect would be eliminated by adding a fairly large amount of electrolyte to a dilute alginic acid solution.

Recently, results of researches on absorption of electrolyte by alginate were reported by WASSERMANN and his collaborators³⁰⁻³²⁾ and it was referred to the cation exchange of insoluble alginate which is concerned with the absorption of electrolyte by fully-swollen alginate fibres.

They reported in a series of papers that a curling up of stretched, chain like, giant molecules, and the accompanying macroscopic effects, could be brought about by stoichiometrically well-defined simple metatheses. The papers dealt with the preparation of fully-swollen, cylindrical alginic acid and alginate gels and with the absorption of simple electrolytes by these materials, but no attention was paid to the matter of algin manufacture.

As already mentioned, algin was first extracted by STANFORD¹⁾ from a seaweed with sodium carbonate and since then a few works on algin manufacture have appeared. DILLON²⁾ proposed to use ammonia, and TAKAHASHI¹⁰⁾ devised a new method for algin manufacture in which he digested with ammonium oxalate. However there are no other available works on manufacturing algin from brown algae.

As is described in this paper, it is established that many sorts of electrolytes can be used for algin manufacture after a proper pretreatment.

III. NEW RECOMMENDED PROCESSES FOR ALGIN MANUFACTURE

Since algin occurs in various combined forms in brown algae as has been described above, it is desirable to pretreat algae with some certain proper reagent solution in order to convert the algin into a definite form, because algin can be extracted by suitable manufacturing process according to the pretreatment. The author discovered that many sorts of electrolytes are available for the extraction of algin from brown algae as described above. From among them two recommendable processes are described in this chapter.

1. Algin Manufacture from Brown Algae Macerated with Sodium Sulfit

It has been customary pretreat brown algae with dilute hydrochloric acid solution. This treatment is used to make algin easily dissoluble in actual practice and to extract potassium salts, iodine, mannit and pigment or other impurities, although little consideration has been given to converting algin into free alginic acid.

Since it is considered that algin in brown algae is converted almost entirely into free alginic acid by pretreating with dilute hydrochloric acid, tests have been conducted to ascertain whether that theory (p. 110) is applied in practice or not. The electrolytes shown in Table 7 have usable for manufacturing algin from brown algae which had been pretreated with dilute hydrochloric acid solution and the applicability of the theory was ascertained as described below :

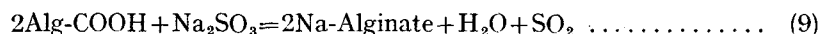
Table 7

M_3PO_4 ;	M_2HPO_4 ;	MH_2PO_4 ;	M_2SO_3 ;	$MHSO_3$;	$M_2S_2O_3$;	Rochelle salt ;
MNO_2 ;	$HCOOM$;	CH_3COOM ;	$M_2B_4O_7$;	M_2CO_3 ;	$MHCO_3$;	MOH ;
M_2S_{2+x} ;	MBO_3 ;	M_2CrO_4 ;	M_2O_2 ;	MCN ;	etc.	

where "M" represents a cation, alkali metal, ammonium radical or magnesium.

It was observed that there are two cases of dissolution of alginic acid with the electrolytes shown in the table. One is the case that the reaction proceeds keeping acid, and the other is the case that the reaction proceeds alkaline. The reason is obvious that the reaction (2) proceeds keeping acid when "A" is an acid radical, while the reaction proceeds keeping alkaline when "A" is OH^- or CO_3^- . Since the latter represents the case of dissolution by neutralization, it may be called a peculiar case of cation exchange reaction which is perfectly irreversible. That is to say, including the dissolution by neutralization, the dissolution of alginic acid by electrolytes takes place through cation exchange reaction.

Among the electrolytes enumerated in the table, Na_2SO_3 is a very highly recommendable one, because the macerated mass or diluted solution is restrained from decomposition by microorganism and algin is bleached by SO_2 which is generated in the process of the reaction (9) :



Furthermore, the reaction is almost perfectly irreversible because the produced SO_2 would escape by heating of the system.

A practical method of manufacturing algin from brown algae with sodium sulfite is briefly described as follows.

In order to convert various types of algin in brown algae into free alginic acid, the algae are treated with 0.1 n hydrochloric acid solution for 15-30 minutes at 50-60°C.

After removal of acid solution, 15 per cent of sodium sulfite by weight to dried algae and 30 times that weight of water are added to the remaining algae and heated for an hour at 60-70°C, then the bodies of the plants are completely disintegrated. Further treatment may be performed as in an ordinary method.

2. Algin Manufacture from Brown Algae Macerated with Sodium Chloride and Sodium Carbonate(SUZUKI'S Method)

The above described theory, in Chapter II, was ascertained as applicable practically for manufacturing algin from brown algae which are pretreated by calcium chloride solution in order to convert algin in the plant body into calcium alginate, and a new recommendable process was established by the author.²⁴⁾

The process is shown in diagrammatically in Fig. 18. The author proposes to name this "SUZUKI'S Method" which was established by fundamental investigation briefly recounted as follows.

The investigation was made with *Laminaria angustata*. The material was first shattered to small pieces, about 5×5 mm in size. After submitting these pieces successively to each treatment described below, frozen microtome sections of them were stained with one per cent methylene blue solution in alcohol and examined under microscope. The results are shown in Figs. 19-31. The structure of the initial material is shown in Figs. 19 and 23. The process of each treatment is described as follows.

(i) *Swelling with water*

The material was treated with boiling water for 30 minutes in order to induce swelling of the tissue and to extract soluble matters, such as potassium chloride, iodine, mannit or impurities. The structure of the material thus treated is shown in Figs. 20 and 24. The parenchymatous cells are rather contracted by this treatment although the sections are swollen as a whole. It is considered that insoluble alginate in the walls of the parenchymatous cells, such as calcium alginate is contracted by boiling in water as described above.

(ii) *Steeping in calcium chloride solution*

After the conclusion of the preceding treatment, the water was drained, and the material was treated with 0.5 per cent calcium chloride solution at 100°C for 30 minutes. The structure of the material thus treated is shown in Figs. 21 and 25. Algin in the cell walls would be converted into calcium alginate almost entirely. The plant tissues are obviously contracted by this treatment and the epidermal tissues are made so firm by calcium chloride solution that they would not be destroyed by further treatment with either sodium chloride or sodium carbonate solution as described later.

(ii) *First digestion with sodium chloride solution*

The liquor used for the second treatment was thrown away, then the material was washed with water and was treated with 0.5 per cent sodium chloride solution at 100°C

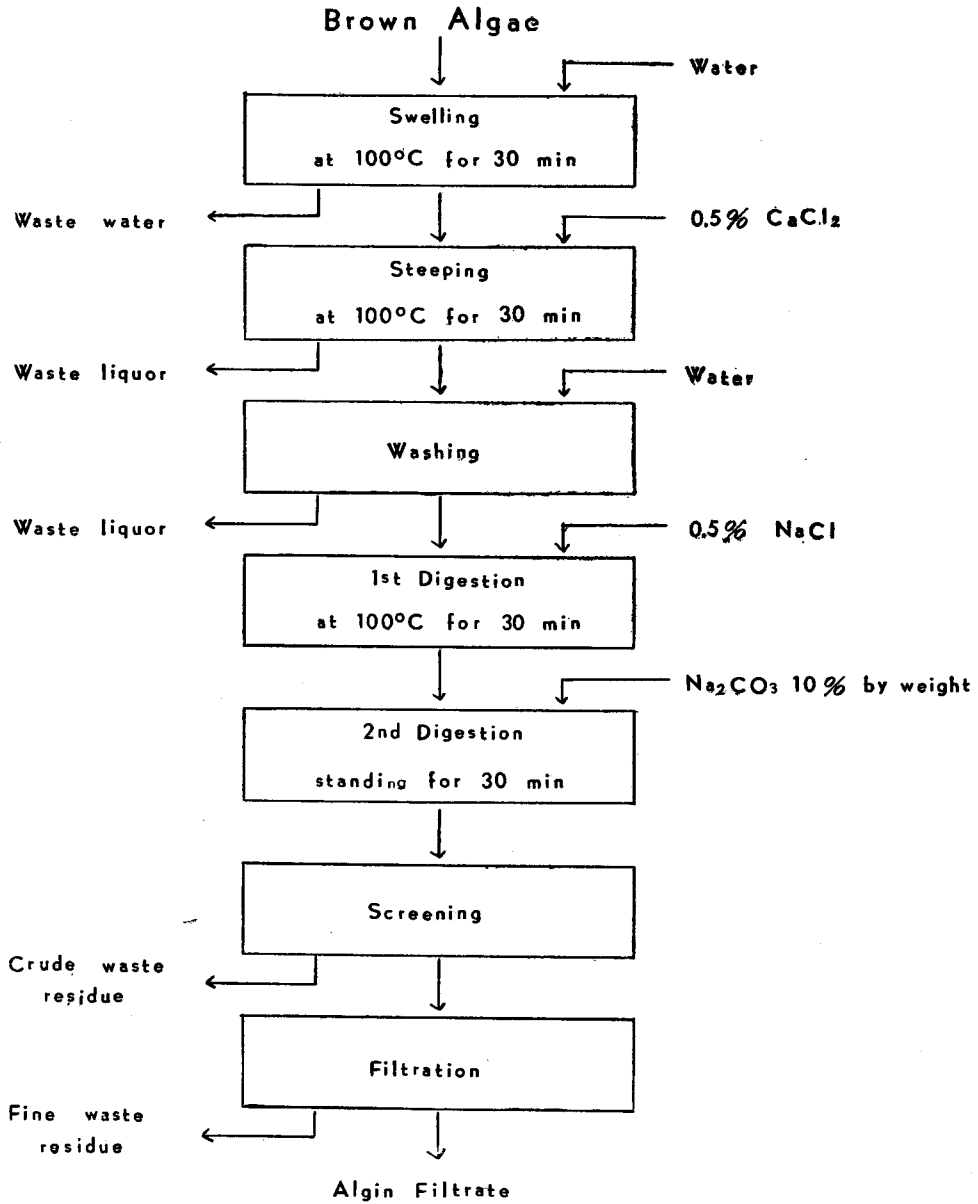


Fig. 18. Diagrammatical illustration of Suzuki's method

for 30 minutes. The tissues became greatly swollen and the cell walls of the inner parenchymatous tissue were partly destroyed. However, the sections continued to maintain their original shapes as a whole as shown in Fig. 22. The reason of this may be explained by above mentioned cation exchange reaction. That is to say, calcium alginate in the cell walls is partly dissolved out from the cells, while the epidermal

cells are made firm by the previous treatment with calcium chloride solution. The epidermal cells do not suffer damage by present treatment whilst viscous algin solution is dissolved out from the material.

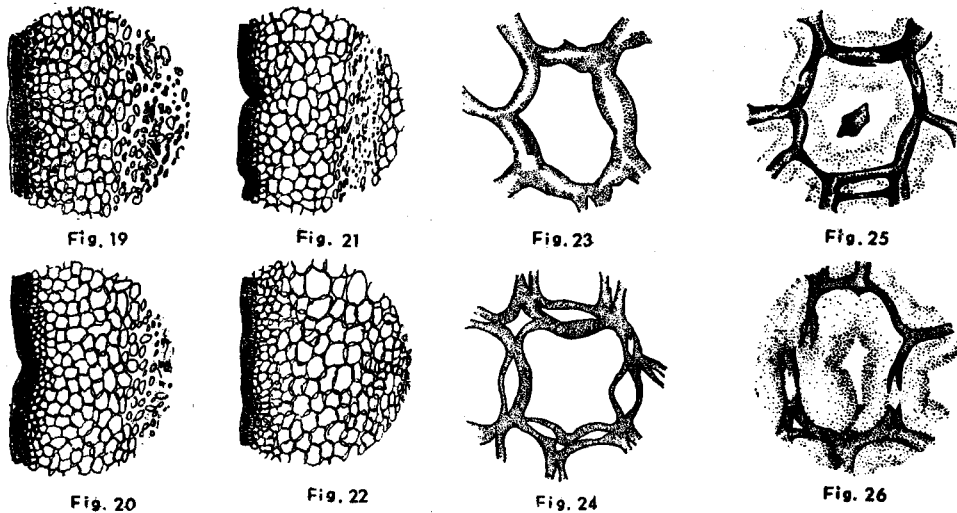
(iv) *Second digestion with sodium carbonate*

The liquor used for preceding treatment was not cast away this time, but 0.1 g of sodium carbonate was added to the digesting mixture at 90°C and the mixture was allowed to stand for 30 minutes. The results are shown in Figs. 27-31. The epidermal tissue suffers hardly any damage by the present treatment as shown in the figures while algin in parenchymatous and filamentous tissues is dissolved. The reason why the epidermal tissue does not suffer damage by treatment with sodium carbonate is considered to lie in the fact that the algin is preliminarily dissolved in part and the algin is well swollen by treating with sodium chloride solution; algin is very easily dissolved by sodium carbonate solution within a short time, because the epidermal tissue is not destroyed under such a mild contact with sodium carbonate.

The same treatments as described above were carried through with *Laminaria longissima* and *L. ochotensis*; the same results as in the case of *L. angustata* were attained also with these species.

Since it is proved that algin can be manufactured by the above described procedure without destroying epidermal tissue and hence without dissolving out the pigment from the assimilatory cells, a practically recommendable method for algin manufacture is developed as follows.

Treat the dried brown algae with boiling water for 15-30 minutes to swell the plant body and remove soluble matters and other impurities. Drain the water, and treat the algae with 0.5 per cent calcium chloride or saturated calcium hydroxide solution for 15-30 minutes at 80-100°C to convert algin in the cell walls into calcium alginate and to make the pigment in the epidermal cells insoluble. Cast away the liquor, and treat the algae with 10 per cent sodium chloride by weight to dried algae and 30 times that weight of water for 30-60 minutes at 80-100°C. Within this period calcium alginate in the cell walls is swollen by cation exchange producing soluble algin, while the pigment is retained in the plant body. Do not cast away the liquor; macerate the remaining bodies of the algae with sodium carbonate added to as large amount as 10 per cent by weight of the original dried algae for 10-20 minutes with gentle stirring. Allow to stand for several hours so as to make maceration complete; the pigment and the epidermal cells would remain undissolved and undamaged. The colour of the algin thus obtained is almost white without any bleaching measures and its viscosity is very high owing to the weak attack of alkali solution. The filtration is very easily performed because epidermis and mucilagenous substance excepting algin are scarcely dissolved.



- Fig. 19. Section through a sample of non-treated material
 Fig. 20. Section through a material treated with boiling water for 30 minutes
 Fig. 21. Section through a material treated further with 0.5% CaCl_2 solution at 100°C for 30 minutes
 Fig. 22. Section through a material treated further with 0.5% NaCl solution at 100°C for 30 minutes
 Fig. 23. Part of the inner parenchymatous tissue in Fig. 19. Magnified
 Fig. 24. Part of the inner parenchymatous tissue in Fig. 20. Magnified
 Fig. 25. Part of the inner parenchymatous tissue in Fig. 21. Magnified
 Fig. 26. Part of the inner parenchymatous tissue in Fig. 22. Magnified

IV. STUDIES ON PRETREATMENT OF ALGAE

It has been made clear that pretreatment of the brown algae for algin manufacture should be made to meet the following two purposes. One is to extract mineral salts, mannit or impurities, and the other is to convert algin in algae into a definite state of combination. To achieve these purposes, two kinds of pretreatments have often be combined, for example, the first treatment aiming at removal of soluble matter from the algae is followed by the second treatment aiming at conversion of algin in brown algae into a definite form of combination.

As for the pretreatment of brown algae, there have been published only a few reports. The above mentioned pretreatment methods³⁾⁻⁶⁾ were originally proposed as a measure to obtain iodine from extracted liquor. According to TAKAHASHI⁹⁾ dilute hydrochloric acid is the most suitable solution to pretreat algae in order to extract mineral salts and mannit but potassium salts are not so well extracted as the other soluble matters are. He suggested that potassium existed party combining with alginic acid.

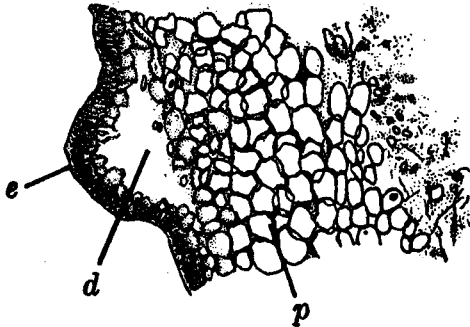


Fig. 27

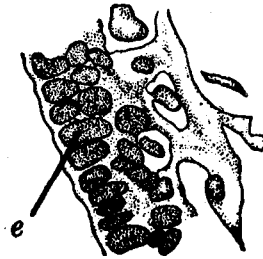


Fig. 28

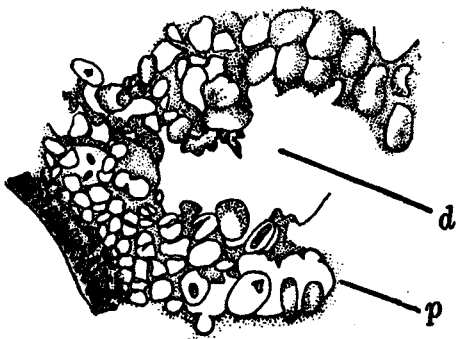


Fig. 29

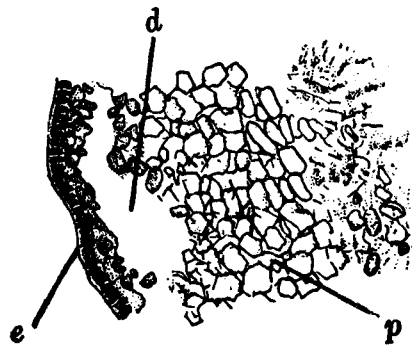


Fig. 30

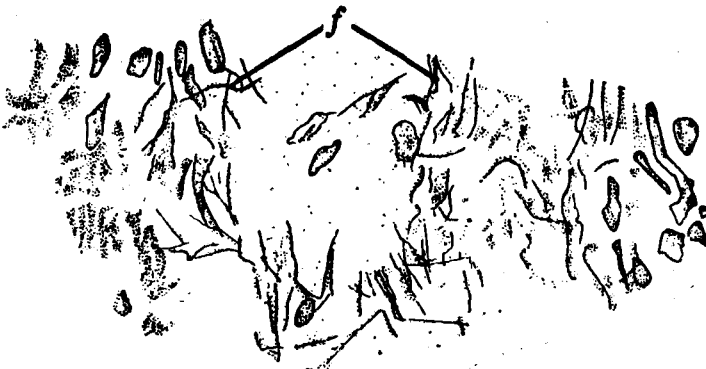


Fig. 31

Figs. 27-31. Section through the material treated further with 0.5% Na_2CO_3 at 90°C for 30 minutes

- d, Damaged spaces due to the digestion of the tissue
- e, Epidermal tissue, not damaged
- f, Filamentous tissue, disintegrated with scattered residues
- p, Parenchymatous tissue, half damaged

The author's own investigation on pretreatment of algae mainly with fresh materials was carried on as described below.

1. Experimental

To learn the swelling of the algal fronds when soaked in water or other solutions the following experiments were made.

(i) Ten grams of dried material and 50 g of wet material of *Laminaria japonica* were respectively placed in a beaker and 150 cc of boiled water was poured into it. The beaker was heated at the boiling point of the contained solution for 5-60 minutes and the water absorbed by the material was determined as shown in Table 8.

Table 8

Material	Duration of treatment (min)	Absorbed water to the material (%)
Wet	5	58
	10	44
	20	18
	30	26
	60	20
Dried	5	280
	10	258
	20	288
	30	336

(ii) Ten grams of dried materials of *Laminaria longissima* and *L. religiosa* respectively were treated with 300 cc of water at 100°C, with 300 cc of 0.1 n hydrochloric acid at 40°C and with 300 cc of 1 per cent calcium chloride solution at 60°C for 15 minutes and the absorption of treated liquor was determined as shown in Table 9.

Table 9

Material	Treatment reagent	Swelling (%)
<i>L. longissima</i>	Water	380
	0.1 n HCl	50
	1% CaCl ₂	340
<i>L. religiosa</i>	Water	310
	0.1 n HCl	30
	1% CaCl ₂	340

From the results is known the fact that wet fresh algae do not absorb the liquor used for treatment so much as dried samples do, and the pretreatment with dilute hydrochloric acid results in minimum swelling of the dried material.

(iii) Wet materials of *Laminaria japonica* 200 g in weight, were placed in a beaker and 600 cc of water or either of the solutions mentioned below were added at 100°C and

boiled for 5 minutes, after which the soaked algae were taken away and fresh materials 200 g in weight, were put into the extract and extraction was repeated in the same way. The extraction was thus repeated from three to seven times, with precaution to keep the volume of the extract constantly at 600 cc by adding water or solution. For this treatment use was made of water or any one of the following solutions, viz., 0.1 n hydrochloric acid, 1 per cent calcium chloride solution, 0.005 per cent NaOH, 0.001 per cent NaOH, 0.01 per cent Na_2CO_3 , sea water, or a mixture of fresh water and sea water. The results of three experiments which were performed independently of each other are shown respectively in Tables 10, 11 and 12.

Table 10

Treated agent	Number of times of treatment	Concentration of extract (%)			Recovery (%)		
		K ₂ O	Iodine	Mannit	K ₂ O	Iodine	Mannit
Water	1	0.319	0.012	0.027	88.2	58.5	37.8
	3	0.714	0.028	0.143	65.7	46.0	66.6
	5	1.100	0.079	0.271	60.8	76.1	75.9
	7	1.309	0.080	0.421	51.7	55.0	84.3
0.1 n HCl	1	0.311	0.009	0.068	85.9	43.8	95.2
	3	0.814	0.024	0.152	74.9	39.6	70.8
	5	1.106	0.044	0.243	61.1	36.3	68.0
	7	1.249	0.051	0.406	49.3	35.4	81.2
1% CaCl ₂	1	0.334	0.020	0.053	92.5	98.7	74.2
	3	0.646	0.036	0.174	59.5	58.5	81.2
	5	1.149	0.076	0.286	63.5	73.2	80.1
	7	1.393	0.067	0.374	54.9	46.0	74.8

Table 11

Treated agent	Number of times of treatment	Concentration of extract (%)			Recovery (%)		
		K ₂ O	Iodine	Mannit	K ₂ O	Iodine	Mannit
Water	1	0.383	0.005	0.050	80.2	100.0	45.8
	3	0.914	0.010	0.299	64.2	66.8	63.9
	5	1.182	0.018	0.347	59.1	56.0	57.6
	7	1.279	0.025	0.395	39.0	—	51.4
0.001% NaOH	1	0.311	0.004	0.060	65.5	92.0	45.8
	3	0.900	0.010	0.248	63.2	66.8	55.7
	5	0.919	0.013	0.277	42.9	76.0	51.4
	7	1.254	0.023	0.416	37.7	60.0	45.4
0.005% NaOH	1	0.359	0.004	0.052	75.6	100.0	44.0
	3	0.993	0.010	0.190	46.7	66.8	87.6
	5	1.062	0.019	0.292	53.2	72.0	61.1
	7	1.120	0.021	0.361	33.7	57.2	49.6
0.01% Na ₂ CO ₃	1	0.426	0.005	0.052	89.7	92.0	52.8
	3	0.890	0.010	0.218	62.5	66.8	72.7
	5	1.240	0.014	0.327	52.2	52.0	48.8
	7	1.350	0.036	0.409	40.6	65.6	52.3

Table 12

Treated agent	Number of times of treatment	Concentration of extract (%)			Recovery (%)		
		K ₂ O	Iodine	Mannit	K ₂ O	Iodine	Mannit
Water	1	0.239	0.035	0.080	71.9	93.0	50.9
	3	0.541	0.109	0.312	44.2	97.2	65.5
	5	0.880	0.087	0.455	53.0	46.7	57.5
	7	1.058	0.129	0.380	45.5	49.4	34.3
Sea water 1: water 2	1	0.320	0.030	0.114	96.4	81.3	71.8
	3	0.550	0.081	0.435	65.1	71.7	91.4
	5	0.981	0.107	0.475	59.0	57.4	60.0
	7	1.172	0.140	0.517	50.4	53.1	46.5
Sea water 1: water 1	1	0.320	0.038	0.072	96.4	100.0	45.6
	3	0.617	0.065	0.339	61.9	58.4	71.3
	5	0.947	0.087	0.558	57.0	46.7	70.3
	7	0.995	0.129	0.668	42.8	49.4	60.1
Sea water	1	0.296	0.035	0.055	89.2	93.0	35.2
	3	0.651	0.065	0.254	65.2	58.4	53.6
	5	1.077	0.118	0.414	64.8	63.2	44.4
	7	1.172	0.118	0.428	50.4	45.1	38.6

2. Discussion

(i) *Swelling of the algae*

The extractive power of a treating solution is greatly affected by its power to cause the algae to swell. It is proved that a dried algal material absorbs water to as much as about three to four times, and calcium chloride solution to as much as 2.5–3.5 times its own weight within the short duration of the treatment. However, a wet material does not absorb solutions above 50 per cent of its weight. Dilute hydrochloric acid is absorbed only as much as 50 per cent of the weight even by a dried algal material.

(ii) *Extractive power of the solution*

The curves of the increase in concentration of K₂O, iodine and mannit in the treated solution are shown in Figs. 32–42. From these figures it is known that the concentration of the extract does not increase linearly by the repetition of the treatment in every case though concentration of the soluble matter is observed to a considerable extent.

The yield of the soluble matter by different treatments of the raw materials are shown in Figs. 43–51. From Figs. 43, 46 and 49 it is learned that each treatment shows remarkable difference of power to extract potassium. In respect to the power of treating solutions to extract iodine some differences are shown amongst them and also there is fluctuation in some cases as shown in the figures. The minimum extractive power is observed in the case of the pretreatment with dilute hydrochloric acid solution and the maximum in the case of alkali treatment. The mannit is known to be less soluble than potassium chloride and iodine under the condition described above.

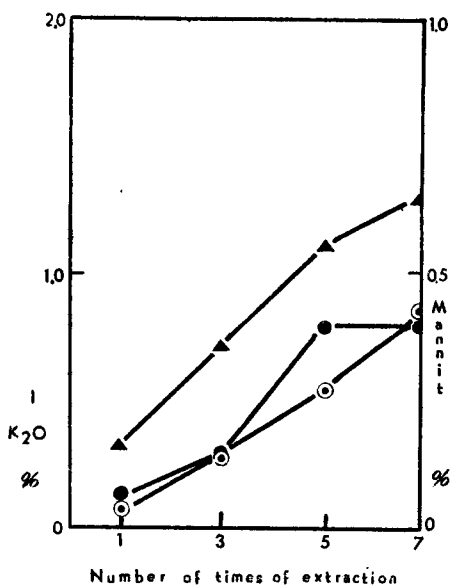


Fig. 32. Extracted with water

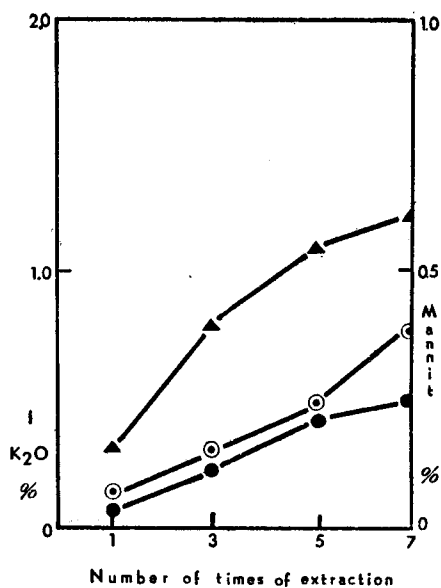


Fig. 33. Extracted with 0.1N HCl

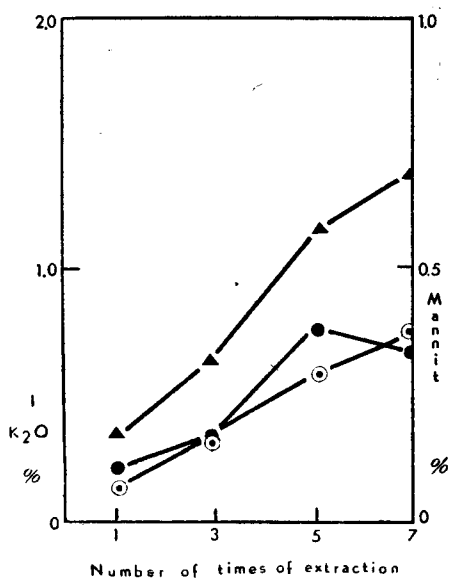


Fig. 34. Extracted with 1% CaCl₂

Figs. 32-34. Concentration of soluble matters in the algal extract (1)

▲----- Concentration of K₂O (%) ●----- Concentration of iodine (% × 10)
 ⊙----- Concentration of mannite (%)

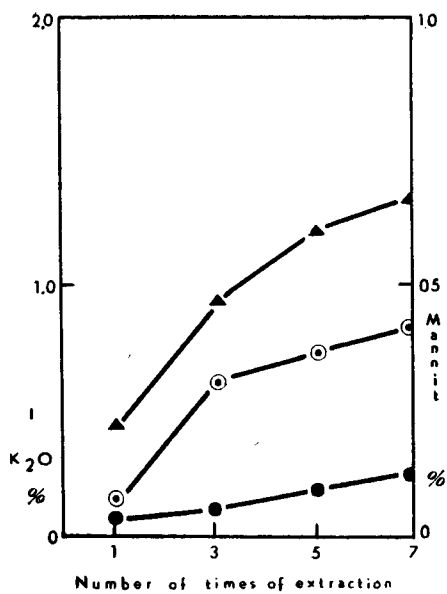


Fig. 35. Extracted with water

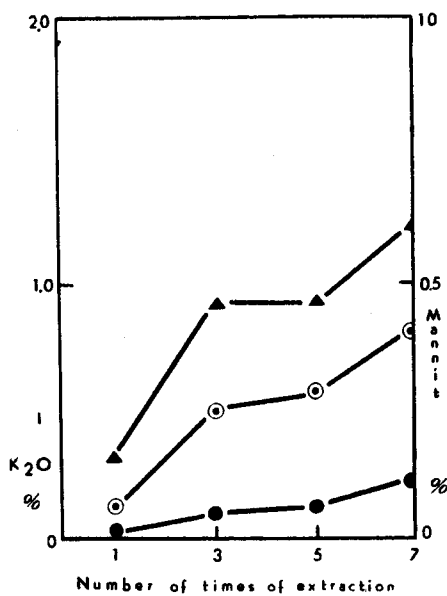


Fig. 36. Extracted with 0.001% NaOH

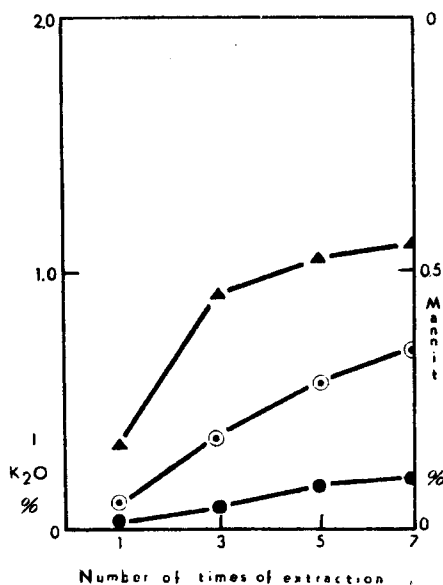


Fig. 37. Extracted with 0.005% NaOH

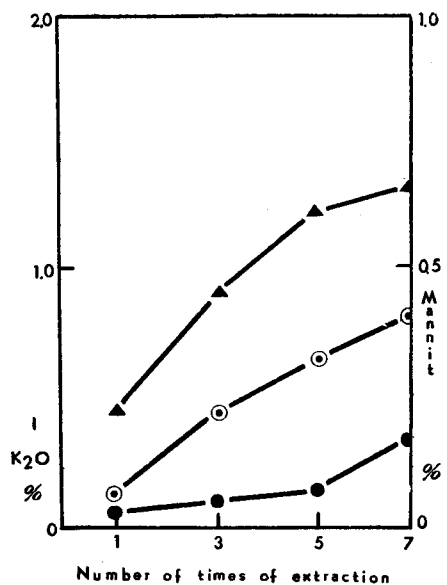


Fig. 38. Extracted with 0.01% Na₂CO₃

Fig. 35-38. Concentration of soluble matters in the algal extract (2)

▲----- Concentration of K₂O (%) ●----- Concentration of iodine (% × 10)
 ⊙----- Concentration of mannit (%)

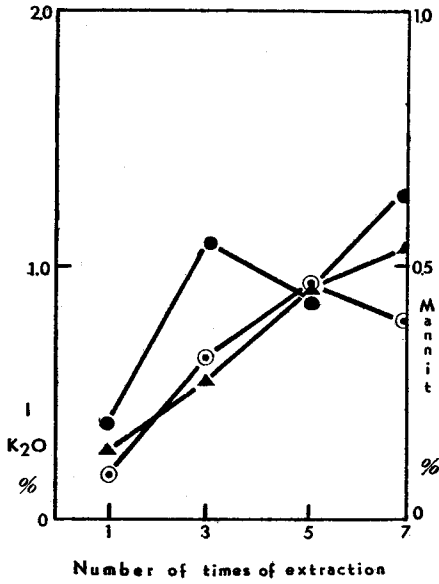


Fig. 39. Extracted with water

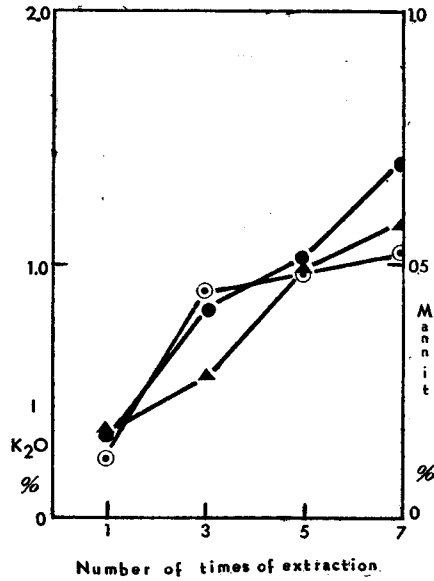


Fig. 40. Extracted with sea water 1 : water 2

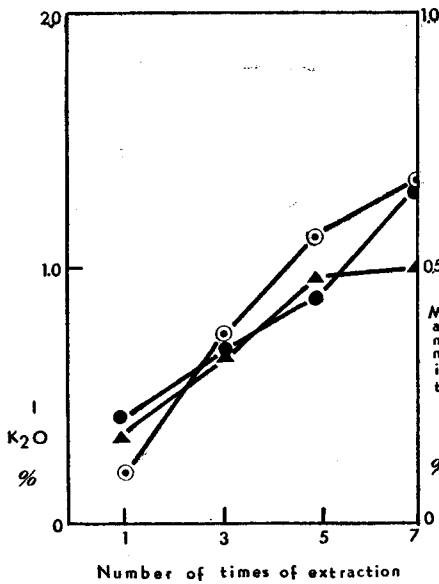


Fig. 41. Extracted with sea water 1 : water 1

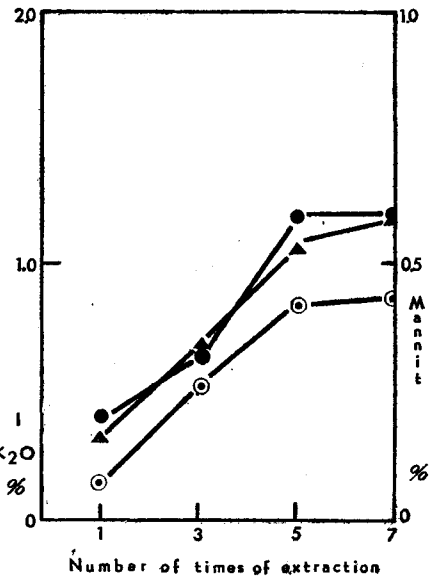


Fig. 42. Extracted with sea water

Figs. 39-42. Concentration of soluble matters in the algal extract (3)

▲----- Concentration of K₂O (%) ●----- Concentration of iodine (% x 10)
 ◎----- Concentration of mannit (%)

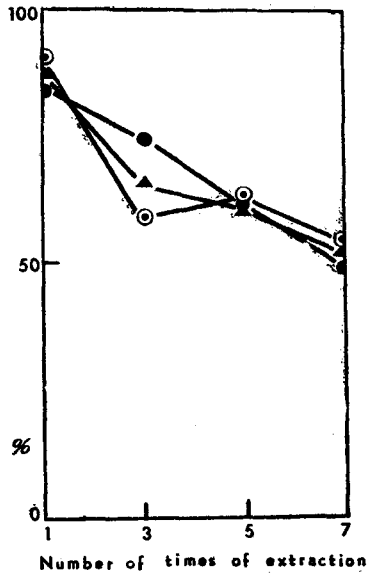


Fig. 43. Recovery of K₂O (%).

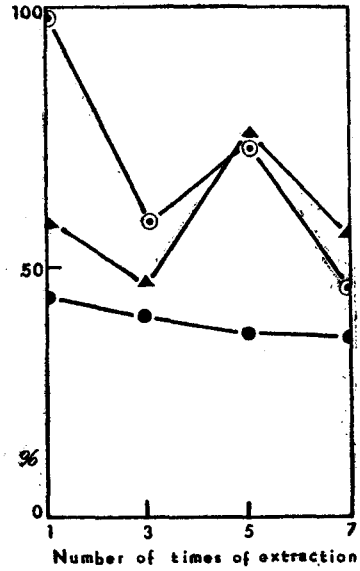


Fig. 44. Recovery of iodine (%).

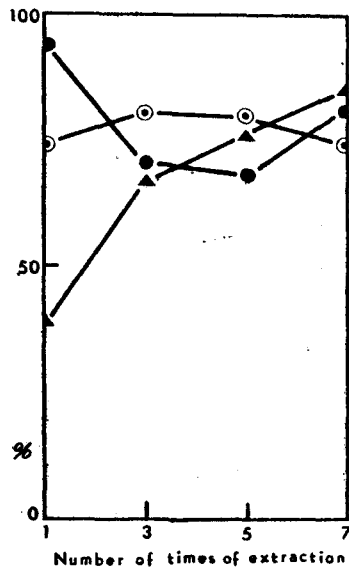


Fig. 45. Recovery of mannit (%)

Figs. 44-45. Recovery of soluble matters (1)

▲----- Extracted with water ●----- Extracted with 0.1N HCl
 ○----- Extracted with 1% CaCl₂

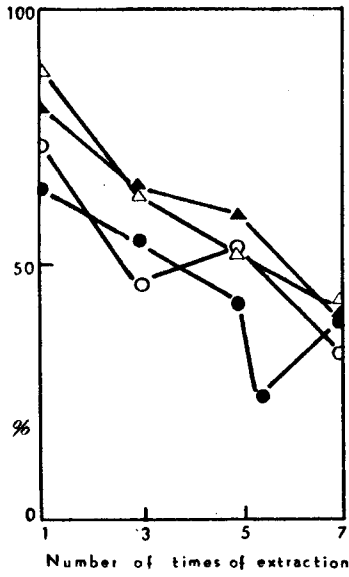


Fig. 46. Recovery of K₂O (%)

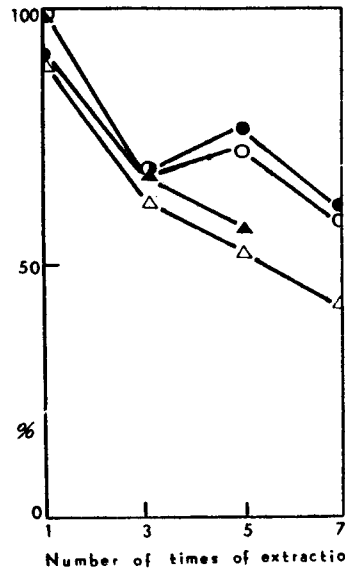


Fig. 47. Recovery of iodine (%)

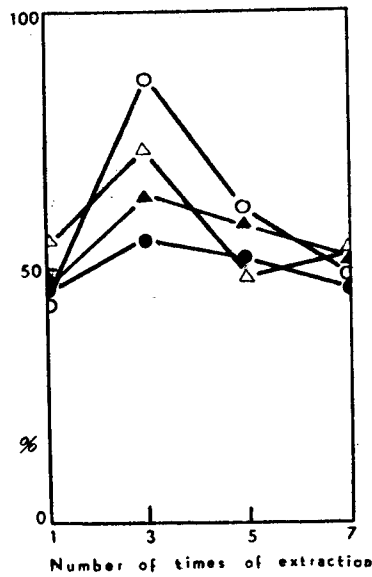


Fig. 48. Recovery of mannit (%)

Figs. 46-48. Recovery of soluble matters (2)

▲----- Extracted with water △----- Extracted with 0.001% NaOH
 ●----- Extracted with 0.005% NaOH ○----- Extracted with 0.01% Na₂CO₃

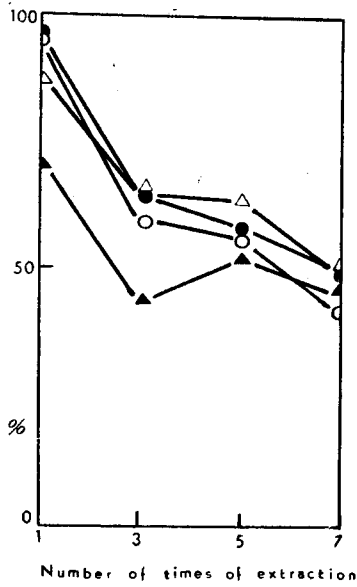


Fig. 49. Recovery of K₂O (%)

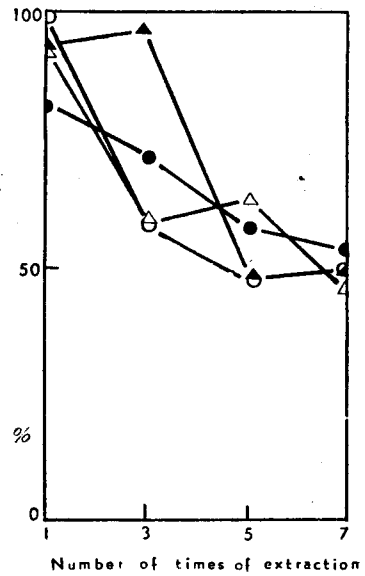


Fig. 50. Recovery of iodine (%)

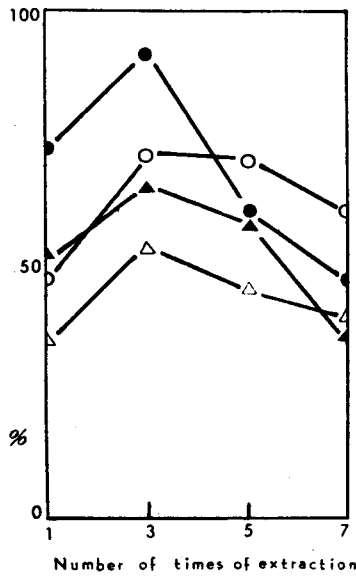


Fig. 51. Recovery of mannit (%)

Figs. 49-51. Recovery of soluble matters (3)

- ▲----- Extracted with water
- Extracted with sea water 1: water 2
- Extracted with sea water 1: water 1
- △----- Extracted with sea water

Since the concentrations of the extracts yielding by the first treatment are very low as shown in Tables 10-12 in every extraction, the repetition of the extraction is desirable in spite of the decrease of the yield. Sometimes a higher concentration of the extracts is required rather than a higher yield of the soluble matter because the recovery of the matter is fairly difficult and expensive when a dilute solution is used.

As no essential difference is found in the extractive powers of the examined pre-treating solutions, the pretreatment of the algae for algin manufacture should be done taking into consideration mainly the conversion of the types of algin in the material, although the extractive power of the solution for pigment and other impurities needs consideration too.

V. RECOVERY OF MANNIT FROM BROWN ALGAE

Mannit has been obtained in laboratory from brown algae by extraction with hot methanol or ethanol and crystallization by cooling. But the solubility of mannit in these alcohols is very low, that is to say, 1.306 per cent in methanol and 0.757 per cent in ethanol even at their boiling points.³³⁾ On account of this fact, these alcohols are not applicable industrially for the extraction of mannit. TAKAHASHI³⁴⁾ reported a procedure to obtain mannit from algae by precipitating it as a copper compound from the dilute hydrochloric acid extract, but his method has not been applied industrially.

An attempt was made by the present author to recover mannit by means of fractional crystallization from other crystallizable matters, such as potassium salts or sodium salts, in the extract. Potassium and sodium chlorides are the most abundant salts in the extract of brown algae, and the solubility of potassium or sodium chloride in water does not vary greatly with the temperature while the solubility of mannit in the water varies extremely with the temperature. That is to say, nearly 70 per cent of mannit dissolves in water at 100°C, whilst it dissolves at the rate of only 10 per cent at 20°C.³³⁾³⁴⁾ An investigation was carried out to ascertain whether fractional crystallization of these substances in the extract could be done or not.

1. Experimental

The solubility of mannit in water has been reported as described above. Its solubility has been reported in concentration with the range of temperature from 20 to 100°C, but no report has appeared up to the present on the crystallization of dissolved mannit by cooling. As it was considered that the amount of crystallized mannit from the water solution by cooling did not agree always with the amount expected from its solubility, the following experiment was performed.

Experiment 1

Mannit was dissolved in water at 90°C so as to get 30, 35, 45 and 50 per cent

solutions; these solutions were allowed to stand for 24 hours at room temperature (20–30°C) and at 0°C. Recovered mannit was as presented respectively in percentages of the dissolved mannit in Table 13.

Table 13

Dissolved mannit (g/dl)	Recovered mannit (%) by cooling	
	20–30°C	0°C
30	24.13	52.03
35	31.14	64.17
45	52.78	71.32
50	57.14	—

From the above result it is proved that mannit would be crystallized by cooling from its solution, although the mother liquor could not be separated from concentrated solution, such as 50 per cent solution, by cooling because mannit would be crystallized together with the mother liquor. It was experienced that mannit could not be separated from the mother liquor when 50 g of mannit was dissolved in 100 cc of water and the solution was cooled at 0°C.

Experiment 2

The excess of mannit, potassium chloride and sodium chloride were put into a tall flask to which was added 50 cc of water, and the mixture was placed in a thermostat with an accuracy of $\pm 0.1^\circ\text{C}$. After the temperature of the mixture reached that of thermostat, the flask was shaken for 90 minutes at the rate of 150 times per minutes; the experiment was operated at the range from -5°C to 80°C . After that the dissolved mannit, potassium chloride and sodium chloride were determined respectively. The determination was made as follows. The mixture was allowed to stand a short while at the experimented temperature, and then 20 cc of supernatant was pipetted into a measuring flask and the flask was filled up with water to 200 cc. Then the mixture was analyzed. A sample solution of lower temperature was taken from the flask and allowed to stand to reach room temperature, then it was pipetted for analysis. In order to avoid crystallization, a sample solution, which was treated under higher temperature, was taken from the flask, a known amount of water of the same temperature was added to it and it was pipetted after it reached the room temperature. The potassium chloride and sodium chloride used in this experiment were MERCK's reagents "for analysis". The mannit used was obtained in the author's laboratory from *Laminaria* fronds extracting with ethanol and recrystallized several times from the ethanol solution; its melting point was 160°C and its Cl content was lower than 0.0001 per cent.

The potassium chloride and sodium chloride content of the solution were calculated from the amounts of total ash and the chloride ion. The mannit content was

Table 14

Treating temperature °C	Dissolved amount (g/dl)				
	NaCl+KCl	Cl ⁻	NaCl	KCl	Mannit
-5	31.15	16.69	22.52	9.13	7.89
0	32.48	18.35	22.29	10.21	10.25
20	34.18	19.11	21.95	12.23	12.65
40	35.13	19.59	22.15	12.98	19.14
60	30.13	16.88	19.57	10.56	28.07
80	25.63	14.11	15.31	10.32	41.08

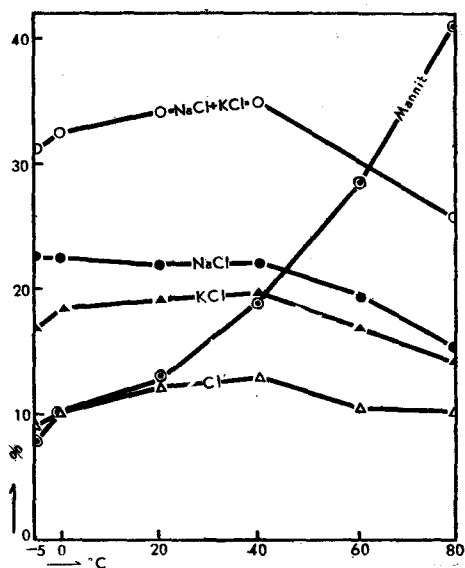


Fig. 52. Respective solubilities of mannit, potassium chloride and sodium chloride when these substances coexist together

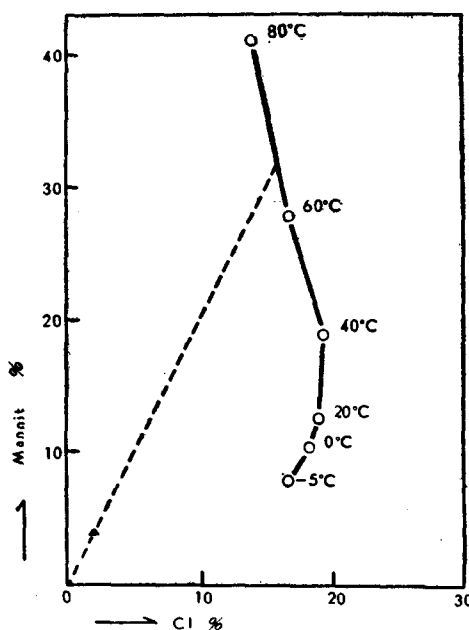


Fig. 53. Relation of solubilities between mannit and chlorides at each temperature, where chlorides are represented as Cl⁻: dotted line; supposed line of evaporation of mannit being 4% and Cl-2%

determined by the author's method.¹⁹⁾ The results are shown in Table 14 and Fig. 52.

From the figure it will be noted that the solubility of mannit varies remarkably with the change of temperature and that the amount of potassium and sodium chloride may be represented by the amount of total chloride ion. The relation between the solubilities of mannit and total chloride ion is shown in Fig. 53. It may be observed from the figure what the saturation point of either mannit or salts of any solution is

reached when the solution is concentrated, and it may be determined which substance would be crystallized in given concentrating or cooling temperatures. For example, if the solution containing 4 per cent of mannit and 2 per cent of chlorides as Cl is evaporated at 80°C, chlorides would be saturated at the concentration of about 16 per cent before mannit reaches its saturation as shown by the straight dotted line in the figure.

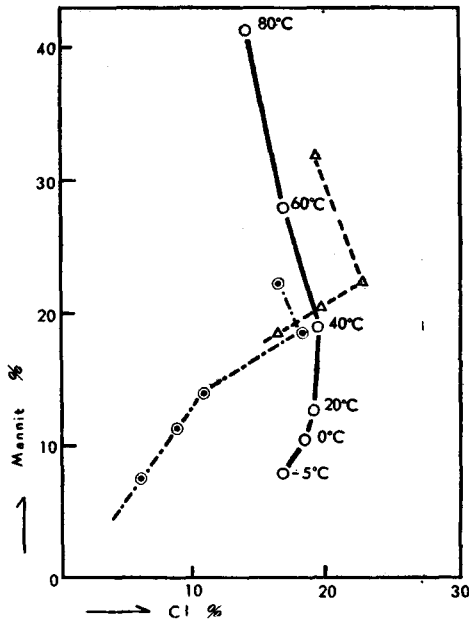


Fig. 54. Solubilities of mannit and chlorides at each temperature and actual concentration by evaporation of the algal extract, where chlorides are represented as Cl⁻

- Solubilities of each temperature
 ⊙ and △----- Stage of actual concentration by evaporation of algal extract

other salts or organic matters existing in the extract.

2. Discussion

Fundamental experiments on the fractional crystallization of mannit from other dissolved matters in the extract of brown algae were made as explained above. It is suggested by the experiments that mannit can be obtained by fractional crystallization, but this can not be recommended as a fully suitable procedure to recover mannit from the extract.

By further evaporation chlorides would be crystallized and the evaporation would proceed along the curve, while mannit would not be crystallized till the evaporation attained the saturation point of the mannit, about 40 per cent, as shown in the figure. If the evaporation is stopped before the saturation point of mannit and the mother liquor is quenched, mannit would be crystallized with good yield but chloride would be crystallized quite sparingly.

Experiment 3

Ten times by weight of 0.1 n hydrochloric acid was added to sample *Laminaria* fronds and boiled for 15 minutes. The extract was neutralized with sodium carbonate and filtered. The obtained liquor was evaporated at boiling point and determined amounts of mannit and chlorides were plotted in Fig. 54 showing two examples. From the figure it may be observed that the evaporation proceeds nearly along the curve after crystallization of chlorides takes place, although it does not proceed exactly on the curve. The reason for that may be the effect of

VI. STUDIES ON THE PROCESS OF MANUFACTURING ALGIN

Works by previous investigators regarding the processes of manufacturing algin from brown algae are scanty. TAKAHASHI has published many papers on the subject, which form the principal part of his book entitled "SEAWEED INDUSTRY".³⁵⁾ His works are all concerned with the manufacture of algin by sodium carbonate or ammonium oxalate. The present author has discovered that there are various processes of manufacturing algin from brown algae using many sorts of chemicals. The following investigations are concerned with new methods of algin manufacture.

As algin is fairly unstable in its colloidal qualities, one should pay careful attention to this fact in every treatment throughout the process. In a factory it is required to manufacture algin having good qualities, that is to say, high viscosity, good yield, no colour and no turbidity. Since the requirements for these qualities are inconsistent with each other, the manufacture of algin is a very difficult undertaking in spite of the fairly simple process. If a higher yield is required, algin should be manufactured using excess of alkali and treating at higher temperature, but under such conditions the viscosity of algin would be decreased and the colour would be deep. The viscosity would also be decreased if bleaching is done in order to obtain colourless product. When the viscosity of the digested solution is high, it would be decreased again by long spells of filtration. In order to meet certain requirements in algin manufacture, one is compelled to sacrifice some others.

1. Temperature of Algin Treatments

As viscosity of sodium alginate would be affected by the temperature of the treatments during the manufacturing process, some fundamental investigations were made as follows.³⁶⁾

Experimental and discussion

Alginate for experiment was prepared as follows:

(1) One hundred grams of dried *Costaria costata* were macerated with 3 litres of 2.0 per cent sodium carbonate solution at room temperature for 24 hours and the liquor was filtered after dilution with water. The residue was macerated again with 2.0 per cent sodium carbonate solution and the liquor was filtered. The two thus obtained filtrates were mixed and the liquor was poured into ethanol in order to obtain sodium alginate. Thus obtained sodium alginate was washed with ethanol several times and next with ether, after which the preparation was dried in desiccator. The preparation is hereafter described as NaA.

(2) Maceration was made at 85-90°C for 2 hours and macerated mixture was allowed to stand for 24 hours. Other treatment was made in the same way as above. The preparation is hereafter described as NaB.

(3) Algin was prepared from the material macerated with 1.0 per cent ammonium oxalate in the same way as (1); the preparation is described hereafter as OxA.

(4) Algin was prepared from the material macerated with 1.0 per cent ammonium oxalate in the same way as (2); the preparation is hereafter described as OxB.

The yield and the specific viscosity of 0.2 per cent solution of these preparations are shown in Table 15.

The 0.2 per cent solution of each preparation was prepared and it was heated at 80°C and 95°C for 30–420 minutes. The specific viscosity of each solution at various stages of treatment was determined. The results are tabulated in Table 16, and illustrated in Figs. 55 and 56.

Table 15

Preparation	Yield (%)	Specific viscosity
NaA	14	7.36
NaB	19	8.64
OxA	15	6.50
OxB	25	9.45

Table 16 Change of specific viscosity by heating

Treated duration (min)	Preparation							
	NaA		NaB		OxA		OxB	
	Treated temperature (°C)							
	80	95	80	95	80	95	80	95
0	7.36	7.36	8.64	8.64	6.50	6.50	9.45	9.45
30	—	6.70	8.50	7.84	5.68	5.41	8.63	6.59
60	6.95	6.45	7.86	7.18	4.58	4.75	8.22	6.45
120	6.59	5.13	7.00	5.80	4.14	3.80	6.95	4.36
180	6.09	4.43	5.91	5.07	3.59	3.02	6.00	3.41
240	—	4.00	5.54	3.98	3.18	2.45	5.36	2.68
300	5.63	3.31	4.91	3.29	2.86	2.05	4.45	2.18
360	5.27	3.13	4.37	2.91	2.55	1.78	3.95	1.86
420	5.09	2.72	4.12	2.48	2.33	1.53	3.55	1.57

From these figures is assumed the following formula.

$$y = Ae^{-\alpha t} \dots\dots\dots (10)$$

where, y represents specific viscosity at each stage of duration of heating, t represents duration of heating and α and A are the constants of the equation concerned with each preparation. Applicableness of the equation (10) is ascertained by means of Figs. 57 and 58. That is to say, the figures are constructed with $\log y$ at ordinate and with t at abscissa, and the erected line is drawn straight. The values of α are calculated and these values are compared for each preparation as shown in Table 17. It is shown in Figs. 57 and 58 that the viscosity of the alginate would be decreased by heating in

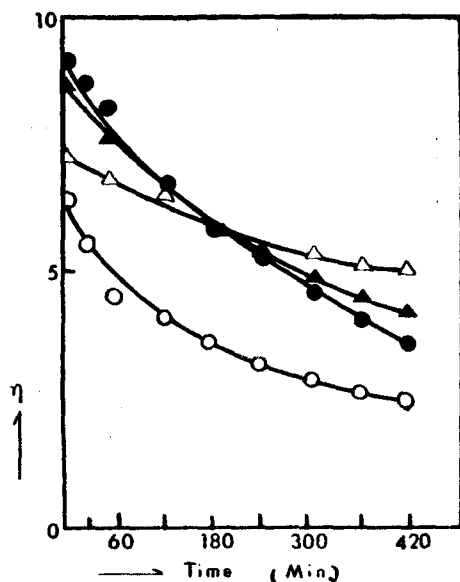


Fig. 55. Decrease in viscosity of algin solution after heating at 80°C

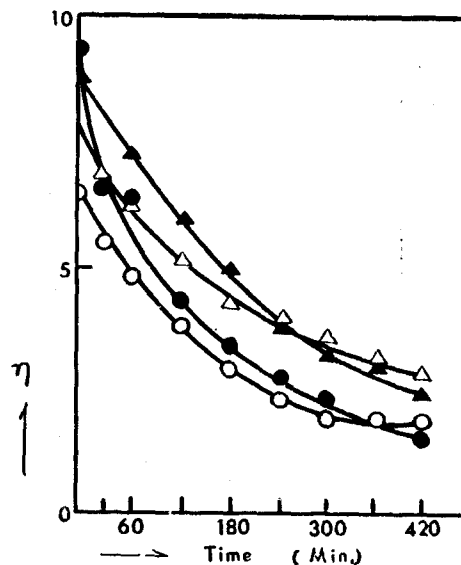


Fig. 56. Decrease in viscosity of algin solution after heating at 95°C

△----- NaA, ▲----- NaB,
○----- OxA, ●----- OxB,

accordance with equation (10). It is clear that the viscosity of algin would be decreased by heating, therefore long spells of heating should be avoided in every process of algin manufacture.

Table 17

Preparation	NaA		NaB		OxA		OxB	
	80	95	80	95	80	95	80	95
Treatment temp. (°C)	80	95	80	95	80	95	80	95
Treatment duration (min)	30-420	30-420	30-420	30-420	60-420	30-420	30-420	120-420
α	0.06	0.17	0.13	0.24	0.17	0.32	0.17	0.35

2. Maceration of Algae

The yield, specific viscosity and colour tone of algin preparation and manufacturing processes were examined the material being macerated with several sorts of chemicals as follows.

(i) Manufacturing processes with different pretreatments

Since the yield, viscosity and colour tone of algin differ with the manufacturing

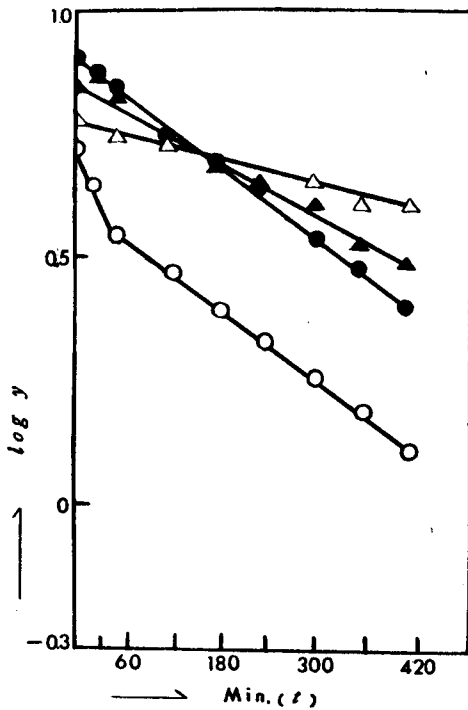


Fig. 57. The relation of $\log y$ to the duration of heating at 80°C

\triangle ----- NaA,
 \circ ----- OxA,

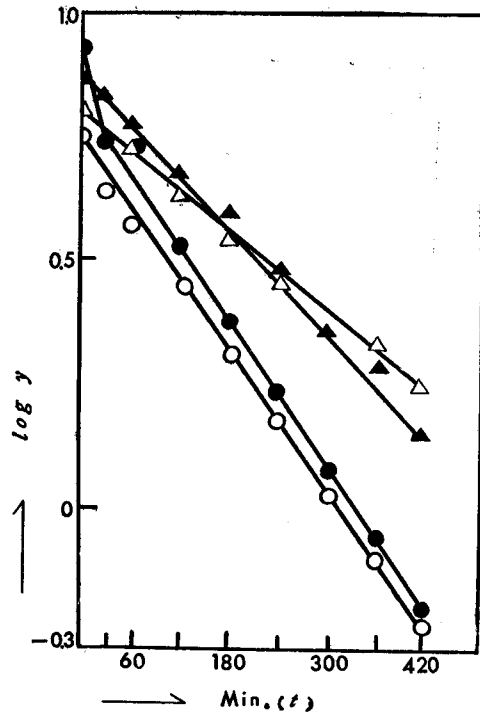


Fig. 58. The relation of $\log y$ to the duration of heating at 95°C

\blacktriangle ----- NaB,
 \bullet ----- OxB,

procedures, the pretreatment process was first examined. Three kinds of pretreatment, namely, (a) pretreatment with water, (b) with calcium chloride and (c) with dilute hydrochloric acid, were compared with each other material being macerated with several chemicals.

These three kinds of pretreatment were carried out respectively as follows: Ten grams of dried *Laminaria longissima* were placed in a beaker and were pretreated (a) with 300 cc of water at boiling point for 15 minutes, (b) with 300 cc of 0.5 per cent calcium chloride solution at boiling point for 30 minutes, or (c) with 0.1 n hydrochloric acid at 50°C for 15 minutes respectively and separately. After the pretreatments, each of the materials was washed with water and macerated with Na_2CO_3 , Na_2SO_3 , $(\text{COONH}_4)_2$, Na_2HPO_4 or NaOH in 300 cc of water at 60°C for an hour with constant stirring. After maceration, 700 cc of water was added and the whole was filtered under reduced pressure. Alginic acid gel was then precipitated by adding 75 cc dilute hydrochloric acid (1:2). The water was separated with silk cloth, and collected gel was put into water at 55–58°C in order to dehydrate the absorbed water in the gel.

Thus obtained gel was washed with water of identical temperature and then with ethanol and ether, and dried in the desiccator. The yield, colour tone and specific viscosity of the algin were determined. The specific viscosity was determined with Ostwald's viscosimeter at 25°C, neutralizing alginic acid with sodium carbonate solution and adjusting the concentration of the solution at 0.2 per cent. The results are shown in Tables 18, 19 and 20.

Table 18. Yield(%) of alginic acid obtained by different processes

Maceration (g)		Pretreatment		
		Water	0.5% CaCl ₂	0.1 n HCl
Na ₂ CO ₃	2.0	21.2	22.2	20.0
Na ₂ SO ₃	3.5	18.0	18.5	20.6
Na ₂ HPO ₄	5.0	19.0	14.0	18.0
(COONH ₄) ₂	1.0	21.0	12.0	—
NaOH	1.0	4.2	2.0	19.0

Table 19. Specific viscosity of sodium alginate solution (0.2 %) obtained by different procedures

Maceration (g)		Pretreatment		
		Water	0.5% CaCl ₂	0.1 n HCl
Na ₂ CO ₃	2.0	69	59	103
Na ₂ SO ₃	3.5	75	57	295
Na ₂ HPO ₄	5.0	68	81	114
(COONH ₄) ₂	1.0	71	49	—
NaOH	1.0	54	—	109

Table 20. Colour tone of alginic acid obtained by different procedures

Maceration	Pretreatment		
	Water	0.5% CaCl ₂	0.1 n HCl
Na ₂ CO ₃	Pale brownish yellow	Brownish yellow	Pale yellowish brown
Na ₂ SO ₃	Pale yellow	Pale brownish yellow	Pale brownish yellow
Na ₂ HPO ₄	Greenish brown	Greenish brown	Pale yellow
(COONH ₄) ₂	Greenish brown	Greenish brown	—
NaOH	Greenish brown	Greenish brown	Pale yellowish brown

From the results the following discussion under three headings is suggested.

Yields :

In the case of pretreatment with water no remarkable difference of the yield can be observed as having connection with the maceration with examined chemicals excepting NaOH. In the case of pretreatment with dilute calcium chloride solution, Na₂CO₃ and Na₂SO₃ give larger yield than other chemicals, while NaOH gives the least yield. In the case of pretreatment with dilute hydrochloric acid solution, no remarkable

difference of yield is observed with the exception of the case of ammonium oxalate.

From the above mentioned facts, the following remarkable phenomena were noted. Sodium hydroxide solution macerates the algae well when the plants are pretreated with dilute hydrochloric acid solution while it hardly macerates the algae at all when the plants are pretreated with water or calcium chloride solution. These facts may be explained as follows: sodium hydroxide solution is able to dissolve free alginic acid into sodium alginate solution while it is scarcely able to dissolve calcium alginate or other insoluble algin in the algae. In other words, the cation exchange reaction between sodium hydroxide and free alginic acid is irreversible whilst the cation exchange reaction between sodium hydroxide and calcium alginate or other insoluble algin in the brown algae is nearly reversible. The practical examination agreed quite well in results with the fundamental experiments which are described above in Chapter II. Another remarkable phenomenon is observed in the case of the maceration with ammonium oxalate solution: it dissolves out algin from the algae when the plants are pretreated with water or calcium chloride solution but not when pretreated with dilute hydrochloric acid solution. This fact may be explained as follows: free alginic acid is not dissolved into colloidal solution by treatment with ammonium oxalate solution but it is dispersed into small particles as noted in Table 3 and for this reason algin can not be dissolved into the solution from the cell wall of the algae.

Viscosity:

The viscosity of sodium alginate obtained by pretreating the material with dilute hydrochloric acid solution is higher than that obtained with other solutions. The reason for this is considered to lie in the fairly mild conditions, under which the pretreatment with hydrochloric acid was done, namely in low concentration of the acid, 0.1 n, and at low temperature, 50°C for 15 minutes. Remarkable high viscosity is observed in the preparation which was pretreated with dilute hydrochloric acid solution and macerated with sodium sulfite solution. The reason may be as follows: the dissolution of algin would follow formula (9) and therefore the reaction would proceed in the weak acid and the decomposition of algin by bacteria would be prevented by the formation of sulfur dioxide during the maceration as described in Chapter III.

Colour tone of the preparation:

The colour tone of alginic acid obtained by macerating the material with sodium sulfite is the faintest among the examined preparations. This fact may be explained as follows: sulfur dioxide produced according to formula (9) bleaches the algin preparation; sulfur dioxide would be liberated by the addition of hydrochloric acid to the macerated solution in order to precipitate alginic acid gel or it would be liberated in the macerating process when the pretreatment is made with dilute hydrochloric acid solution.

(ii) *Filtration in cases of different maceration*

Ten grams of *Laminaria longissima* were placed in a beaker and pretreated with

300 cc of water at boiling point for 15 minutes. After throwing the liquor away, residues were washed with water, and then 300 cc of water and various amount of several chemicals were added. The mixture was macerated at 60–70°C for an hour with continuous stirring. After that 700 cc of water was added at 60°C in order to assist the filtration. Filtration of macerated mixture was made under reduced pressure till the filtrate amounted to 600 cc. After filtration 75 cc of dilute hydrochloric acid (1:2) was added to the filtrate in order to precipitate alginic acid gel. Precipitated gel was collected in a silk cloth and was put into water at 55–58°C in order to dehydrate the gel. Thus obtained wet gel was washed with water at the same temperature, then washed with ethanol and ether, and dried in the desiccator. Yield of alginic acid and the velocity of filtration were determined. The velocity of filtration was measured as the length of time needed for filtrating the macerated solution until 600 cc of filtrate was obtained. The results are shown in Table 21.

Table 21. Yield of alginic acid and the velocity of filtration, pretreating algal material with water

Maceration (g)	Yield of alginic acid (%)	Velocity of filtration (min/600 cc)	Specific viscosity of filtrate
Na ₂ CO ₃	1.0	62	53.0
	2.0	51	51.6
	3.0	47	105.5
Na ₂ SO ₃	2.5	57	52.3
	3.5	126	68.6
	4.5	83	77.7
(COONH ₄) ₂	0.3	16	18.5
	1.0	83	92.3
	2.0	75	114.5
Na ₂ HPO ₄	3.0	44	48.9
	5.0	67	99.0
	7.0	59	109.2
NaOH	0.5	28	35.5
	1.0	20	63.3
	2.0	8	50.4

No remarkable differences concerning the maceration can be found when algae are pretreated with water, but NaOH can not macerate the algae well. It is considered that characteristic effects of the macerating chemical can not be found when the algae are pretreated with water because the algin in brown algae is not settled into a definite form.

The following experiments were carried out in order to learn the effect of the pretreatment with hydrochloric acid solution. Processes were almost the same as in the former experiments, except that the pretreatment was made with 150 cc of 0.1 n hydrochloric acid solution at 50°C for 15 minutes and that the material was *Laminaria religiosa*. The velocity of filtration was measured as the length of time needed for

filtration till 600 cc of filtrate was obtained. The results are shown in Table 22 and Fig. 59.

Table 22. Yield of alginic acid and the velocity of filtration, pretreating algal material with 0.1 n HCl

Maceration (g)	Yield of alginic acid (%)	Velocity of filtration (min/600 cc)	Specific viscosity of filtrate
0.5	—	157	1.7
1.0	17.3	346	33.6
1.5	20.0	311	50.8
2.0	21.0	325	54.7
2.5	22.0	305	51.8
3.0	21.0	333	59.3
3.5	23.2	270	58.3
4.0	23.0	305	46.9
1.0	—	47	1.2
1.5	—	38	1.1
2.0	—	48	3.6
2.5	15.0	62	7.7
3.0	14.2	125	11.8
3.5	17.2	90	19.0
4.0	17.0	115	25.7
4.5	18.2	61	10.0
5.0	19.0	—	—

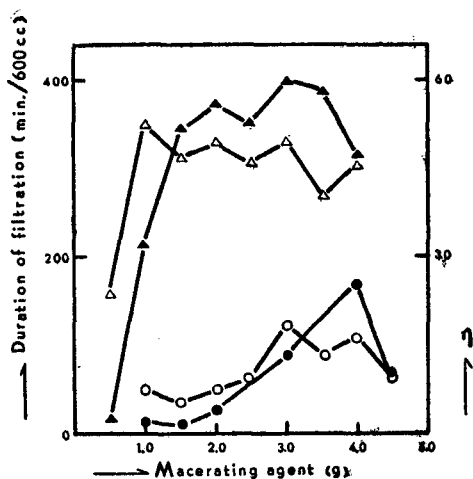


Fig. 59. Duration of filtration (min./600 cc) and specific viscosity of filtrate macerated with Na₂CO₃ and Na₂SO₃

- △----- Duration of filtration in the case of macerating with Na₂CO₃
- Duration of filtration in the case of macerating with Na₂SO₃
- ▲----- Specific viscosity in the case of macerating with Na₂CO₃
- Specific viscosity in the case of macerating with Na₂SO₃

As shown in the table and the figure, the filtration of measured liquor obtained by macerating with sodium sulfite is remarkable easier than that of the liquor obtained by macerating with sodium carbonate. The fact may be explained as follows: reaction proceeds the material being always acidic in the case of maceration with sodium sulfite while it proceeds in alkaline state in the case of maceration with sodium carbonate.

The fluctuation of the data is due to the circumstance that the experiments were carried through by two assistants who worked alternatively on every maceration; the maceration process is furthermore affected by the condition of stirring, treating temperature and other unavoidable factors and constant results would not be expected even if close attention were paid in each treatment. Nevertheless, a remarkable difference of filterability is observed, on the whole, between the two macerating procedures as described above.

Viscosity of filtrate:

The filtrability of the macerated liquor would be affected by the viscosity of the filtrate. Specific viscosity of the liquor resulting from maceration with sodium sulfite is lower than that from sodium carbonate. The particularly low viscosity in the case of maceration with 0.5 g of Na_2CO_3 or 1.0–2.0 g of Na_2SO_3 is dependent upon imperfect maceration as is clearly shown in the yield of alginic acid. Remarkable ease of filtration is observed when the algae are pretreated with dilute hydrochloric acid and macerated with Na_2SO_3 .

Yield of alginic acid:

The yield of alginic acid is higher in the case of maceration with sodium carbonate solution. The reason may be considered to lie in the circumstance that the cation exchange reaction between alginic acid and sodium carbonate would be perfectly irreversible because the reaction is a neutralization, while the cation exchange between alginic acid and sodium sulfite would be partly reversible because sulfurous acid is a stronger acid than alginic acid. It is recommended to use sodium sulfite for maceration of algae after the pretreatment by dilute hydrochloric acid solution in view of the ease of filtration, though it somewhat reduces the yield of algin.

Viscosity of the product:

The viscosity of thus obtained algin products was compared as follows: the preparations of alginic acid by both methods were neutralized with sodium carbonate solution and specific viscosity of thus obtained sodium alginate solution was measured. The results are shown in Table 23.

Table 23

Concentration of sodium alginate (%)	Macerated with Na_2CO_3 1.5 g	Macerated with Na_2SO_3 3.0 g
0.500	100.3	112.0
0.400	59.6	65.1
0.300	39.9	45.3
0.250	25.2	31.0
0.150	13.5	15.8
0.075	7.1	9.0
0.040	3.2	4.2
0.025	2.0	2.2

It is proved that the viscosity of algin preparation obtained by macerating with sodium sulfite is rather higher than that of the preparation obtained by macerating with sodium carbonate although the viscosity of filtrate macerated with sodium sulfite is remarkably lower than that of filtrate macerated with sodium carbonate.

Generally speaking, it would be a recommendable procedure for algin manufacture to pretreat algae at first with dilute hydrochloric acid solution in order to convert algin in the algae into free alginic acid and then to macerate them with sodium sulfite solution.

3. Process of Filtration of Macerated Liquor

The filtration of macerated liquor is one of the most difficult processes in algin manufacture. In practice, it is usually done by filter bag, filter press or centrifuge. Some investigations on the filtration were carried out with filter bags as described in the following.

The filter bags used for this study were made of cotton; they measured 17 cm in diameter and 245 cm in length. Dried *Laminaria* fronds were pretreated with 0.1 N hydrochloric acid solution added to the amount of 15 times the weight of algal fronds, at 50°C for 30 minutes and macerated with as much sodium carbonate as 15 per cent by weight of the fronds at 60°C for an hour. The macerated mass was diluted with water to 7 times by volume and poured into the bags. The experiments were made in August in order to learn the effect of decomposition by microorganisms. The results are shown in Table 24; the figures are mean value of the data from 10 bags. The specific

Table 24

Duration of filtration (min)	Filtrate at each period (cc)	Filtrate in total (cc)	Specific viscosity of filtrate	Yield of alginic acid (%)	Alginic acid in filtrate at each period (g)	Alginic acid in filtrate in total (g)
30	3950	3950	54.8	0.24	9.45	9.45
60	1200	5150	44.8	0.17	2.25	11.70
90	600	5750	43.2	0.24	1.44	13.14
120	400	6150	30.4	0.33	1.32	14.46
150	350	6500	24.0	0.13	0.52	14.98
180	250	6750	20.8	0.18	0.45	15.43
210	215	6965	17.0	0.16	0.34	15.77
240	175	7140	16.6	0.17	0.30	16.07
270	150	7290	15.0	0.15	0.23	16.30
300	125	7415	14.2	0.14	0.18	16.48

viscosity of the filtrate and the yield of alginic acid from the filtrate per dl. were also determined. The results are illustrated in Fig. 60.

The amount of filtrate is distinctively decreased with the duration of the filtration as shown in the table and figure, over 80 per cent of the total filtrate is obtained within 120 minutes. Alginic acid is also obtained to as much as 80 per cent of its total within 90 minutes. The specific viscosity of filtrate is remarkably decreased by the duration

of the filtration, therefore it is clearly indicated that decomposition of algin does take place during the process of filtrating the macerated liquor.

It is considered from the above described results that the decomposition of algin would be caused to occur by the long spells of filtration, and therefore the filtration of macerated liquor should be done mechanically in order to avoid the decomposition of the algin in the filtrate.

It has been reported by the author³⁷⁾³⁸⁾ that alginic acid is decomposed by natural fermentation into acetic acid, so the application of some antiseptic treatment to the filtrate is worth considering in order to prevent the decomposition of algin due to microorganisms. However the use of such antiseptic treatment should be avoided in a factory, where algin is used as a food stuff, for instance as the stabilizer of ice-cream.

4. Gelling of Alginic Acid

Though it has been customary to get gel of alginic acid from the filtrate by adding hydrochloric acid solution, there has appeared no report regarding the formation of alginic acid gel by adding hydrochloric acid to the filtrate of macerated liquor. Some fundamental investigations on the conditions of gel formation were carried out as follows. To be used for these investigations alginic acid powder was prepared by the author's method and purified by ordinary method; 0.3-0.7 per cent solutions of sodium alginate were prepared by neutralizing alginic acid with sodium hydroxide solution because gel of alginic acid is customarily made in the per cent of 0.3-0.7.

(i) Experimental

Ten cc of sodium alginate solution were pipetted into a test tube, to which was added 10 cc of hydrochloric acid solution of various concentrations. Then the mixture was shaken well. Produced alginic acid gel was collected on the filter paper under

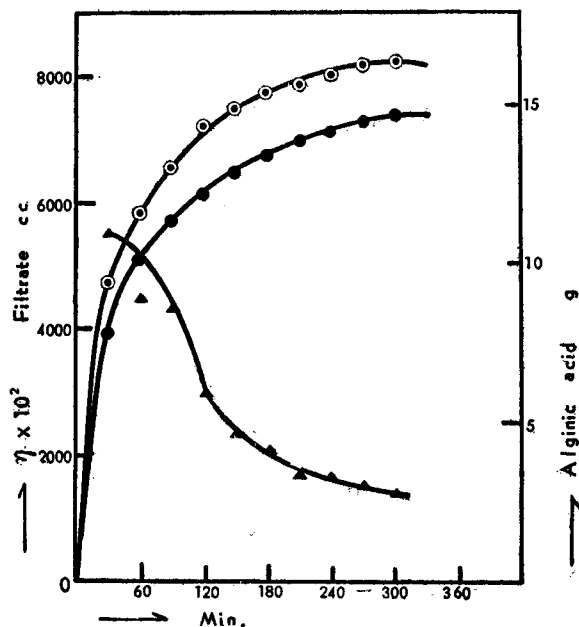


Fig. 60. Filtrate (cc), specific viscosity of filtrate and yield of alginic acid (g) in the filtrate in relation to the duration of filtration of macerated liquor

- Yield of alginic acid in total (g)
- Filtrate in total (cc)
- ▲----- Specific viscosity

Table 25. Yield of alginic acid (%) gelling with hydrochloric acid

HCl in the mixed solution (n)	Concentration of sodium alginate (%)				
	0.3	0.4	0.5	0.6	0.7
2.0	90.2	96.4	96.3	96.4	85.3
1.75	93.5	95.2	96.7	93.0	79.0
1.5	97.1	96.0	92.3	86.1	70.1
1.25	—	95.0	90.0	—	—
1.0	97.6	93.0	85.0	72.3	67.8
0.75	91.3	95.3	78.6	68.0	57.0
0.5	94.5	89.5	76.0	61.0	53.5
0.25	78.3	68.0	57.1	40.0	36.0
0.125	60.7	46.5	37.3	32.0	20.0
0.063	39.4	27.1	15.8	10.5	—
0.030	21.3	9.0	—	—	—

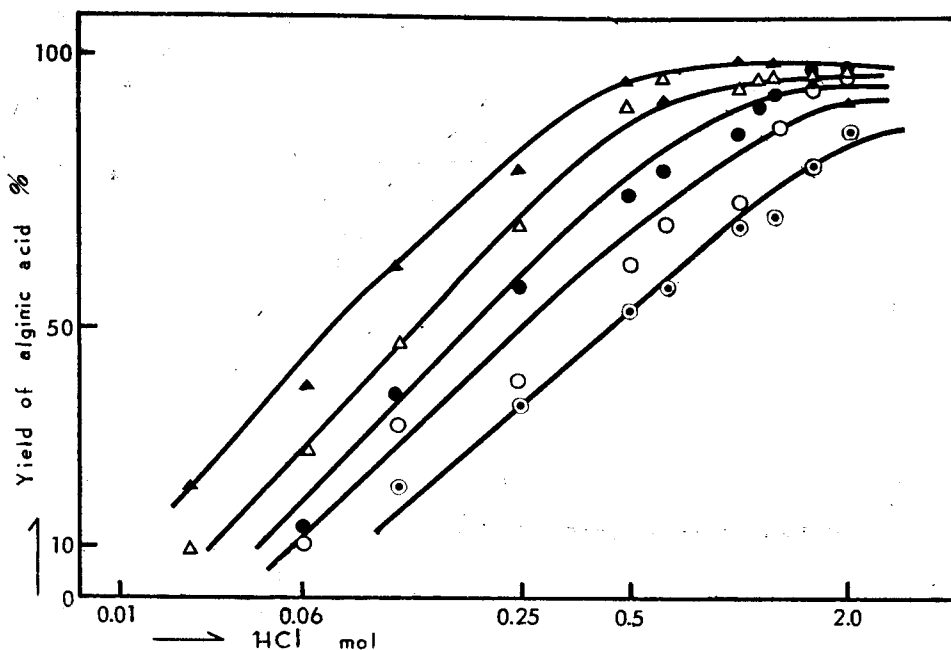


Fig. 61. Yield of alginic acid by gelling with HCl from sodium alginate solution

- ▲----- Gelled from 0.3% sodium alginate solution
- △----- Gelled from 0.4% sodium alginate solution
- Gelled from 0.5% sodium alginate solution
- Gelled from 0.6% sodium alginate solution
- ⊙----- Gelled from 0.7% sodium alginate solution

reduced pressure and washed with hot water and then with ethanol and ether. Thus obtained alginic acid gel was dried at 95°C and weighed.

(ii) Results

The results are shown in Table 25 and Fig. 61.

It is shown that more hydrochloric acid solution is needed for getting gel from fairly concentrated sodium alginate solution.

The data shown in Table 25 and Fig. 61 are calculated in Tables 26-30 and Fig. 62 as to give the equivalent ratio of added hydrochloric acid for dissolved sodium alginate and to give the yield of alginic acid recovered by gelling with hydrochloric acid solution.

Table 26. Yield of alginic acid (%) by gelling with HCl from 0.3% sodium alginate solution

Added HCl (n)	Added HCl (mol) to dissolved Na-alginate (mol)	Yield of alginic acid (%)
4.0	248	90.2
3.5	217	93.5
3.0	196	97.1
2.0	124	97.6
1.5	93	91.3
1.0	62	94.5
0.5	31	78.3
0.25	15.5	60.7
0.125	7.8	39.4
0.063	3.9	21.3

Table 27. Yield of alginic acid (%) by gelling with HCl from 0.4% sodium alginate solution

Added HCl (n)	Added HCl (mol) to dissolved Na-alginate (mol)	Yield of alginic acid (%)
4.0	186	96.4
3.5	163	95.2
3.0	134	96.0
2.5	116	95.0
2.0	93	93.0
1.5	67	95.3
1.0	46.5	89.5
0.5	23.8	68.0
0.25	11.6	46.5
0.125	5.8	27.1
0.063	2.9	9.0

Table 28. Yield of alginic acid (%) by gelling with HCl from 0.5% sodium alginate solution

Added HCl (n)	Added HCl (mol) to dissolved Na-alginate (mol)	Yield of alginic acid (%)
4.0	149	96.3
3.5	130	96.7
3.0	112	92.3
2.5	93	90.0
2.0	74.4	85.0
1.5	55.8	78.6
1.0	37.2	76.0
0.5	18.6	57.1
0.25	9.3	37.3
0.125	4.7	15.8

Table 29. Yield of alginic acid (%) by gelling with HCl from 0.6% sodium alginate solution

Added HCl (n)	Added HCl (mol) to dissolved Na-alginate (mol)	Yield of alginic acid (%)
4.0	124	96.4
3.5	109	93.0
3.0	93	86.1
2.5	78	—
2.0	62	72.3
1.5	46.5	68.0
1.0	31	61.0
0.5	15.5	40.0
0.25	7.8	32.0
0.125	3.9	10.5

Table 30. Yield of alginic acid (%) by gelling with HCl from 0.7% sodium alginate solution

Added HCl (n)	Added HCl (mol) to dissolved Na-alginate (mol)	Yield of alginic acid (%)
4.0	106	85.3
3.5	93	79.0
3.0	80	70.1
2.5	66.5	—
2.0	53.2	67.8
1.5	40	57.0
1.0	26.6	53.5
0.5	13.3	36.0
0.25	6.7	20.0

(iii) *Discussion*

From the above data is clearly learned the fact that an enormous quantity of hydrochloric acid is needed in order to make gel of alginic acid from sodium alginate solution. In a comparatively higher concentrated solution of sodium alginate, the equivalent ratio of hydrochloric acid would need to be still large. That is to say, about 60 mol of hydrochloric acid is required for 1 mol of alginate in 0.3 per cent sodium alginate solution, but over 100 mol of hydrochloric acid is required in 0.7 per cent sodium alginate solution in order to recover 90 per cent of algin in the solution. Moreover, a considerable amount of hydrochloric acid is consumed in a factory for neutralizing alkali which was used to macerated algae, so that requirement of hydrochloric acid amounts to a very large quantity in algin industry.

5. Drying of Algin

Alginic acid obtained by the above described methods should be washed and dehydrated. However, the dehydration of swelling water in alginic acid gel is very difficult by washing with water. The author proposes to wash the gel with hot water at 55–58°C, in accordance with what he was learned by actual experience. Such a

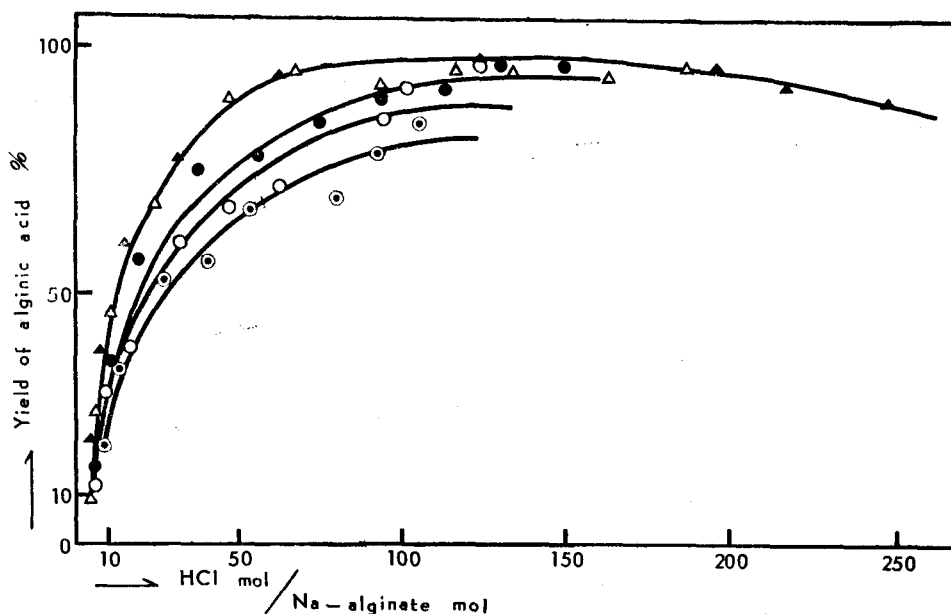


Fig. 62. Yield of alginic acid and equivalent ratio of HCl to sodium alginate (HCl mol/Na-alginate mol) when alginic acid is gelled with HCl from sodium alginate solution

- ▲----- Gelled from 0.3% sodium alginate solution
- △----- Gelled from 0.4% sodium alginate solution
- Gelled from 0.5% sodium alginate solution
- Gelled from 0.6% sodium alginate solution
- ⊙----- Gelled from 0.7% sodium alginate solution

mild heating would make the dehydration of the gel very easy and at the same time would not decrease its viscosity. Further dehydration of thus obtained alginic acid gel can easily be done by centrifuging or pressing. The contracted gel thus obtained contains ordinarily about 80-90 per cent water, while alginic acid gel obtained without heating contains swelling water 200 times as much as its weight.

Algin is marketed in various types of combination prepared by various drying procedures. Sodium alginate and alginic acid are the commonest products of algin which are on the market. The drying process in the preparation of these two products of algin were investigated in the present study.

There are two ways for producing sodium alginate in a factory. One is by drying sodium alginate paste itself by some drying method, and the other is by preparing at first sodium alginate gel in 30-40 per cent ethanol solution by neutralization of alginic acid gel with sodium carbonate or caustic soda and drying thus obtained solid sodium alginate gel by some drying process. However, both methods are accompanied by a process of drying sodium alginate. The effects on viscosity of alginate by heating sodium alginate paste are investigated in connection with the preparation of sodium alginate.

(i) *Drying of sodium alginate*

Sodium alginate preparation was made from sodium alginate solution by repeating dissolution with water and gelling with ethanol several times. Then the gel was washed with ether and dried in desiccator.

Twenty five cc of 1 per cent sodium alginate was pipetted into each of number of petri-dishes and heated at 70, 80, 90 and 100°C respectively. After heating for a certain period 0.25 per cent solution of sodium alginate was prepared and its specific viscosity was measured. The relative values of the viscosity were calculated adjusting initial viscosity of the solution to 10.00, where observed initial viscosity of the solution was 90.00. The results are shown in Table 31.

Table 31. Viscosity of 0.25% Na-alginate solution

Duration of heating (min)	Temperature of drying (°C)			
	100	90	80	70
0	10.00	10.00	10.00	10.00
30	7.23	7.15	9.10	9.28
60	4.55	5.97	8.69	—
90	3.23	4.39	7.60	8.80
120	2.69	4.50	7.66	—
150	2.77	3.92	7.07	—
180	—	—	—	8.22

It is considered that the colloidal quality of sodium alginate would not be affected very markedly by heating at 70°C, however, the effect of heat upon dried film of sodium alginate should be examined because the heat attack upon dried film of sodium alginate does not occur under such a condition.

Undesirable effect of heat upon the viscosity at lower temperature in concentrated solution of sodium alginate was investigated as follows. Twenty grams of 2.5 per cent sodium alginate was weighed in a petri-dish, the change of specific viscosity and the weight of the paste were determined and calculated to initial values 10.00; the results are shown in Table 32 and Figs. 63 and 64.

Table 32

Duration of heating (min)	Temperature of drying (°C)						
	40		50		60		70
	Viscosity	Weight	Viscosity	Weight	Viscosity	Weight	Weight
0	10.00	10.00	10.00	10.00	10.00	10.00	10.00
30	10.20	9.33	10.00	8.31	8.35	6.95	5.87
60	10.67	9.08	9.70	7.85	8.04	4.28	4.05
90	11.15	9.02	9.10	7.23	—	3.25	2.55
120	14.13	8.59	5.58	6.85	—	1.87	1.38
150	14.75	8.18	5.13	5.89	—	—	—
180	17.05	7.74	3.78	—	—	0.75	0.38

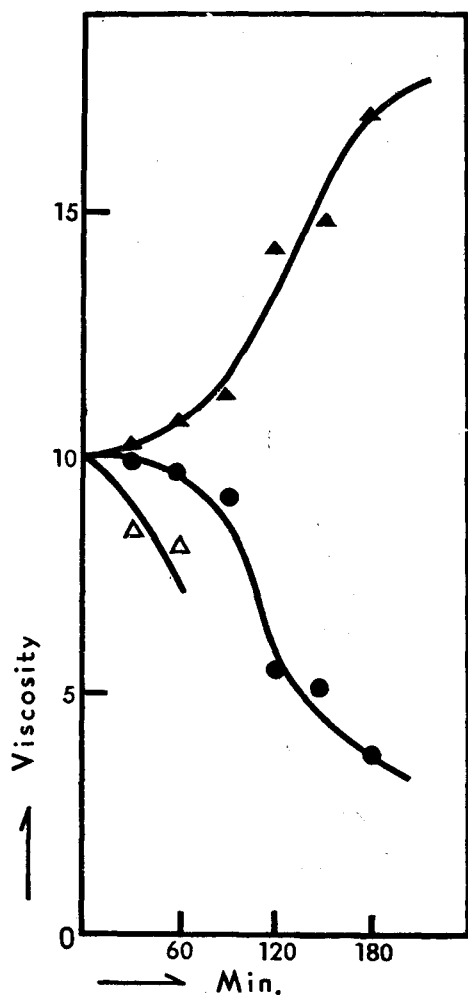


Fig. 63. Change of specific viscosity of 2.5% sodium alginate solution by heating

- △----- Heated at 40°C
- Heated at 50°C
- △----- Heated at 60°C

to increase at lower temperatures, sometimes even at lower than 40°C. The reason of this increase is not clearly known yet.

As it is suggested that the colloidal quality of sodium alginate is attacked especially by heating after it is dried in the form of film. The heat attack to solid sodium

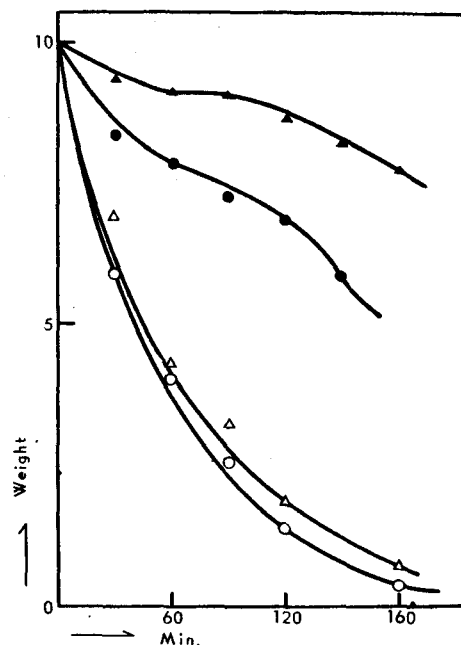


Fig. 64. Reducing of the weight of 2.5% sodium alginate solution by heating

- △----- Heated at 40°C
- Heated at 50°C
- △----- Heated at 60°C
- Heated at 70°C

The decrease of viscosity caused by heat attack is observed more distinctly as is shown in the table and in Fig. 63 than in the case of heating of dilute solution, though the viscosity of preparations treated at 40°C is increased by heating. The viscosity of concentrated sodium alginate solution is often observed

alginate was investigated as follows.

Solid sodium alginate, 1.00 g was heated at 40–70°C for 30–150 minutes and specific viscosity of each treated preparation was determined. The measured values are shown in Table 33 and Fig. 65 adjusting to initial value 10.00.

Table 33. Change of viscosity by heating to solid sodium alginate

Duration of heating (min)	Temperature of heating (°C)			
	40	50	60	70
0	10.00	10.00	10.00	10.00
30	9.90	10.00	9.25	7.10
60	9.70	9.40	8.85	6.22
90	9.15	8.80	8.52	5.47
120	9.07	8.22	7.77	5.15
150	9.07	7.37	7.15	5.05

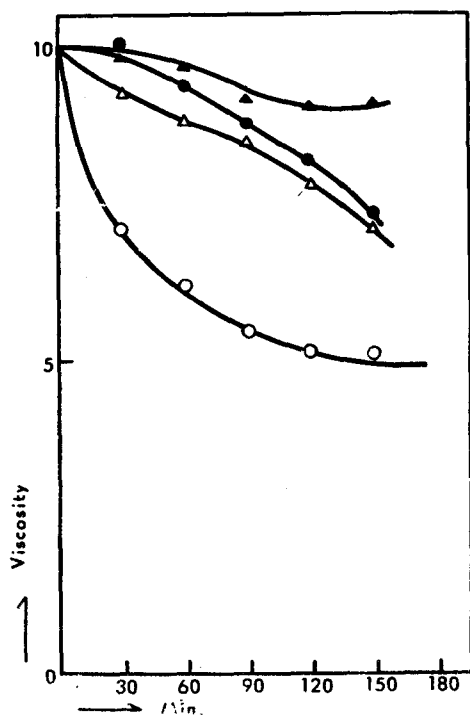


Fig. 65. Change of viscosity of solid sodium alginate by heating

- ▲----- Heated at 40°C
- Heated at 50°C
- △----- Heated at 60°C
- Heated at 70°C

Exposure to heat over 50°C is proved to cause the decrease of the viscosity of solid sodium alginate.

(ii) *Drying of alginic acid*

The alginic acid gel is swollen by water very largely. Actually, the swelling water amounts 200 times the weight of the original gel substance. Consequently the dehydration of the gel is very difficult as described above. However, the author has succeeded to dehydrate the swelling water of alginic acid gel, after many experiments by means of heating the gel in water at 55–58°C as described above. The alginic acid gel thus dehydrated, containing 82.15 per cent water, was used for the investigations of drying. Each 5.0 g portion of alginic acid gel was placed on petri-dish and dried at 40, 50, 60, 70 and 80°C respectively for a certain period ranging from 30 to 180 minutes. The changes of viscosity were measured on 0.25 per cent sodium alginate solution prepared from heated alginic acid gel. The results are shown in Tables 34 and 35 and Figs. 66 and 67.

Table 34. Change of weight of alginic acid gel by drying

Duration of heating (min)	Temperature of heating (°C)				
	40	50	60	70	80
0	10.00	10.00	10.00	10.00	10.00
30	8.05	5.78	5.35	4.82	2.45
60	6.17	2.90	2.85	2.83	1.27
90	5.88	2.40	1.73	1.34	1.24
120	4.80	2.02	1.71	1.27	1.24
150	4.08	1.73	1.34	1.27	1.24
180	3.95	1.42	1.27	1.27	1.27

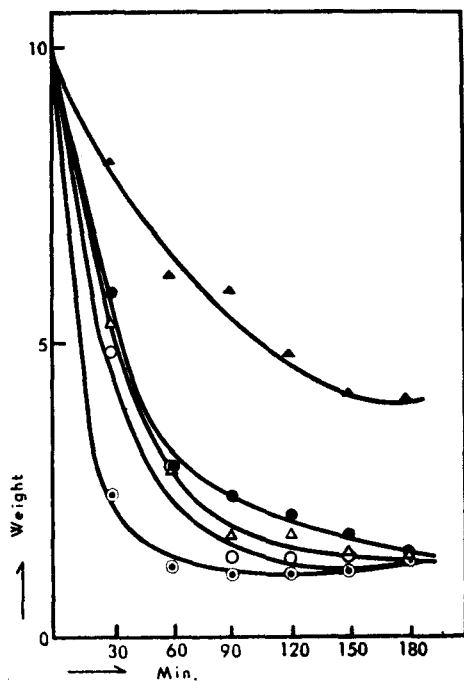


Fig. 66. Change of the weight of alginic acid gel by drying

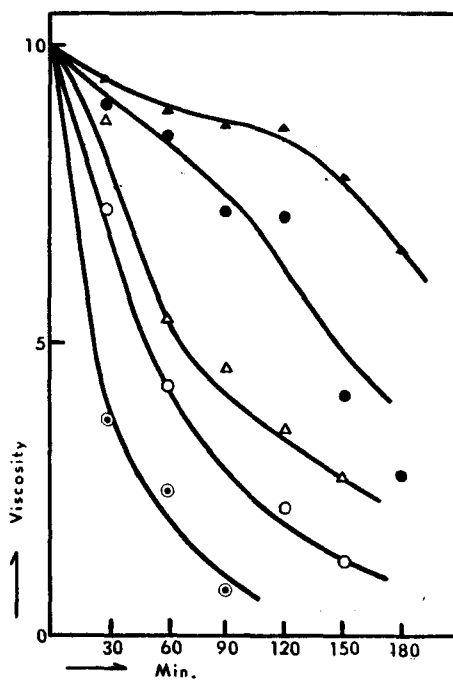


Fig. 67. Change of 0.25% sodium alginate by drying

▲----- Heated at 40°C ●----- Heated at 50°C △----- Heated at 60°C
 ○----- Heated at 70°C ⊙----- Heated at 80°C

It is clearly that the viscosity of alginic acid is remarkably decreased by heating at higher temperature and heating the dried surface of alginic acid gel. In order to prevent this effect of heating, alginic acid should be shredded into small pieces

Table 35. Change of viscosity of alginic acid gel by drying

Duration of heating (min)	Temperature of heating (°C)				
	40	50	60	70	80
0	10.00	10.00	10.00	10.00	10.00
30	9.40	8.95	8.75	7.30	3.67
60	8.90	8.42	5.38	4.23	2.43
90	8.65	7.14	4.57	3.14	0.79
120	8.57	7.14	3.49	2.15	0.67
150	7.76	4.08	2.65	1.25	—
180	6.50	2.71	—	—	—

uniformly and dried at temperatures lower than 50°C in presence of relatively wet air aiming to prevent the heat attack upon the dried surface of alginic acid gel.

CONCLUSION

Since there have been published only a few works on the theories involved in the mechanism of manufacturing algin from brown algae, the author made these investigations.

Through microchemical examinations it was proved that algin exists in various types of combination. The existence of calcium alginate, even partly or mostly, is supposed in the cell wall of the *Laminaria* fronds. The algin which would be converted into calcium alginate when it is treated with calcium chloride solution seemed to exist in the cell wall of the brown algae. The existing state of algin in brown algae is considered to be not so simple but very complicated, therefore studies on the mechanism of algin manufacture would be impossible without converting algin into a certain definite combined form.

The author analysed a number of samples of brown algae which would be used as raw materials in algin manufacture and investigated seasonal, local and specific differences of their components. Since mannit, potassium or iodine would be obtained as by-products of algin industry, their contents were also examined. The obtained remarkable results are briefly described as follows.

Because the seasonal variations of the chemical components in the material effect each other in respect to their contents, the absolute amount of each component in the material can not be discussed, but their relative contents can be. Very obvious seasonal variations are observed in the amounts of iodine, mannit, protein and fat. The former two components show their minimum amounts in spring and evidently increase toward autumn. On the contrary, the latter two show their maxima in spring and minima in autumn. This relationship is considered to be significant in the life of brown algae, because the photosynthesis of plants is at its maximum in summer and the metabolic processes are at their minima in winter. The contents of ash, potassium and alginic acid vary seasonally to some degree, but the variations could not be

distinguished from those which may be caused by alternative relation of other obviously varying components.

Alginic acid, from the above described consideration, would be considered a standing component of brown algae because it does not vary much with season or species. Alginic acid is considered to exist as an intercellular substance in the cell wall of brown algae, to play some important role in their skeleton. As for the role of algin in brown algae, further studies should be undertaken.

The brown algae should be harvested in autumn, at their reproductive period, because their growth reaches its maximum and the mannit and iodine contents show their maxima in that season, although algin contents do not vary so remarkably by season.

Algin, mannit, iodine and potassium are generally contained in larger amount in Laminariaceae than in Fucaceae. Therefore, it may be said that most suitable raw materials for algin manufacture and its by-products are the algae belonging to the Laminariaceae, though it should be taken into consideration in a factory that the Laminariceous algae are rather expensive.

Theoretical and practical studies on algin manufacture were carried out by converting algin in brown algae into free alginic acid and calcium alginate, pretreating algae with dilute hydrochloric acid or calcium chloride solution. Algin in brown algae exists in various types of combination as above described, so it is desirable to convert algin in brown algae into some definite combined form in order to manufacture algin by means of most suitable manufacturing process.

For the purpose of learning the actual mechanism of manufacturing processes, fundamental investigations were made using purified free alginic acid and calcium alginate, and several properties of both kinds of algin are also studied as described next.

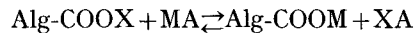
Alginic acid, from the results of determination of dissociation degree, pH, ionization constant and pK of the saturated alginic acid solution, is proved a fairly weak acid.

The titration curve of saturated alginic acid solution with sodium hydroxide is very characteristic and the reason of the fact is considered to be the existence of carboxyl radicals having various dissociation constants or some certain colloidal effect. The characteristic of titration curve does not disappear by adding electrolyte to alginic acid solution, therefore, the existence of carboxyl radicals having different dissociations is considered to be fairly possible although further investigations are needed because the colloidal effect would be eliminated by adding a fairly large amount of electrolyte to such a dilute colloidal solution.

Calcium alginate preparations were made by adding various amounts of 5 per cent calcium acetate solution to sodium alginate solution. Preparates were dialyzed in distilled water until Ca ion reaction in dialyzing water had disappeared. Since any

remarkable difference was not recognized concerning their solubilities and other properties in the range of calcium content in calcium alginate, 9.7 to 10.5 per cent, the preparates containing calcium in the range of 9.8 to 10.5 per cent were used for learning the mechanism of algin manufacture from calcium alginate, where the theoretical calcium content in calcium alginate calculated from the formula $C_6H_7O_6 \cdot 1/2Ca$ is 10.25 per cent.

It has been made clear by studies reported in this paper that insoluble algin such as free alginic acid or calcium alginate, is dissolved into soluble alginate by cation exchange. The cation exchange equilibrium between insoluble algin and an electrolyte concerned with the dissolution of algin is presented in the following formula ;



where, "X" represents a cation which forms an insoluble algin combining with Alg-COO^- , such as H or Ca, and "M" represents a cation which forms a soluble algin combining with Alg-COO^- , such as an alkali metal, ammonium radical or magnesium, and "A" represents an anion.

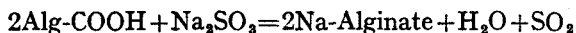
An insoluble algin is dissolved into soluble algin when the above mentioned reaction is almost or completely irreversible. That is to say, the dissolution of algin takes place in the special case of cation exchange reaction. The electrolytes which are able to be used for dissolving free alginic acid and calcium alginate are described respectively in Tables 7 and 4.

Since algin occurs in various states of combination in brown algae as described above, it is desirable to pretreat algae with a certain proper reagent solution in order to convert algin in brown algae into a definite form, because algin would be extracted by suitable manufacturing process according to the pretreatment. The author discovered that many sorts of electrolytes are available to extract algin from brown algae as described above. Among them two recommendable processes are described as follows.

1) *Algin manufacture from algae macerated with sodium sulfite*

It is observed that there are two cases in dissolution of free alginic acid into soluble alginate with the electrolyte in Table 7. One is the case where reaction proceeds with the material keeping acid, and the other is the case where the reaction proceeds with the material keeping alkaline. The reason is obvious that the reaction (2) proceeds keeping acid when "A" is an acid radical, while the reaction proceeds keeping alkaline when "A" is OH^- or CO_3^{--} . This is true since the latter is a case of dissolution by neutralization, and it may be said a peculiar case of cation exchange reaction which is perfectly irreversible.

Among the electrolytes enumerated in Table 7, Na_2SO_3 is a very highly recommendable one, because the macerated mass or diluted solution is protected from decomposition by microorganisms and algin is bleached by SO_2 which will be generated in the process of the reaction :



Furthermore, the reaction is almost perfectly irreversible because the produced SO_2 would escape from the system by heating.

A practically manufacturing method of algin from brown algae with sodium sulfite is briefly described as follows.

In order to convert various types of algin in brown algae into free alginic acid, the algae are treated with 0.1 n hydrochloric acid solution for 15–30 minutes at 50–60°C. After removal of acid solution, 15 per cent of sodium sulfite by weight to dried algae and 30 times that weight of water are added to the remaining algae and heated for an hour at 60–70°C, then the bodies of the plants are completely disintegrated. Further treatment may be done as in an ordinary method.

2) *Algin manufacture from algae macerated with sodium chloride and sodium carbonate (SUZUKI'S method)*

It is proved that algin can be manufactured by following procedure without destroying epidermal tissue and hence without dissolving out the pigment from the assimilatory cells; the author proposes to name this method "SUZUKI'S method." The method is established by the fundamental investigation which is shown diagrammatically in Fig. 18, the method is developed as follows.

Treat the dried brown algae first with boiling water for 15–30 minutes to swell the plant body and to remove soluble matter and other impurities. Drain the water, and treat the algae with 0.5 per cent calcium chloride or saturated calcium hydroxide solution for 15–30 minutes at 80–100°C to convert algin in the cell walls into calcium alginate and to make the pigment in the epidermal cells insoluble. Cast away the liquor, and treat the algae with 10 per cent sodium chloride by weight to dried algal materials and 30 times that weight of water for 30 minutes at 80–100°C. Within this period, calcium alginate in the cell walls is swollen by cation exchange reaction producing soluble algin, while the pigment is retained in the plant body. Do not cast away the liquor, macerate the remaining bodies of algae with sodium carbonate added to as large amount as 10 per cent by weight of the original dried algae for 10–20 minutes with gentle stirring. Allow to stand for several hours so as to bring about complete maceration; the pigment and the epidermal cells would remain undissolved and undamaged. The colour of the algin thus obtained is almost white without any bleaching and its viscosity is very high owing to the weak attack of alkaline solution. The process of filtration is very easily carried out because epidermis and mucilagenous substances excepting algin are very slightly dissolved.

Further investigation regarding each treatment process in the manufacture of algin and its by-products is made as follows.

The pretreatment of brown algae should be done for two purposes, namely, first for the purpose of extracting mineral salts, mannit or soluble impurities and second

for the purpose of converting algin in brown algae into definite state of combination. Since only a few works are available for pretreatment of brown algae, several investigations were carried out in the present study. The extracting power of the pretreating solution is greatly affected by its effect in causing the algae to swell. It is proved that algae absorb water as much as about 3-4 times their weight and they absorb 1 per cent calcium chloride solution as much as 2.5-3.5 times their weight within a short duration of the treatment, while wet algae do not absorb water as much as 50 per cent of their weight. Dilute hydrochloric acid is absorbed only as much as 50 per cent of the weight of algae even the dried material. The extractive power of several solutions, such as water, 0.1 n hydrochloric acid, 1 per cent calcium chloride, 0.005 per cent and 0.001 per cent NaOH, 0.01 per cent Na_2CO_3 , sea water and the mixture of fresh water and sea water was investigated. No remarkable difference of power to extract potassium was shown amongst the treatments. Some difference of power to extract iodine was observed amongst the treatments; the minimum power to extract iodine was observed in the case of pretreating with dilute hydrochloric acid solution and the maximum is shown in the case of alkali treatment. The yield of mannit showed fairly great fluctuation and it is suggested that mannit is less soluble than potassium chloride or iodine under the conditions above described. At all events, no essential difference in respect to extractive power could be found amongst the examined pretreatment, therefore, pretreatment of algae may be done only considering the conversion of type of algin, though the extractive power as having effect upon pigment and other impurities should be taken into consideration. It is considered that high concentrations would be required in spite of lowering the yield by repeating pretreatments because the recovery of the matter is generally difficult and expensive when the solution is dilute.

Some fundamental investigations were made on the recovery of mannit by fractional crystallization from other soluble mineral matter.

Since only a few works are available on the processes of manufacturing algin from brown algae, the author investigated each process of manufacturing stages as follows concerning with the new methods which have been perfected by him.

As algin is fairly unstable in its colloidal qualities, close attention must be paid to every step throughout the process of manufacturing algin.

The viscosity of the soluble alginate is decreased by heating according to the following formula,

$$y = Ae^{-\alpha t}$$

where, y represents specific viscosity of each stage of the duration of heating, t represents duration of heating and α and A are the constants of the equation. It is clear that the viscosity of algin would be decreased by heating, therefore, long spells of heating and treatment with high temperature should be avoided on every step in the

process of manufacturing algin.

The macerating processes with several chemicals were investigated pretreating algae with three solutions, water, dilute hydrochloric acid and dilute calcium chloride solution. The maceration was promoted by using sodium carbonate, sodium hydroxide, sodium sulfite, sodium dibasic phosphate and ammonium oxalate; the yield of alginic acid, specific viscosity of the preparations and the colour tone of alginic acid were examined. From the results, it is proved that sodium carbonate, sodium sulfite and sodium dibasic phosphate are able to macerate algae with every one of the used pretreatments, sodium hydroxide can macerate algae with dilute hydrochloric acid pretreatment only and, on the contrary, ammonium oxalate can not macerate algae with hydrochloric acid pretreatment. Those facts may be explained by the fact that the cation exchange reaction between sodium hydroxide and free alginic acid is irreversible but the cation exchange reaction between sodium hydroxide and calcium alginate or other insoluble alginate in the brown algae is almost reversible, and free alginic acid is not dissolved into colloidal solution by treating with ammonium oxalate solution although it is dispersed into small particles as described in Table 3. For this reason it is that algin is scarcely dissoluble into the solution from the cell wall of the algae. From the examination of the yield, viscosity and colour tone of the algin preparations, it may be said that the most excellent way of algin manufacture among the experiments is to macerate algae with sodium sulfite after pretreating with dilute hydrochloric acid solution.

Filtrability of the macerated liquor was studied after maceration with above mentioned chemicals in the case of pretreating with water and dilute hydrochloric acid solution. It is also proved that maceration with sodium sulfite after pretreatment with dilute hydrochloric acid solution is the most excellent.

From the examination concerning the filtration of macerated liquor it is suggested that decomposition of algin would be caused by long spells of filtration and, therefore, the filtration of macerated liquor should be done mechanically in order to avoid the decomposition caused by long spells of filtration.

It is clear that an enormous amount of hydrochloric acid for alginic acid is needed to gel alginic acid from sodium alginate solution. That is to say, about 60 mol of hydrochloric acid is needed for gelling 1 mol of alginic acid from 0.3 per cent sodium alginate solution and over 100 mol of hydrochloric acid is needed for gelling 1 mol of alginic acid from 0.7 per cent sodium alginate solution. Moreover, in a factory, hydrochloric acid is expended for neutralizing alkali which is used for macerating algae, therefore, the maceration agent must be used as economically as possible in algin industry.

The viscosity of algin is remarkably decreased by heating, especially decreased by heating the dried surface of solid algin. Therefore, algin should be dried with careful management in order to prevent the above mentioned effect.

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Table 1

No.	Name of seaweed	Locality of collection	Date	Ash	K ₂ O	I	Cl	Crude fat	Crude protein	Alginic acid	Mannint
Scytosiphonaceae											
1	<i>Scytosiphon Lomentarius</i> (LYNGB.) J. AG.	Hakodate-Bay	May 24	39.51	4.06	—	4.74	1.466	—	—	8.26
2	"	Toi-Setarai	June 19	44.60	8.61	0.107	9.74	—	13.48	21.93	1.73
Laminariaceae											
3	<i>Laminaria japonica</i> ARESCH.	Muroran	Mar. 22	38.61	13.86	0.110	—	0.992	20.04	27.51	5.97
4	"	"	Apr. 26	45.87	14.45	0.256	—	0.491	18.16	18.16	4.63
5	"	"	June 8	46.70	19.28	0.205	—	0.467	16.31	20.33	6.93
6	"	"	July 18	41.60	15.65	0.309	15.46	0.187	7.82	28.73	20.71
7	"	"	Sept. 5	32.30	19.88	0.351	—	—	8.49	24.94	22.27
8	"	Hakodate-Bay	May 24	50.48	16.27	0.229	17.10	0.752	9.70	21.50	14.00
9	"	Tachimachimisaki	May 23	41.52	14.84	0.399	13.15	—	7.38	24.05	13.04
10	"	Toi-Setarai	June 19	44.94	16.86	0.248	14.85	—	6.23	23.84	16.57
11	"	Toi-Datemachi	June 22	39.41	12.14	0.076	12.08	—	8.27	21.37	24.00
12	"	"	June 22	38.41	12.63	0.306	14.21	—	6.34	27.95	16.04
13	"	Hakodate-Yamasedomari	June 16	27.11	8.05	0.171	6.91	—	3.12	26.95	27.83
14	<i>L. ochotensis</i> MIYABE	Esashi-Kamomejima	June 2	21.76	7.23	0.221	—	0.328	4.08	25.03	21.83
15	"	Otobe-Honson	July 28	18.60	3.70	0.169	6.55	—	6.24	22.31	30.29
16	<i>L. angustata</i> KJELLM. (1st year)	Muroran	Mar. 22	44.15	16.45	0.100	—	1.628	22.55	24.48	2.42
17	"	"	Apr. 26	40.95	19.73	0.144	—	0.345	17.53	21.70	4.49
18	"	"	June 8	47.09	19.17	0.128	20.01	0.459	11.51	22.79	7.85
19	" (2nd year)	"	Mar. 22	17.64	7.95	0.099	—	0.801	16.38	18.18	5.94
20	"	"	Apr. 26	29.24	8.12	0.117	—	0.822	17.54	27.19	4.77
21	"	"	June 8	37.61	11.70	0.132	—	0.112	10.15	25.32	6.93
22	"	"	July 18	21.10	6.77	0.142	7.75	0.167	7.31	26.68	11.02
23	"	"	Sept. 5	27.60	11.51	0.361	—	—	10.54	21.59	18.31
24	"	Kojohama	June 9	32.55	9.29	0.119	—	0.299	8.02	24.08	4.72
25	"	Toi-Datemachi	June 22	35.29	10.76	0.107	7.82	—	11.22	25.78	16.95
26	<i>L. religiosa</i> MIYABE	Esashi-Kamomejima	June 2	41.42	15.77	0.264	—	0.179	13.06	23.00	5.10
27	"	Matsumae	June 3	35.54	14.56	0.223	—	0.694	9.24	25.68	6.73
28	"	Futuro-Kawajiri	July 3	33.63	8.35	0.536	9.20	0.299	4.82	32.57	21.29
29	"	Udomari	July 3	33.93	10.35	0.391	9.85	0.271	7.67	29.26	23.03
30	"	"	July 3	30.30	9.59	0.083	8.60	0.165	9.09	26.32	28.49
31	"	Kudo	July 4	34.72	7.02	0.166	6.98	0.240	5.15	32.29	27.51
32	"	Tsugunai	July 4	33.88	10.42	0.527	9.52	0.219	4.24	31.46	22.90
33	"	"	July 4	34.86	9.37	0.322	10.30	0.267	4.21	32.16	22.17
34	"	Baikatsu	July 4	39.83	11.46	0.388	11.31	0.291	5.51	31.18	12.86
35	"	Ota	July 5	42.90	16.04	0.368	15.09	0.449	9.00	27.33	11.97
36	"	Kamiura	July 5	35.96	13.53	0.424	13.39	0.269	6.19	28.67	9.84
37	"	Otobe-Shiomi	July 28	28.11	—	0.665	—	—	5.55	25.36	16.28
38	"	Otobe-Mitsuya	July 28	31.61	—	0.853	—	—	4.11	25.24	20.29
39	"	Otobe-Motowa	July 28	31.58	—	0.811	—	—	4.62	27.61	15.71
40	<i>Costaria costata</i> (TURN.) SAUND.	Kaitorima	July 28	32.01	—	0.442	—	—	6.07	29.42	13.74
41	"	Muroran	Mar. 22	39.92	—	—	—	2.081	23.10	—	0.73
42	"	"	Apr. 26	36.39	7.27	0.024	—	0.729	14.94	25.80	8.15
43	"	"	June 8	33.99	10.45	0.006	—	0.721	12.52	25.79	9.22
44	"	"	July 18	34.32	10.23	0.099	10.30	0.338	11.48	29.52	11.55
45	"	Toi-Setarai	June 19	32.37	11.12	0.123	8.77	—	13.52	21.99	4.65
46	"	Toi-Datemachi	June 22	42.13	13.66	0.044	10.38	—	14.01	28.17	8.54
47	<i>Alaria crassifolia</i> KJELLM.	Muroran	Mar. 22	36.31	11.79	0.038	—	0.950	33.56	23.08	5.74
48	"	"	Apr. 26	35.68	9.94	0.045	—	0.837	22.30	25.52	6.50
49	"	"	June 8	33.05	10.29	0.018	12.56	0.553	14.74	27.98	13.16
50	"	"	Mar. 22	44.98	6.34	0.022	—	1.007	18.32	27.08	3.26
51	"	"	Apr. 26	28.34	6.65	0.024	—	0.680	18.87	31.71	5.29
52	"	"	June 8	25.46	8.19	—	—	0.514	12.85	28.97	8.22
53	"	"	July 18	30.23	7.54	0.043	8.60	0.195	13.57	30.75	14.24
54	"	"	Sept. 5	24.84	3.87	0.012	—	—	11.34	26.91	10.98
55	"	Tachimachimisaki	May 23	33.56	6.96	0.012	7.73	—	19.68	22.18	4.74
56	"	"	May 23	39.66	9.28	0.024	10.37	—	19.66	25.86	7.60
57	"	Toi-Setarai	June 19	32.20	7.04	0.066	7.44	—	12.21	34.86	3.32
58	"	Toi-Datemachi	June 22	29.88	8.15	0.119	8.14	—	16.01	35.04	7.19
59	<i>Undaria pinnatifida</i> SURING.	Tachimachimisaki	May 28	44.51	8.38	—	—	—	5.64	25.69	3.14
60	"	Udomari	July 3	36.94	3.76	0.012	9.17	—	7.66	23.76	14.11
Fucaceae											
61	<i>Fucus evanescens</i> C. AG.	Muroran	June 8	39.50	4.52	0.012	10.71	0.486	7.75	13.31	10.86
62	<i>Pelvetia Wrightii</i> (HARV.) YENDO	"	June 8	28.21	3.67	0.167	5.34	0.501	13.85	18.74	11.65
63	"	Toi-Setarai	June 19	31.12	4.58	0.072	2.28	—	6.56	25.97	11.14
64	"	Muroran	July 18	29.57	5.81	0.031	4.47	—	5.85	30.85	15.93
65	"	"	Sept. 5	30.54	5.25	0.113	—	—	8.49	27.98	6.50
66	<i>Hijikia fusiforme</i> (HARV.) OKAM	Hakodate-Yamasedomari	June 16	48.02	15.84	0.041	14.34	—	5.68	18.70	14.65
67	<i>Cytophyllum hakodatense</i> YENLO	Muroran	June 8	29.49	8.39	0.032	10.78	0.487	11.90	16.17	12.20
68	"	Toi-Setarai	June 19	27.13	9.33	0.048	9.28	—	11.26	13.01	4.09
69	"	Muroran	July 18	36.47	8.53	0.080	9.29	0.372	6.85	18.64	23.94
70	"	"	July 18	35.27	8.53	0.038	8.96	0.467	7.53	13.91	13.69
71	<i>Sargassum Horneri</i> (TURN.) C. AG.	Esashi-Kamomejima	June 2	36.37	7.48	0.042	9.80	0.197	6.42	15.59	20.08
72	"	Matsumae	June 3	45.50	6.57	0.061	10.54	1.280	10.87	17.68	7.86
73	"	Udomari	July 3	29.95	6.63	0.068	8.71	—	5.01	13.91	23.98
74	"	Ota	July 5	39.09	8.80	0.097	—	—	5.89	14.79	13.71
75	<i>S. tortile</i> C. AG.	Kudo	July 4	28.65	6.04	—	12.09	—	—	14.02	14.70
76	"	Otobe-Honson	July 28	23.59	3.51	0.031	10.80	—	4.43	18.42	22.72
77	<i>S. sagamianum</i> var. <i>yezoensis</i> YAMADA	Ota	July 5	21.08	4.78	0.024	5.23	—	6.66	27.72	17.05
78	"	Esashi-Kamomejima	July 27	34.13	4.71	0.043	7.88	—	5.92	22.80	12.95
79	"	Udomari	July 3	35.08	6.24	0.056	6.27	—	4.36	19.71	17.48
80	<i>S. confusum</i> AG.	Muroran	June 8	29.87	7.77	0.041	9.22	0.841	11.48	18.85	12.37
81	"	Esashi-Kamomejima	June 2	25.94	6.43	0.035	7.43	0.720	8.25	19.67	11.12
82	"	"	June 2	25.67	8.87	0.009	8.26	0.792	6.89	21.16	11.61
83	"	Hakodate-Bay	May 24	33.24	5.70	0.006	5.39	1.418	22.38	20.36	12.59
84	"	Toi-Setarai	June 19	35.77	7.69	0.059	7.19	—	11.15	23.19	3.40
85	"	Hakodate-Yamasedomari	June 16	36.72	6.09	—	7.00	—	13.83	19.16	13.89
86	"	Futuro-Kawajiri	July 3	34.34	13.01	—	5.71	—	4.54	22.67	14.79
87	"	Kudo	July 4	28.27	7.82	0.057	7.13	—	—	21.67	13.06
88	"	Udomari	July 3	33.04	7.74	0.043	8.50	—	8.47	20.80	13.63
89	"	Muroran	July 18	40.73	8.98	0.025	10.08	—	10.54	22.29	5.64
90	"	Otobe-Honson	July 28	29.90	4.58	0.025	3.70	—	6.07	21.37	15.59
91	"	Otobe-Shiomi	July 28	25.38	8.84	0.057	—	—	5.36	22.84	14.82
92	"	Kumaiishi	Aug. 2	30.76	5.56	0.012	—	—	4.18	25.28	9.38
93	"	Otobe-Honson	Aug. 5	37.72	4.26	0.013	—	—	2.87	24.90	3.95
94	<i>S. Thunbergii</i> (MERT.) KUNTZE	Muroran	June 8	41.91	9.14	0.173	9.89	0.596	11.04	10.87	8.94
95	"	Matsumae	June 3	44.76	8.28	0.024	10.40	0.571	12.53	12.53	8.77
96	"	Toi-Setarai	June 19	37.31	8.06	0.046	7.00	—	9.71	17.49	6.15
97	"	Hakodate-Yamasedomari	June 16	41.00	10.57	—	9.36	—	11.21	18.46	10.02
98	"	Ota-Kawajiri	July 3	31.88	6.71	—	7.75	—	—	17.41	