SEASONAL VARIATION IN SOME OF THE PROPERTIES OF A PHYCOCOLLOID FROM GRACILARIA CONFERVOIDES

by

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ABSTRACT

The investigation was carried out in order to determine the seasonal variation in some of the properties of agar from British Columbia Gracilaria confervoides. Optimum conditions for the preparation of agar were determined. The methods employed in the preparation of the seaweed for extraction were found to affect the properties of the agar. A very marked seasonal variation in the gelation property of the agar was obtained and the maximum gel strength values were found to vary with the source of the seaweed. Maximum gel strength values obtained during the investigation were found to be 100% and more higher than that of commercial Difco Bacto agar. The other significant physical properties of the agar prepared in this investigation were found to be comparable to those reported by other investigators working with Gracilaria confervoides in other parts of the world.

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I. INTRODUCTION

A. Historical

The preparation of agar-agar in the dehydrated form originated in Japan about 300 years ago (54). It is believed the first preparation was obtained by the accidental freezing and thawing of agar-agar jellies. Because of this it was called "kanten" which means in Japanese, "cold sky" (58). This substance was extracted in Japan principally from the red seaweed Gelidium amansii (54).

The name agar-agar, of Malayan origin, refers to certain East Indies seaweeds, chiefly Eucheuma muricatum, and occasionally to the jelly made from this seaweed. Tseng (54) surmised that Chinese immigrants to the East Indies imported Japanese "kanten" and rather than use the Japanese name, they adopted the name agar-agar already in use in the East Indies. For some years now, agar-agar has been shortened to agar.

The Japanese agar industry soon developed after it was found possible to prepare the substance in a pure dry form. However, it remained an Oriental commodity, except for small quantities exported from Japan to Europe for making jellies or blanc mange (26). Hitchens and Leikind (22) have recorded the story of how, in 1882, the wife of an associate of Koch, the famous German bacteriologist, suggested that agar be tried as a substitute for gelatin as a medium for culturing bacteria.

At the beginning of the twentieth century, agar export from Japan became an important industry. Cheap labour per-

mitted Japan to maintain a monopoly on agar (49). However, during World War II supplies were cut off from Japan and in the United States, agar became one of the first commodities to be designated a "critical war material" by the War Production Board (26). The loss of the Japanese product during the war stimulated interest in agar production in practically all countries bordering the sea. Many attempts were made also to find a substitute for agar. The most suitable substitutes have been prepared, by special treatment, from such Rhodophyceae as Chondrus crispus and Gigartina stellata.

B. Terminology and Definitions

The term phycocolloid was proposed by Tseng (54) to designate the hydrophilic colloidal polysaccharide constituents extracted from the brown and red algae. There are a number of important phycocolloids present in various genera of the brown and red algae. Tseng (53) has proposed a tentative systematic arrangement of usefull phycocolloids. In this scheme he has included laminarin, algin and fucoidin as being derivatives of the brown algae. He has divided the red algae into three groups characterized by the general nature of the phycocolloids obtained from each group. The following are the groups as suggested by Tseng:

(a) Agarophytes
(b) Carrageens
(c) Others

Tseng (53) suggested that the term "agarophyte" be used to refer to the agar-bearing seaweeds and to restrict the use of the word "agar" exclusively to the dried extract.

The term "agar" will be used with this meaning throughout the experimental work of this investigation. He has also proposed that agars be classified according to seaweed source and country, for example, Gelidium-agar (Japan) and Pterocladia-agar (New Zealand). In the second group Tseng has included the carrageenin extracts from Chondrus crispus and Gigartina stellata. In the third group, Phyllophora, Gloiopeltis and Iridophycus were placed and they produced extracts called respectively agaroid, funorin and iridophycin.

The Russian worker, Kizevetter, used the term agaroid to describe the agar-like substance extracted from Phyllophora rubens. Agaroid was not suitable for all purposes for which agar is used and it apparently had properties similar to the carrageenins. Humm (26) suggested that the term agaroid was suitable for any agar-like extractive that formed gels too weak to be regarded as agar. According to this concept there would be two general types of extracts from the red algae. First, the agars which, in concentrations of about 1%, give firm gels and second, the agaroids which at much higher concentrations, give weak gels only. Another essential difference between the types is that agar sols are not viscous, whereas agaroid sols are extremely viscous. It should be pointed out at this time that the agaroids can be given physical characteristics similar to the agars by the addition of electrolytes (18,55) or special extraction procedures (17,27,36).

Tseng (53) also suggested that the term "gelose", proposed by Payen, be kept to designate all algal extracts which

are able to form a more or less firm jelly in aqueous solutions.

Several attempts have been made to find a suitable definition for agar. Fellers (19) stated that agar was the commercial name applied to the dried and more or less purified stems of certain kinds of marine algae. The U.S. Pharmacopoeia (42) defined agar as "the dried mucilaginous substance extracted from Gelidium corneum and other species of Gelidium and closely related algae".

Stoloff stated (48) that the War Production Board (U.S.) in its enforcement of Order M-96 defined agar as: "any mucilaginous substance, whether dried or in other forms, extracted from Gelidium corneum, Gelidium cartilagineum, Gelidium amansii, Gracilaria confervoides, Gracilaria lichenoides, Euchema spinosum, Euchema isiforme, Euchema denticulatum, Gigartina spinosa, Gigartina mamilosa, and from other species of the genera named above and closely related algae of the class Rhodophyceae". The two latter definitions include both agars and agaroids under the term "agar".

In 1944, Tseng (51) defined agar as, "the dried amorphous gelatine-like non-nitrogenous extract from Gelidium and other red algae, being the sulphuric ester of a linear galactan, insoluble in cold but soluble in hot water, a dilute neutral solution of which sets upon cooling to a firm gel solidifying at 35-50°C. and melting at 90-100°C.". A year later, Tseng (53) modified this definition as follows, "the dried amorphous, gelatin like, non-nitrogenous extract from Gelidium and other agarophytes, being the sulfuric acid ester of a linear galactan, insoluble in cold water but

soluble in hot water, a 1% neutral solution of which sets at 35° to 50°C. to a firm gel, melting at 80°C. to 100°C.". This modification excluded the agaroids.

Wood (58) defined agar in such a way as to include the agaroids. He stated, "agar is derived from certain members of the Rhodophyceae and consists of the sulphuric ester of a galactan complex, which, with water, forms a lyophilic colloidal solution liquefying between 70° and 100°C. and setting between 30° and 50°C.".

Stoloff (48) stated that agar should be standardized as to temperature of gelation, gel strength, elasticity, syneresis, viscosity, transparency, ash content, and content of impurities. Humm and Wolf (25) considered that an agar, so standardized, should be regarded as "bacteriological agar", because such a product would be quite different from the generally accepted meaning of the word "agar". These authors further stated that an unstandardized agar might, and does, satisfactorily meet many of the other requirements for which agar is employed. They suggested because agar was not a single complex but a group of closely related entities, that the term "agar" should remain generic.

C. Agarophytes used in the manufacture of agar

A large number of genera and species of red algae have been used for commercial production of agar. An attempt has been made to gather together the majority of the red algae genera and species which have been used in the manufacture of agar. The scientific names (as reported) are tabulated

in Table 1, along with the countries using the particular species and the literature reference. The table also includes those algae which have been reported as suitable for agar manufacture but may not have been used, as yet, because of limited quantities. The algae which produced the agaroid type extract have been omitted from the table. Other red algae have been investigated, but the published information did not supply sufficient data to enable classification as an agar or an agaroid type seaweed. Therefore, such algae have been omitted from the table.

TABLE 1. Agarophytes used in the manufacture of agar

Scientific Name	Country	Reference
Acanthopeltis japonica Okam.	Japan	59
Ahnfeltia plicata (Hud.) Fries	Russia	54
" " Huds. var.	Japan	59
Ceramium boydenii Gepp	Japan	59
" hypnoides (J.Agh.) Okam.	Japan	59
" rubrum T. Agh.	Japan	59
Eucheuma gelatinae (Esp.) J. Agh.	Japan	59
" spinosum (L)J.Agh.	Japan	59
Gelidium amansii Lam.	Japan	59
" "	United States (W. Coast)	48
" cartilagineum	South Africa	28
" "	United States (W. Coast)	48
" caulacantheum	New Zealand	38
" corneum	Spain	36

Table 1 (continued)

Scientific Name	Country	Reference
Gelidium coulteri	United States (W. Coast)	1
" japonicum	Japan	59
" latifolium	Ireland	36
" pacificum Okam.	Japan	59
" pulchellum	Ireland	36
" subcostatum	Japan	59
" vagum Okam.	Japan	59
Gracilaria chorda Holm	Japan	59
" compressa Agh.	Japan	59
" confervoides	New Zealand	38
" "	Australia	58
" "	India	3
" "	South Africa	29
" "	United States (E. Coast)	24
" " (L) Grev.	Japan	59
" gigas Haro	Japan	59
" multipartita (Clemente) J. Agh.	United States (E. Coast)	24
Hypnea cervicornis J. Agh.	Japan	59
" musciformis (Wulf) Lamour	Japan	59
" seticulosa J. Agh.	Japan	59
Pterocladia capillacea	New Zealand	38
" lucida	New Zealand	38
" tenuis Okam.	Japan	59
Suhria vittata (L) J. Agh.	South Africa	28

D. Production and Consumption of Agar

At the outbreak of World War II Japan was supplying about 95% of the world's supply of agar (58). From 1930 to 1940 the average production in Japan was 2,476 metric tons per year (59). Slightly less than half of this was exported and the remainder used for home consumption (59). The United States imported between 600,000-700,000 pounds annually (49). Canada imported from Japan an average of 25,000 lbs. (approximately) during the years 1934 to 1940 inclusive (59).

In 1945, Japan began exporting agar again and during that year the total exported was 75 metric tons. However, during the war years other countries were working towards independence in agar production. In 1940 United States had only one agar factory which produced 24,000 lbs. of agar (53). By 1945 there were four factories operating and three more in plant stages with the expected production capacity of 200,000 lbs. per annum (53).

In 1946, Australia and New Zealand hoped to obtain a production of 60 to 100 tons and 15 tons per annum respectively. India was producing commercial and bacteriological grades of agar (6). Considerable work has been carried out by Great Britain and the United States to extend the usefulness of agaroids. By special treatment the agaroids were given properties which made the product suitable for many purposes for which agar was formerly used. Figures for Britain's expected production were not available at time of writing. The United States produced about 500,000 lbs. of carrageeninin 1944 (53).

E. Uses of Agar

In the Orient, agar was used primarily as a food. After agar was introduced to Europe and America, many uses were found for it. The purposes for which agar has been used depend principally upon the peculiar properties of its gel, particularly its marked stability, high viscosity, swelling with water, hysteresis, and its stabilizing effect on emulsions (58). Agar is most commonly known for its use in the preparation of culture media. However, in the United States, only about 20% was used for this purpose (48).

No attempt will be made to list all the uses which have been reported for agar. Uses of agar have been extensively reviewed by Tseng (52), Wood (58), Humm and Wolf (25), Humm (26) and Okamura (41). An adequate summary of agar uses can be obtained from Tseng's figures (51) for the uses of agar in the United States, as shown in Table 2.

TABLE 2. Uses of Agar in the United States

Industry	Lbs. per annum
Laxative	100,000
Microbiology	100,000
Bread, etc.	100,000
Confectionery	100,000
Dental impression material	75,000
Meat packing	50,000
Emulsification	50,000
Cosmetics	25,000
Miscellaneous	50,000

F. Methods of agar manufacture

1. Australia

The following method of agar manufacture in Australia was suggested by Wood (58). The seaweed was removed from air-dried storage and washed for a short time in a tumbler or dolly, drained and placed in a non-corrosive boiling vat. Extraction was carried out with live steam in acid medium (at or below pH 6.5) with a water/weed ratio of 25/1. The weed was boiled twice and the liquor from the second boiling was used as the mother liquid for the raw material. The recommended times for boiling were 1-3 hours for the first boiling and ½ hour for the second boiling with the specific times dependent upon the rate of agitation and the nature of the weed. The liquor was then filtered through a coarse canvas, either in a press, a filter bag, or a centrifuge and the filtrate run into a steam-heated vat, neutralized, and filter aid or activated carbon added if needed. It was then passed into a second filter or super-centrifuge for clarification. The clear liquor was evaporated and allowed to set in shallow vessels, frozen at 15-20°F. and thawed out in running water or in air. The resulting gel was centrifuged, washed in alcohol and dried by batteries of infra-red lamps.

2. Japan

In 1946, Dr. Carter (Director Fisheries Experimental Station, Vancouver,B.C.) made a survey of some of the agar manufacturing centres of Japan under the direction of the Supreme Allied Command for Occupied Japan. Dr. Carter has kindly given the author permission to make use of notes

from his "Memorandum of a field trip made 15th July 1946, around the coast of Izu Peninsula, Shizuoka Prefecture, Japan". In his notes he described the harvesting of Gelidium. The following is a brief description of harvesting as reported in his memorandum.

Seaweed collections are made chiefly between early May and early September. After the seaweed is collected by divers it is washed in fresh water. The individual collections are then weighed, recorded and pooled for drying by being spread out in a layer about three inches thick on open air bamboo racks. The seaweed is turned over once during the drying period.

In the sorting shed the seaweed clumps are pounded with a wooden mallet on a wooden block to loosen bits of shell, coral, etc., which are picked out. The seaweed is separated into three piles, two representing a separation into two species groups (species yielding different grades of agar) and the third pile representing mixed seaweed with considerable foreign matter entangled in it. Sometimes portions of the weed become bleached during drying and, if so, it is segregated as being of higher quality than unbleached weed. The dried seaweed is stored for baling.

Wood (59) has reported, in minute detail, the manufacturing process used in Japan at the present time. He stated that the usual procedure in agar manufacture was to use a blend of seaweeds and Table 3 shows a typical mixture used.

TABLE 3. Proportions of Seaweeds Usually Blended in Japanese Agar

Group Name	Percent
1) Tengusa (*Gelidium amansigp*)	45
2) Oni (*Gelidium japonicum*)	10
3) Torisachi (*Acanthopoltis japonica*)	5
4) Ego (*Ceramium hypnaeoides*)	10
5) Ogo (*Gracilaria* spp)	15
6) Igisu (*Ceramium* and *Gelidium* spp)	5
7) Hirakusa (*Gelidium subcostatum* sp)	10

1,2,3,7 - Hard weeds
4,5,6 - Soft weeds

The following is a summary of Wood's report.

The seaweed is taken from the bales, placed in cement tanks, and covered with soft water not warmer than 10°C. The tanks are outside so that the sun will bleach the weed which is turned over frequently. The hard weeds are soaked for about 24 hours, the soft weeds from 12-15 hours. The weed is next rinsed in a special washing machine for about ½ hour, the time depending on the amount of foreign matter present. After washing, the weed is drained and when about 225 kg. has been washed, it is rinsed for about two hours and placed in the boiling vat.

The boiling vats are large iron pots with an outside flange which sits on a brick hearth and the base of the pot is in the flue of a wood fire. The upper exposed portion of the pot is surrounded by a wooden cask with a lid through which a stirring paddle can be wielded. The usual charge is about 225 kg. of weed and 3,400 litres of water. A wood fire is

started under the pot. When the water is boiling, the hard seaweeds are added. This stops the boiling and when boiling resumes about 740 gms. of sulfuric acid are added to adjust the pH to between 6.3 and 7.0. The hard weeds are boiled for an hour to an hour and a half, then the soft weeds, igisu and ogo, are added. After 15 minutes boiling the softest weed, ego, is added. The liquor is allowed to simmer for twelve hours with the temperature dropping about 1^{o} per hour. At this stage the liquor from the second boiling of the previous batch (see below) is added and the whole boiled for four hours.

The addition of the second boiling lowers the temperature about 3^{o} so that the temperature at the end of the cook is 80^{o} to 83^{o}C. The solids slowly settle so that the upper liquor becomes relatively clear. This is dipped out with wooden dippers into filter vats. The bottom of the vats are perforated and lined with thin bamboo rods. A cotton cloth, 20 mesh, is placed in the filter box and the agar strains through.

The residue is placed in a second boiling pot, about 1230 litres of water is added, and boiled for ten hours, then strained through a separate filter. The liquor is the second boiling and is added to the first boiling as described above. The residue from the second boiling is dried and used as fertilizer.

The filtrate runs from the filter box into a collecting tank. It has a sloping floor and at the lower corner is a second box. The end is solid but the side has five holes arranged vertically. These holes are plugged as the tank

fills. After two hours the top plug is removed and the upper liquor flows into the box and is removed in a wooden bucket. Then it is poured into shallow wooden trays for setting. As the collecting tank liquor falls, the other plugs are successively removed. During the removal the solids not removed by the filter, sink to the bottom. The lower layer is kept separate and sold as lower grade agar.

The setting trays are stacked on racks and left for the jelly to set. The solidified jelly is cut with special knives into blocks about 2 1/4" x 3/8" x 18". The drying trays are stacked in the field for a few days so that the agar is slowly dehydrated and the strips can be easily handled. They are then spread out separately on trays or mats and left in the open at a slight angle so that the water will run off. The agar is frozen every night and the water thawed out each morning. After two to three weeks the freezing and thawing process is complete and the dried agar is sorted according to size, shape and color and tied in bundles. The yield of agar (20% moisture) is about 23%.

3. New Zealand

Moore (39) has reported the manufacturing process which has been taken up as a side-line by a gelatin and glue manufacturing industry in New Zealand. The dried seaweed was washed in cold water in wooden vats until freed of salt, sand, and the majority of the corallines, sponges and other foreign matter. Hydrolysis was carried out in a specially designed digestor operated under pressure, with the extraction spread over several waters.

The extracted liquor was drawn off into a mixing-vat, bleached and then filtered. The filtered liquor was run into enamelled cooling-pans, cooled until set, cut up and dialysed in a series of operation, frozen and then thawed. After thawing, the gel was spread on wire-gauze trays and dried in a cabinet through which hot air was passed. The dried agar was ground in a hammer mill to a fine powder, sifted and blended into a standard product.

4. Russia

Wood (58) has also reported the Russian method of manufacture, the technology of which was originally described by Kizevetter. Extraction was carried out in 8% lime solution with three extractions used, boiled 6 hours, 4 hours, and 2 hours respectively. The large material of the residue was removed by decanting and the fine material was removed by filtration through conical filters of cheap cotton material. The filtered liquor was decolorized, allowed to set in water-cooled moulds, frozen and thawed in water. Drying was carried out by drum drying below 80°C in vacuo.

5. United States

Several manufacturing processes have been used in the United States. They are essentially the same; therefore the individual processes will not be described. A summarized account as reported by Humm (26) is as follows. A weighed quantity of dried seaweed was placed in a large tank of fresh water and washed by propellor agitation. It was then conveyed to pressure cookers (California) and wooden tanks heated by live steam (North Carolina). After 2-6 hours cooking, the

agar solution was separated from the residue by straining and run into a storage tank in which a high temperature was maintained by steam coils. The seaweed residue was usually cooked again and the extract used as liquor in which to cook the next batch of seaweed. Filter aid was added to the hot solution and then it was forced through a filter press. Some factories added a hypochlorite bleach before filtration and others added the bleach later.

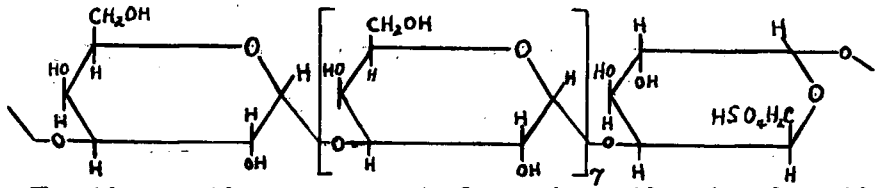
The filtrate was run into ice cans to cool and gel. The gel was either chopped and then frozen, or frozen without chopping. The frozen agar was fed through a crusher and thawed in water. In North Carolina the water was drained off through a sieve and the wet agar was spread on screens and dried by circulating hot air. After drying, it was hammermilled and pulverized. In California the thawed agar-water mixture was pumped to a "dewaterer" which drew off the free water by vacuum. The wet agar was conveyed to a cylindrical stack drier through which a hot air current passed. When the moisture content fell to 20% the agar particles were buoyant enough to be blown to the top of the stack and be picked up by a screw-conveyor. The agar was either marketed as flakes or granulated by hammermill.

G. Chemical Nature of Agar

1. Structure

In 1937, Percival and Somerville (43) established the fundamental features of the structure of agar. They showed that the major part of the molecule consisted of d-galactose residues united by 1:3-glycosidic linkages chiefly of

the β-type. In 1942 Jones and Peat (30) concluded that agar was the sulfuric acid ester of a linear polygalactose in which the repeating unit consisted of nine d-galactopyranose residues terminated at the reducing end by one residue of l-galactose. The l-galactose residue was attached to the rest of the chain by a 1:4-glycosidic linkage and it was esterified at carbon 6 with sulfuric acid. The following was the repeating unit of agar proposed by Jones and Peat (30).



Further, they suggested an hypothesis for the biological synthesis of agar. They stated that the sulfate fulfilled a role similar to that of phosphate in the enzymatic synthesis of starch from glucose-1-phosphate. The mechanism proposed was that the chain of d-galactose residues was formed by d-galactose-1-sulfate and when 10 of these residues had united a change took place with the tenth residue undergoing an intramolecular transformation which resulted in a 1-galactose residue esterified at carbon 6.

The above structural formula was criticized by Barry and Dillon (4) because they found that agar, prepared from Gelidium latifolium, contained a sulfur content corresponding to one sulfate group per 38 galactose units. Further, when the agar was redissolved, frozen, and thawed the ratio changed from 1 to 53. Barry and Dillon (4) also found that the agar was completely stable to periodic acid for a period of six months. They therefore concluded that carbon 4 of the

l-galactose residue could not be involved in the glycosidic linkage.

Percival (45) also found that samples of agar which he prepared did not contain enough sulfate to account for one l-galactose residue per nine d-galactose residues. He stated that there was no direct evidence to assign the ethereal sulfate group to carbon 6 rather than to carbon 3.

McFarlane (27) reported results obtained by Araki, who had found evidence that the l-galactose residue did exist in a 1:4 glycosidic linkage. He isolated the disaccharide agarobiose after dilute sulfuric acid hydrolysis. Agarobiose was composed of a d- and l-galactose residue united by a 1:4 glycosidic linkage.

In order to account for the variable results obtained by different workers, Percival and his collaborators (45) suggested that there was a gradual removal of the sulfate during the life of the plant with the formation of the 3-6-anhydro ring in the l-galactose residue. This could account for a variation in sulfate content and the remarkable stability towards periodic acid found by Barry and Dillon.

Percival and Thomson (44) reported that hydrolysis of methylated agar yielded some dimethyl galactoses. Dimethyl derivatives indicated, either the presence of a certain amount of branching in the agar molecule or incomplete methylation. They prepared the δ and λ hydrato-kantens of Takahashi and Shirahama (54) and found the molecular weight of the δ fraction to be in the region of 15,000 and the λ fraction about 3,000 to 5,000.

2. Evidence that Agar Consists of at Least Two Components

Humm (26) stated that it is probably a mistake to speak of agar as a single substance of certain chemical structure. He also said that it must be assumed and, until it has been shown otherwise, that each species of seaweed synthesized a chemically different type of phycocolloid.

Tseng (54) reported that in 1931, Takahashi and Shirahama separated agar into two fractions which they called hydrato-kanten $\delta+\lambda$. The separation was carried out by autoclaving for two hours at 130°C. Upon cooling, a white jelly was obtained. This was called hydrato-kanten δ and it was soluble in hot water and precipitated on cooling. A precipitate was obtained from the filtrate by the addition of ethanol. This was called hydrato-kanten λ and it was soluble in water and dilute alcohol.

In 1935, Lipatov and Morozov (34,35) showed the existence of an insoluble or "highly associated" fraction and a soluble "poorly associated" fraction of agar. They showed that the latter fraction exerted on the insoluble fraction a stabilizing effect towards dehydration and gelation. They further stated that the properties of gelation and swelling resided in the "highly associated" fraction. The "poorly associated" fraction caused a lowering of the structural viscosity of the "highly associated" fraction and a diminution of the total volume of the dispersed phase.

Neuberg and Schweitzer (40) obtained two fractions from agar by shaking crude agar with water for 20 hours. The filtrate contained a fraction, almost sulfur free, which

formed a gel like ordinary agar. The other fraction contained about 5% sulfur and would not gel. Stoloff (48) found that Gracilaria agar showed two sudden rises in viscosity, one at about 60°C and the other at about 40°C. He considered this to indicate the presence of two fractions in the agar. He was not able to separate the two fractions. In 1945, DeLoach et al (18) attempted to separate agar fractions from Gracilaria confervoides. The results obtained, based upon gelatin temperatures, indicated the presence of a mixture of several compounds.

3. Characteristics of agar and agar gels

a. Mechanism of gelation and gel structure

Hoffman and Gortner (23) stated that the gelation of agar was the gelation of a salt and not the gelation of a complex polysaccharide. However, in 1937, Percival and Somerville (43) showed that the deacetylation of a sulfur-free agar acetate gave a product that gelled in the usual fashion thus establishing that the ester sulfate was not necessary for gelation.

The mechanism of gelation and gel structure of the natural emulsoids, such as gelatin and agar, has received considerable attention for the past 100 years. It is impossible to review all the individual theories that have been proposed, but Bradford (10) reviewed many of them and in 1923 he made the following statement: "The present condition of uncertainty is illustrated by the multitude of mutually incompatible ad hoc theories of gel structure that are current". He said there was no direct evidence to support any hypothesis other

than those which assumed the presence of a solid phase. Further, he stated that gels were composed of molecular complexes or micellae with crystalline properties, in the interstices within and between which the water was held by molecular attraction. (This was the original suggestion of K. von Nageli, 1858).

Bradford (7) stated that in 1862 Nageli wrote, "Organized substances are composed of crystalline doubly refractive molecules aggregated from many atoms but which lie closely in definite arrangement with one another. In the moist condition, in consequence of the unbalanced attraction, each is surrounded with a skin of water". Bradford (9) did not believe the hypothesis of a network could be supported with the known facts. He considered that the natural emulsoid gels had a granular structure like that of a pile of shot. Finally he considered the gelation of gelatin and agar to be a process of crystallisation. (10)

Krishnamurti (31) stated that as an agar sol cooled, the hydration of the colloidal particles increased thus reducing the amount of "free water" in the intermicellary solution. Then, as the temperature continued to drop, a decrease in solubility of agar caused the intermicellary liquid to become supersaturated. As a result, fresh colloidal particles would form, and when these were sufficiently numerous, the further supersaturation would be relieved by the condensation of the dissolved agar on the particles already in the sol. This brought about an increase in the size of the particles, and tended to make them more spherical in shape. He also

stated, "The phenomenon of gel-formation is essentially due to the union of the hydrated particles, enmeshed in the intermicellary liquid. This confers on the system the rigidity which is characteristic of a gel".

Dean (16) stated, "The term 'plastic' is applied to any substance which can be changed from a solid to a liquid and back again to a solid by the application of force and heat". According to this definition, an agar gel is a plastic. It is of interest, therefore, to consider some of the points which he has discussed with respect to this type of substance and to high polymers in general. Dean stated that gels were rigid structures, therefore, the solid component had to extend continuously through the system.

He also said that a high polymer which was dispersed in solution would form a gel when its solubility was reduced, and the chains attracted each other more than they attracted the solvent molecules. In the case of agar, the bonds between the molecules were quite weak and hydrogen bonds or even molecular friction might be sufficient to produce a gel upon cooling.

It seems to be a generally accepted view, at present, that gels have a crystalline structure. Ward (56) stated the following:

"The sharp change in properties at the gelation temperature has been interpreted as showing that portions of the long chain molecules form crystallites (micelles). A single molecular chain may run through a crystallite, be free for a further part of its length and then take part in a second

crystallite. The existence of these imbound chains linking the crystallites explains the easy elastic deformation of jellies."

b. Physical Properties

Hysteresis

Hysteresis has been defined as, "the lagging of response behind the application of some change causative of the response" (26). That is to say, an agar gel melts at a much higher temperature than that at which it sets. The temperature at which agar sols gel and the temperature at which the gels melt varies with the source of agar. However, the hysteresis range will vary with other factors as well, such as time, presence of electrolytes and concentration, according to Wood (58). He also stated that stirring a sol or sowing with a preformed gel would reduce hysteresis.

Syneresis

When agar gels are protected against evaporation, the process of contraction results in the exudation of some of the liquid phase. This process was named syneresis, by Graham. Wood (58) has stated that the cause of this is not known but that it is considered to be due to a decrease in the surface area of the internal phase. Humm (26) reported that gels of Gracilaria confervoides agar typically exhibited greater syneresis than those of Gelidium agar.

Imbibition

Clarke (14) has shown that imbibition by agar gels was greatest when the water content was approximately 400 mg. per gram of dry agar. Humm (26) stated that totally dehydrated

agar swells relatively little.

Solubility

According to Humm (26), agar precipitated in alcohol, could be redissolved in cold water if the precipitate was not permitted to dry, and a gel would form immediately following dispersion of the agar. Also, agar was soluble in glycerin, ethylene glycol and other polyhydroxy alcohols but it would not gel in these substances. However, if a small amount of water was present gels would form.

H. Ecology of Gracilaria confervoides

1. Australia

Wood has stated (57) that there were three varieties of Gracilaria which had to be distinguished from the economic point of view. They are as follows:

(1) A short plant, little branched and seldom longer than 6 inches, growing on whelks, cockles, sticks, stones and other substrates.

(2) Plants grew up to 12 inches in brackish areas. These plants contained low agar content and were difficult to extract.

(3) Plants 18 inches to 12 feet long. Usually found in estuaries on sandy or sand and mud bottom. There was some evidence that these plants were sensitive to reduced salinity and that the seaweed disappeared in wet years and was at its best in dry years.

2. New Zealand

Moore (39) reported that Gracilaria confervoides in New Zealand rarely attained a length of more than one foot. It

was an estuarine plant growing on sandy mud-flats, attached to small pebbles, cockle-shells, etc. It was more abundant in shallow hollows left damp from about half-tide downwards, and also in the pools at and above half-tide on rocky coasts.

3. South Africa

Isaac et al (29) reported that Gracilaria confervoides was sub-littoral in habit, grew in calm water rooted in sand. It was washed ashore in considerable amounts after heavy seas so that harvesting was easy and cheap.

4. East Coast of United States

Causey et al (13) stated that Gracilaria confervoides in the vicinity of Beaufort, North Carolina, grew in shallow water which was protected to a degree against wave action. They stated that the sterile plants always grew unattached and the sexual plants grew attached to shells or other objects. They also reported that salinity did not seem to be a limiting factor on growth but low temperature and low light intensity might retard or inhibit growth. Maximum rate of growth took place between 25° and 28°C and temperatures as low as 10°C permitted growth.

5. British Columbia

Gracilaria plants have been reported in areas where the maximum summer temperatures of the water exceeds 16°C. The plants are found only near the mouths of streams. They grow on sandy or pebbly beaches between zero tide level and seven fathoms depth. The plants are also found in pools or in tidal or fresh water streams to nearly the high water level. Four kinds of plants, which differ in size, have been found. The

male plants grow attached to pebbles and reach a length of about 6 inches. The female plants also grow attached to pebbles etc., and reach a length of 2 feet. The asexual or tetrasporic plants are also attached to pebbles and vary in size up to 3 feet. The vegetative plants are attached to such objects as tube worms below the surface of the sand. These plants grow up to 12 feet in length and during favorable conditions may increase five feet in length in a period of twenty days.

I. Nature of the Investigation

During and following the last war, considerable interest was shown in the commercial possibilities of utilizing British Columbia red seaweeds, in particular Gracilaria confervoides, for the production of agar. Prior to this investigation some work had been carried out on the determination of some of the properties of the agar-like extract from Gracilaria. However, it was considered that these investigations were not extensive enough insofar as the study of optimum conditions for extraction of agar were concerned. Further, samples of seaweed had never been collected at regular intervals over a period of at least one year; thus the seasonal variations in the properties of the agar-like extract from Gracilaria confervoides growing in British Columbia coastal waters had not been adequately investigated.

It was a well known fact that methods of preparation of agar, even from the same species of seaweed, differed in various parts of the world. It was necessary, therefore, to determine optimum conditions for the preparation of agar from

the seaweed species used in the investigation. This constituted the first phase of the investigation. It was impossible from the outset to investigate adequately optimum conditions in the time available. Since the properties of agar vary with time of year and with locality, it follows that there may be a different set of optimum conditions for different times of the year and different localities. With this is mind, homogeneous seaweed samples were used to determine optimum conditions for preparation of agar and a procedure, based on optimum conditions was developed. Some variations of this procedure were then used in order to determine if the chosen procedure was, in reality, the most suitable for other seaweed samples.

Considerable emphasis has been placed upon the necessity for producing agar with low ash and low nitrogen content. From the point of view of suitability for bacteriological work, the reason for this is quite apparent. However, it was not known whether the ash and nitrogen content of the agar had any marked effect upon gel strength. To obtain information with respect to this, percent ash and percent total nitrogen values were determined for as many agar samples as time permitted.

Another phase of the investigation dealt with the seasonal variations in some of the properties of agar prepared from seaweed which had been harvested at approximately monthly intervals, over a period of one year, from three different localities. Also some variation in the method of treatment of the seaweed, after harvesting, was used. Such factors as (a) air drying and artificial drying, (b) washing and not washing the seaweed, and (c) washing in fresh water and washing in salt water only, were

investigated.

In addition, other information was obtained with respect to the monthly collections. Such information included data relative to the: temperature, pH, salinity and chlorinity of the seawater at the time of harvesting, percent moisture and percent total nitrogen in the seaweed. Finally, some work was carried out on the water soluble fractions of Gracilaria confervoides.

II. MATERIALS AND APPARATUS

A. Identification of the seaweed

On the basis of the description given in Harvey's Nereis Boreali-Americana, the red alga used in this investigation has been identified as Gracilaria confervoides Grev. Figs. 1, 2 and 3 are photographs of portions of plants collected at Cherry Point, Mill Bay and Ganges, respectively.

B. Sources of Gracilaria confervoides used in the investigation.

The seaweed was collected from Cherry Point and Mill Bay, located on the East Coast of Vancouver Island, and from Ganges Harbour, located on Salt Spring Island. Fig. 4 shows the particular location of the Gracilaria beds from which the seaweed harvests were made.

C. Seaweed collections used in the investigation

Stock A Gracilaria confervoides. When the work on optimum conditions for preparation of agar was begun, there was not sufficient seaweed available from one harvest. It was therefore necessary to mix different collections of Gracilaria, in spite of the fact that such a procedure was not ideal. However, steps

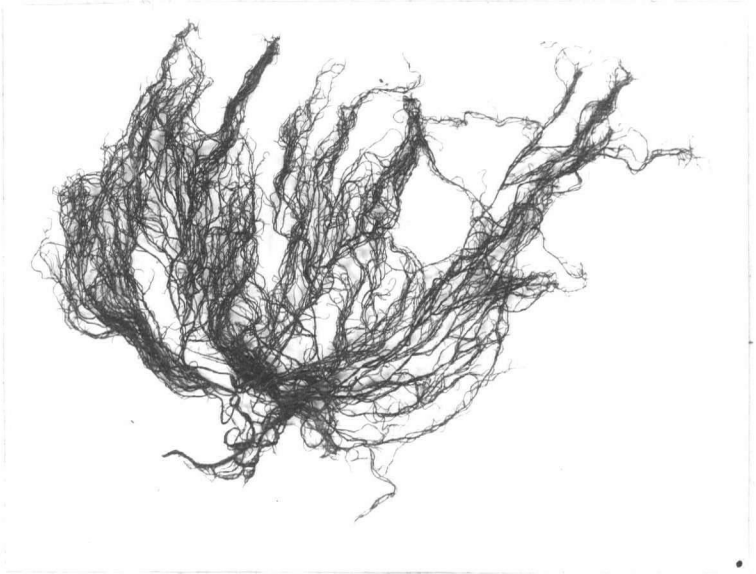


Fig. 1

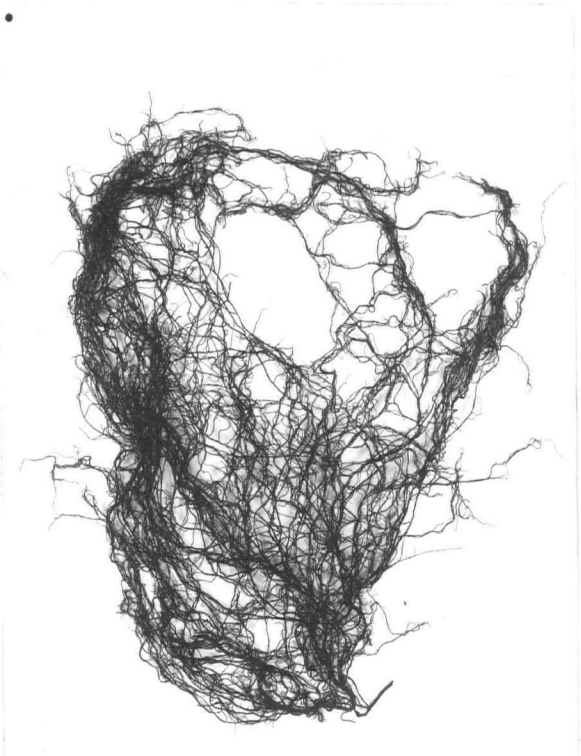


Fig. 2

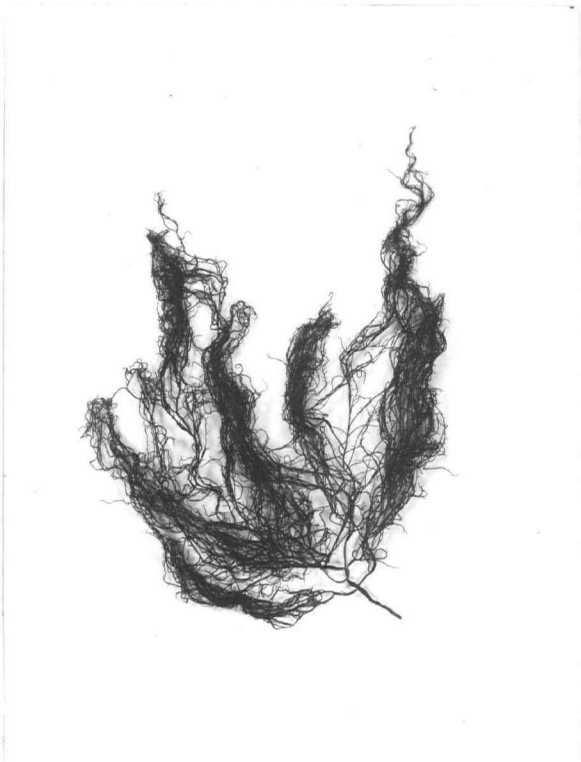
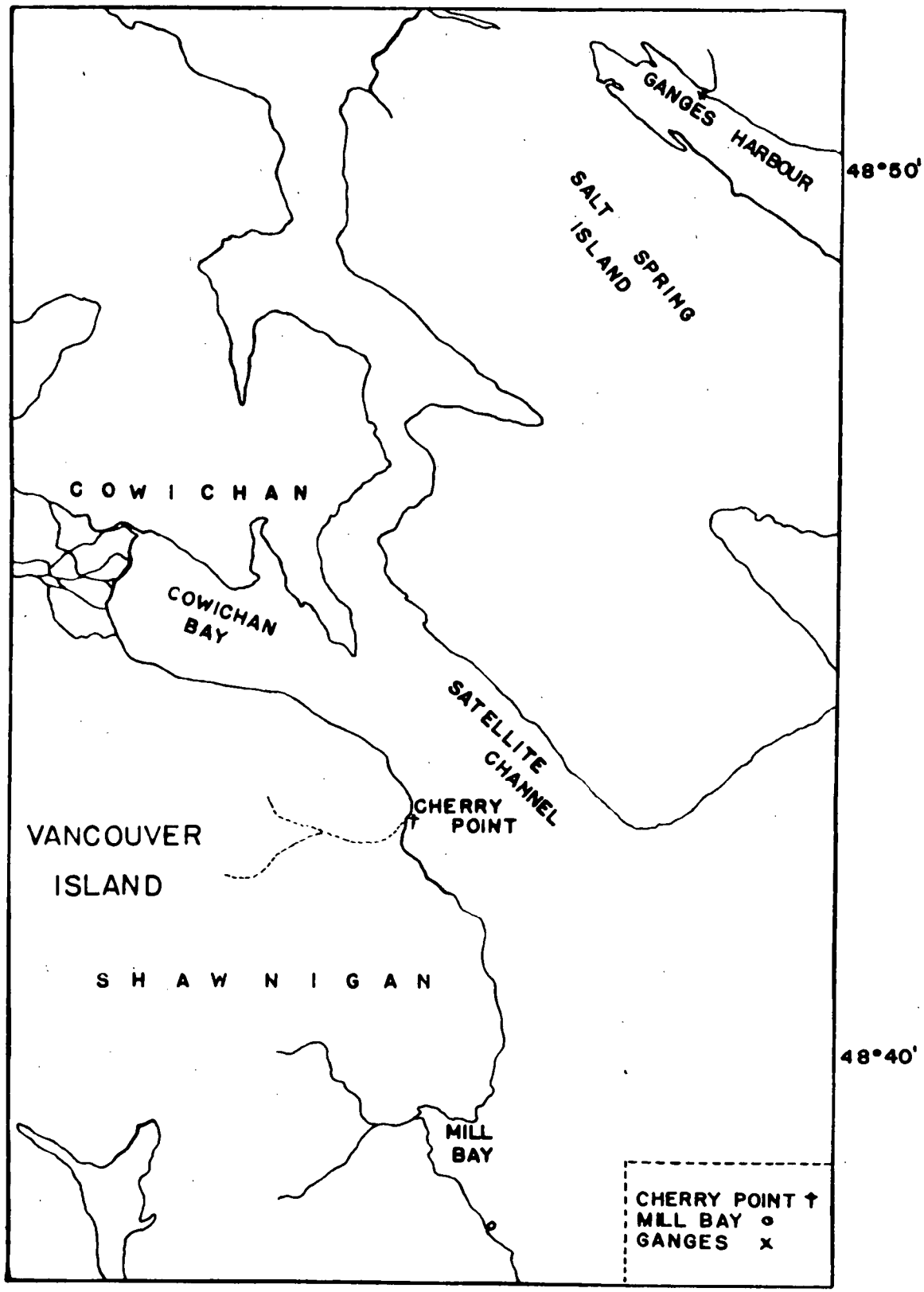


Fig. 3

Fig. 1,2 and 3. Portions of Gracilaria confervoides plants collected from Cherry Point, Mill Bay and Ganges, respectively.

FIG. 4. MAP SHOWING GRACILARIA CONFERVOIDES HARVESTING SITES.



were taken to assure homogeneous sampling. The seaweed used was a mixture of February and July, 1947 Gracilaria collected at Ganges. The dried, stored seaweed was rewashed to remove as much foreign material as possible, and then oven dried at 70-75°C. After it was dried, it was ground twice in a corn mill, thoroughly mixed and stored in a metal container. This ground Gracilaria will be referred to as Stock A Gracilaria.

Stock B Gracilaria confervoides. Seaweed collected December 29, 1948 at Cherry Point was used in some of the experiments on optimum conditions for the preparation of agar. This seaweed was a portion of one of the monthly collections and was treated as described later under "Harvesting and treatment of seaweed" (p. 29). This Gracilaria will be referred to as Stock B Gracilaria.

All other work was carried out on the Gracilaria collected at approximate monthly intervals from June, 1948 to May 1949. In the text, these samples will be identified by stating the place and date of collections.

D. Apparatus

The apparatus used in the investigation and its description is listed below.

(1) Bloom gelometer. Precision Scientific, improved model, Serial No. B-9.

(2) Klett Summerson Photoelectric Colorimeter, Serial No. 3753.

(3) Transformer. Hammond 12 volt, type 267x60

(4) Refrigerator. General Motors frigidaire, model L-6.

(5) Oven. National Appliance. Serial No. 5575.

(6) MacBeth pH meter, model A.

(7) Wire screening. 20 mesh brass screen.

(8) Corn mill. Climax "52"

(9) Glass plates. Plate glass, 10½ x 15 inches.

(10) Liquor glass. Inside top and bottom diameters, 4.6 cm. and 2.4 cm. respectively, and inside depth, 4.2 cm.

III. PROCEDURES AND ANALYTICAL METHODS

A. Procedures relative to seaweed collections

1. Harvesting and treatment of seaweed

Gracilaria confervoides collections were made, at or near zero tide level, at approximate monthly intervals. In each collection the seaweed was harvested on the same part of the Gracilaria beds. Collections were made at Cherry Point and Mill Bay by Mr. O.W. Maxwell, and at Ganges by Mr. C.W. Baker.

As the seaweed was collected, it was rinsed in salt water. It was then spread on wire netting to allow removal of excess surface moisture. The drained seaweed was packed in paper sacks within a burlap sack or wooden box, and shipped to the University by express. Upon arrival at the University, the seaweed was washed in cold running tap water. The length of time in the water was dependent on the amount of contaminants present with the Gracilaria. The average time in the water was approximately 15 minutes. During the washing, contaminating seaweeds, pebbles, shells and animal life were removed by hand. After this preliminary washing, the seaweed was placed in a small wire sieve and the full force of cold tap water was run over it for about 5 minutes. This removed a great deal of

the adhering sand. If sufficient seaweed had been collected, it was divided into two portions. One portion was air dried at room temperature (18-25°C) and the other portion was oven dried at 50°C. If sufficient seaweed was not obtained oven drying was omitted.

On occasions, sufficient seaweed was obtained to permit other methods of treatment. Variations in treatment included drying, without washing and with washing to remove pebbles, shells, etc. but not seaweed contaminants. Where either variation was used the seaweed was air dried at room temperature.

2. Temperature, pH, chlorinity and salinity of seawater

At the time of collection, the water temperature was recorded and a seawater sample was bottled and shipped, with the seaweed, to the University. Upon arrival at the University, the pH was taken and specific gravity was obtained with hydrometers (accurate to 3 decimal places). Chlorinity and salinity values were obtained with the specific gravity data, from a momograph prepared in 1942 by J.P Tully, Pacific Biological Station, Nanaimo, B.C.

3. Percent moisture determinations

After the seaweed was washed, two 50 gram samples (approx.) were blotted with absorbing paper to remove excess surface moisture. The weighed samples were then dried to constant weight at 50°C.

4. Preparation of agar (see IV E and IV G)

B. Procedures and analytical methods used to determine the properties of agar and agar gels

1. Gel strength

No standard method has been adapted to measure the strength

of agar gels. Thus, many types of instruments have been used which gave values in arbitrary units only. Lampitt and Morris (32) investigated methods used for the determination of the strength of gelatin gels. They found that there were four main types of instruments used and that the type of instrument which measured the load required to produce a definite depression in the gel, was very satisfactory. Campbell (12) reported that the British Standards Institution prescribed in the case of gelatin gels, either (a) the use of relative determinations by any suitable instrument, using an agreed reference sample as standard, or (b) the use of the Bloom gelometer in cases where there is no reference standard. No definite policy has been agreed upon concerning agar but some investigators have used Difco Bacto agar as a reference standard. In this investigation, Difco Bacto agar, control no. 395714, was used as a reference standard.

The Bloom gelometer was used to determine gel strengths. It measures the weight of lead shot required to produce a standard depression of a plunger, one cm^2 in area. The instrument was operated under the following standard conditions:

1. a constant depression of 4 mm.

2. a rate of flow of lead shot of 200 grams per 5 seconds.

Gel strength is defined, therefore, in terms of the number of grams per cm^2 required to produce a depression of 4 mm at a rate of increase in weight of 200 grams per 5 seconds.

All agar gels were prepared for testing in the following manner. Sufficient cold distilled water was added to a weighed

amount of agar, in a 250 ml erlenmeyer, to give the required weight percent gel. The flask was corked with a cotton plug and left standing overnight (14-15 hours), then autoclaved for 30 minutes at 121°C. Enough agar sol was prepared each time to make five 25 ml samples. While the erlenmeyers were agitated quite vigorously, 25 ml samples were pipetted from the flask and were run into the liquor glasses. The liquor glasses were covered with stender lids, the sols were cooled at room temperature, and gel strength determinations were made 5 hours after pipetting into the liquor glasses.

A simple statistical method was used to determine the significance of the gel strength results. Because the error was not homogeneous, more refined technique for the comparison of differences between means was not warranted. However, it was desirable to have some measure of experimental error, even if only approximate. Therefore standard errors were calculated for several samples at various levels of gel strength. A rough test of significance may be applied within a given level and a difference, to be significant must be at least twice the recorded average standard error. The average standard error and maximum standard error within each level are given in Table 4.

TABLE 4. The approximate standard errors at various levels of gel strength

Levels (in grams)	Average Standard Error	Maximum Standard Error
1000 - 500	±5.0	±8.7
500 - 400	±2.9	±4.1
400 - 300	±2.2	±3.3
300 - 200	±1.4	±2.8
200 - 100	±1.3	±1.8
100 -	±0.4	±0.6

2. Temperatures of gelation and melting

A number of methods have been used for the determination of the temperatures of gelation and of melting. To determine the temperature of gelation, Wood (58) used a method which allowed a bubble of air to escape at regular intervals from a capillary tube into an agar sol. The temperature at which the bubbles of air stopped rising to the surface was recorded as the temperature of gelation. The same principle was used to determine the melting temperature. Marshall et al (36) trapped air in the top of a small corked glass tube containing an agar sol. The temperature was gradually lowered and at each degree or half degree the tube was inverted and at the gelation point the air bubble stopped moving. The melting temperature could be obtained by inverting the tube containing an agar gel with an air bubble at the bottom. The temperature was raised and at the melting point the bubble rose rapidly to the top of the tube. This method was not considered very satisfactory because of the difficulty in getting air bubbles the same size. Humm and Williams (27) used a Stormer viscosimeter and recorded the temperature of gelation as the temperature at which the viscosimeter suddenly began to rotate very slowly or not at all. To determine the melting temperature, a test tube containing an agar gel was heated in a water-bath. A few bird shot were placed on the surface of the gel and the temperature at which the shot sank to the bottom was taken as the temperature of melting. The Japanese used a similar method, reported by Wood (59), but mercury was added to the surface of the gel rather than bird shot.

Ostwald (46) stated that all colloidal changes of state require a certain time. For example, the temperature at which a gelling solution gels or melts depends essentially upon the rate of temperature change. The more slowly a gel is cooled, the higher is the temperature at which it solidifies: the more slowly a gel is warmed, the lower is the temperature at which it melts. It follows, therefore, that the terms "gelation temperature" and "melting temperature" have no exact meaning unless standard conditions and apparatus are used. Thus it is impossible to compare results of different investigators.

With this in mind, it was considered that there would be no advantage in using one or the other of the methods described above. Rather, a method was developed based upon the fact that Difco Bacto agar sol was transparent and the same agar, when gelled, was opalescent. Casual observation indicated that the change from sol to gel was accompanied by a marked increase in opalescence. It was decided to use the photo electric colorimeter in an attempt to characterize the nature of the change which took place. A procedure was worked out and Table 5 shows the nature of the results obtained for the determination of gelation temperature.

TABLE 5. Data obtained for determination of temperature of gelation: 2.0% Difco Bacto agar

Time (in minutes)	Temperature (°C)	Colorimeter Reading	Increment
0	50.0	0	
1	48.1	0	
2	46.6	0	
3	45.2	2.0	

Table 5 (continued)

Time (in minutes)	Temperature (°C)	Colorimeter Reading	Increment
4	43.8	5.6	
5	42.8	6.8	
6	41.8	6.8	
7	40.7	6.8	
8	39.8	6.8	
			1.2
9	39.1	8.0	
			2.0
10	38.4	10.0	
			3.2
11	37.9	13.2	
			4.0
12	37.4	17.2	
			6.8
13	37.0	24.0	
			11.5
14	36.6	35.5	
			12.5
15	36.2	47.0	
			14.3
16	35.7	61.3	
			17.5
17	35.3	78.8	
			20.2
18	35.0	99.0	
			23.0
19	34.6	122.0	
			24.0
20	34.2	146.0	
			27.0
21	33.9	173.0	
			32.0
22	33.6	205.0	
			29.0
23	33.2	234.0	
			26.0
24	32.9	260.0	
			26.0
25	32.7	286.0	
			22.0
26	32.5	308.0	
			20.0
27	32.2	328.0	
			19.0
28	32.0	347.0	
			17.0

Table 5 (continued)

Time (in minutes)	Temperature (°C)	Colorimeter Reading	Increment
29	31.8	364.0	
			17.0
30	31.6	381.0	
			16.0
31	31.4	397.0	
			15.0
32	31.2	412.0	
			11.0
33	31.0	423.0	

In Table 5, the figures to the right of the colorimeter readings are the increments in colorimeter readings obtained at minute intervals. The maximum increment obtained was 32.0, and this indicated the greatest increase in opalescence during any minute interval. The temperature at which this greatest increment occurred was called the temperature of gelation, and in this particular instance the temperature was between 33.9 and 33.6°C. The nature of the results was such that the exact temperature of gelation could not be ascertained, so the higher of the two values was arbitrarily chosen a the temperature of gelation. It was found that the results were very readily reproducible, to within 0.5°C, even though there was a slight variation in the rate of cooling. The value, 33.9°C, was slightly lower than a value reported by Marshall et al (36) of approximately 36°C, for 2.0% Difco Bacto agar. Humm and Williams (27) also reported 36°C for 2.0% Difco Bacto agar.

The procedure adopted to obtain the results shown in Table 5 was as follows. Agar sols (2.0%) were prepared in the same manner as for gel strength determinations. When the hot agar sol was cooled to 70°C it was poured into a rectangular

pyrex colorimeter absorption cell. The cell was then placed in the colorimeter with the greater depth of the cell parallel to the path of light, and a thermometer was put in the agar. The instrument was zeroed when the temperature dropped to 50°C. At intervals of one minute, the temperature and the colorimeter readings were recorded until it was certain that the temperature had dropped below the temperature at which the greatest increment had occurred. A blue filter was used for Difco Bacto agar and a green filter was used for Gracilaria agar.

A modification of the same apparatus and procedure was used to determine the melting temperature. A heating coil, wound on sheet mica, was constructed so it would lie flat against the wide inside walls of the rectangular pyrex absorption cell. Since the heating coil was constructed to operate on 12 volts, a transformer was used in the 120 volt circuit. Table 6 shows a typical series of results obtained for the temperature of melting.

TABLE 6. Data obtained for determination of temperature of melting: 2.0% Difco Bacto agar

Time (in minutes)	Temperature (°C)	Colorimeter Readings
0	31.0	423
3	48.0	407
6	60.6	345
7	64.3	312
8	67.8	272
9	70.8	230
10	73.8	188
11	76.8	149

Table 6 (continued)

Time (in minutes)	Temperature (°C)	Colorimeter Readings
12	79.3	122
13	82.2	98
14	85.0	81
15	87.6	69
16	89.5	59
17	90.3	53.4
17.5	90.6	51.7
18	90.8	49.8
18.5	91.0	48
19.0	91.2	46.5
19.5	91.3	45
20.0	91.4	44
20.5	91.6	44
21.0	91.7	44
21.5	91.8	44
22.0	91.9	44

The results were obtained in the following manner. After the temperature of gelation had been obtained and the temperature of the gel had dropped to about 30°C, the heating coil was turned on. The temperatures and colorimeter readings were recorded at regular time intervals until the colorimeter readings remained constant. The temperature at which the colorimeter readings remained constant for the test shown in Table 6 was 91.4°C. The colorimeter readings indicated a decrease in opalescence and a corresponding increase in light transmission.

The temperature at which maximum light transmission took place and remained constant (i.e. constant colorimeter reading) was taken as the temperature of melting. Again, the results were reproducible even though the rate of heating varied slightly with each test. The value, 91.4°C was slightly higher than a value reported by Marshall et al (36) of 90°C, for 2.0% Difco Bacto agar and considerably higher than a value of 76°C reported by Humm and Williams (27).

Since the foregoing procedure was worked out, it was found that a similar principle was used by Cook and Axtmayer (15) in 1937 to determine the gelatinization temperature of starches. In 1947, Beckord and Sandstedt (5) adapted a spectrophotometer to make a similar study on starch.

3. Percent ash

Agar samples were oven dried to constant weight at 50°C and duplicate percent ash determinations were made using the procedure of Loomis and Shull (35).

4. Percent Total Nitrogen

Duplicate samples were oven dried to constant weight at 50°C and determinations were made by the standard Kjeldahl procedure.

5. Slope holding capacity

The method used by Marshall et al (36) was followed in order to determine the slope holding capacity of agar gels. About 10 ml agar sol was poured into a test tube and allowed to set, with the test tube inclined at an angle such that the surface of the agar just reached the beginning of the curve near the bottom of the tube. It was left in this position

overnight and then after standing it upright for half an hour it was transferred to a constant temperature chamber at 40°C for 24 hours. All tests were made at 1.5% concentration.

6. Syneresis

No analytical method was used to determine the amount of syneresis exhibited by agar gels. Where results are recorded regarding syneresis they are based upon observation only.

7. Viscosity

Viscosity determinations were not made on any agar samples. Results given concerning viscosity were based upon observations made while pipetting hot agar sols in preparation for gel strength, or when the seaweed extract was being filtered. Remarks concerning viscosity therefore are based upon only very obvious differences.

8. pH

The pH of agar was determined on the agar gels after the gel strength determinations had been made. Unless otherwise stated, all pH determinations were made with the MacBeth pH meter.

IV. RESULTS

A. Harvesting and receiving dates of seaweed

Table 7 shows the dates on which Gracilaria was harvested at Cherry Point, Mill Bay and Ganges and the dates on which the harvested seaweed arrived at the laboratory. There was a variation in time, from harvesting to receiving, of one to seven days.

TABLE 7. Harvesting and Receiving Dates of Seaweed

Cherry Point		Mill Bay		Ganges	
Harvested	Received	Harvested	Received	Harvested	Received
22.6.48	23.6.48	22.6.48	23.6.48	21.6.48	23.6.48
19.7.48	21.7.48	19.7.48	21.7.48	21.7.48	27.7.48
7.8.48	9.8.48	7.8.48	9.8.48	6.8.48	9.8.48
2.9.48	9.9.48	2.9.48	9.9.48	1.9.48	3.9.48
27.9.48	30.9.48	27.9.48	30.9.48	30.9.48	1.10.48
2.11.48	5.11.48	2.11.48	5.11.48		
1.12.48	3.12.48	1.12.48	3.12.48	4.12.48	6.12.48
29.12.48	4.1.49			30.12.48	4.1.49
15.2.49	22.2.49				
11.3.49	15.3.49				
16.5.49	17.5.49				

Although some of the seaweed collections spent several days on the way to the laboratory, they arrived in apparent good condition. Severe winter conditions made it impossible to continue collection, after December at Mill Bay and Ganges.

B. Data obtained concerning environmental factors

The temperature, pH, chlorinity and salinity are recorded for each sample of sea water obtained from Cherry Point, Mill Bay and Ganges in Tables 8, 9, and 10, respectively. Results were not obtained for June 1948 collection.

TABLE 8. Cherry Point: temperature, pH, chlorinity and salinity of seawater

Date Collected	Temperature (°C)	pH	Chlorinity	Salinity
19.7.48	16	8.21	17.10	30.90
7.8.48	16	7.57	16.10	29.00
2.9.48	13	8.15	17.49	31.60
27.9.48	13	8.28	15.56	28.12
2.11.48	6	7.93	16.08	29.05
1.12.48	6.5	7.25	9.24	16.70
29.12.48	4	7.55	13.45	24.32
15. 2.49	0	7.40	16.56	29.90
11. 3.49	8.5	7.47	12.53	22.63
16. 5.49	18	7.60	15.17	27.40

TABLE 9. Mill Bay: temperature, pH, chlorinity and salinity of seawater

Date Collected	Temperature (°C)	pH	Chlorinity	Salinity
19.7.48	17.5	8.07	14.40	25.90
7.8.48	17	7.73	15.70	28.30
2.9.48	13	7.06	17.40	31.42
27.9.48	14	8.19	15.05	27.20
2.11.48	8	7.79	15.81	28.57
1.12.48	7.5	7.60	15.65	28.28

TABLE 10. Ganges: temperature, pH, chlorinity and salinity of seawater

Date Collected	Temperature (°C)	pH	Chlorinity	Salinity
21.7.48	17	7.10	15.60	27.70
6.8.48	18	7.86	15.70	28.30
1.9.48	14	8.06	15.82	28.58
30.9.48	12	7.68	15.26	27.57
4.12.48	5	7.58	11.28	20.40
30.12.48	4	7.45	12.22	22.10

The data obtained showed that the temperature of the water was maximum during the summer months with the temperature decrease beginning in September. Observations made by Mr. Maxwell at Cherry Point and Mill Bay indicated that even after the temperatures had dropped during September and October, the rate of growth of the seaweed was just as great, or greater, than during July and August.

At Cherry Point conditions of maximum pH, chlorinity and salinity occurred during September and the minimum conditions occurred in early December. Conditions at Mill Bay were somewhat similar. Maximum pH occurred at the end of September and maximum chlorinity and salinity occurred at the beginning of September. At Ganges maximum pH, chlorinity and salinity occurred at the beginning of September and minimum pH in July. Mimimum chlorinity and salinity occurred in December.

It is considered inadvisable to draw specific conclusions about the environmental factors because the data represented very isolated recordings. The results did show that water temperature at the three localities was above 16°C, a condition considered necessary for growth of Gracilaria in B.C. coastal waters. Furthermore, chlorinity and salinity values were maximum in September, the time of year when the run-off from streams in the vicinity of the Gracilaria beds was at or near a minimum. Harvey (21) stated that there was a tendency for diluted water (where rivers and streams enter the sea) to remain banked up near the coast during the winter and then run out as a surface layer during the summer. One would expect, then, to find a higher salt concentration in summer than in winter. Since pH changes could take place in the sample bottles, while on the way to the laboratory, the values obtained are of doubtful significance. The results did, however, indicate that the pH was generally higher in summer than in winter.

C. Percent Moisture in Gracilaria

The results obtained for percent moisture are shown in Tables 11, 12 and 13 for Cherry Point, Mill Bay and Ganges Gracilaria, respectively. Results were not obtained for June collections.

TABLE 11. Percent moisture in Cherry Point Gracilaria

Date Harvested	% Moisture
19.7.48	88.9
7 .8.48	89.4
2.9.48	87.9
27.9.48	87.9
2.11.48	90.6
1.12.48	86.6
29.12.48	86.5
15.2.49	85.6
11.3.49	84.0
16.5.49	86.6

TABLE 12. Percent Moisture in Mill Bay Gracilaria

Date Harvested	% Moisture
19.7.48	87.5
7.8.48	87.6
2.9.48	88.4
27.9.48	88.4
2.11.48	88.3
1.12.48	87.6

TABLE 13. Percent Moisture in Ganges Gracilaria

Date Harvested	% Moisture
21.7.48	88.7
6.8.48	98.2
1.9.48	87.1
30.9.48	87.1
4.12.48	86.1
30.12.48	85.0

The results showed that Cherry Point Gracilaria had a maximum moisture content from July to early November and it then dropped throughout the remainder of the winter and spring. Mill Bay Gracilaria had a fairly constant moisture content and no seasonal trend was apparent. The moisture content in Ganges Gracilaria was highest during July and August and dropped off from early September to the end of December.

D. Seasonal variation of total nitrogen in Gracilaria confervoides

Butler (11) reported an investigation on the seasonal variation of total nitrogen in Chondrus crispus. It was found that the percent nitrogen reached a maximum of about 4% in December, remained practically constant until March, decreased to a low of approximately 1.5% in August and increased again to about 3% in November.

Total nitrogen determinations were made on the air dried Gracilaria monthly collections. The results, based on oven dried weight at 50°C are given in Table 14 and are graphed in Fig. 5.

TABLE 14. Seasonal variation of total nitrogen in Gracilaria confervoides

Cherry Point		Mill Bay		Ganges	
Date Harvested	% Total Nitrogen	Date Harvested	% Total Nitrogen	Date Harvested	% Total Nitrogen
22.6.48	3.26	22.6.48	3.41	21.6.48	3.02
19.7.48	2.49	19.7.48	2.41	21.7.48	2.79
7.8.48	2.46	7.8.48	3.84	6.8.48	2.96
2.9.48	4.38	2.9.48	3.18	1.9.48	3.80
27.9.48	4.86	27.9.48	4.66	30.9.48	3.60
2.11.48	4.63	2.11.48	4.77	4.12.48	3.31
1.12.48	3.61	1.12.48	4.83	30.12.48	4.83
29.12.48	4.87				
15.2.49	3.49				
11.3.49	4.08				
16.5.49	4.10				

The results showed that the percent total nitrogen in Cherry Point Gracilaria was minimum during July and the early part of August and rose to a maximum at the end of September. This was followed by a decrease until the beginning of December then a rise again to the maximum at the end of December. Mr. Maxwell reported that the seaweed bed at Cherry Point was almost completely covered by sand when he made the 1.12.48

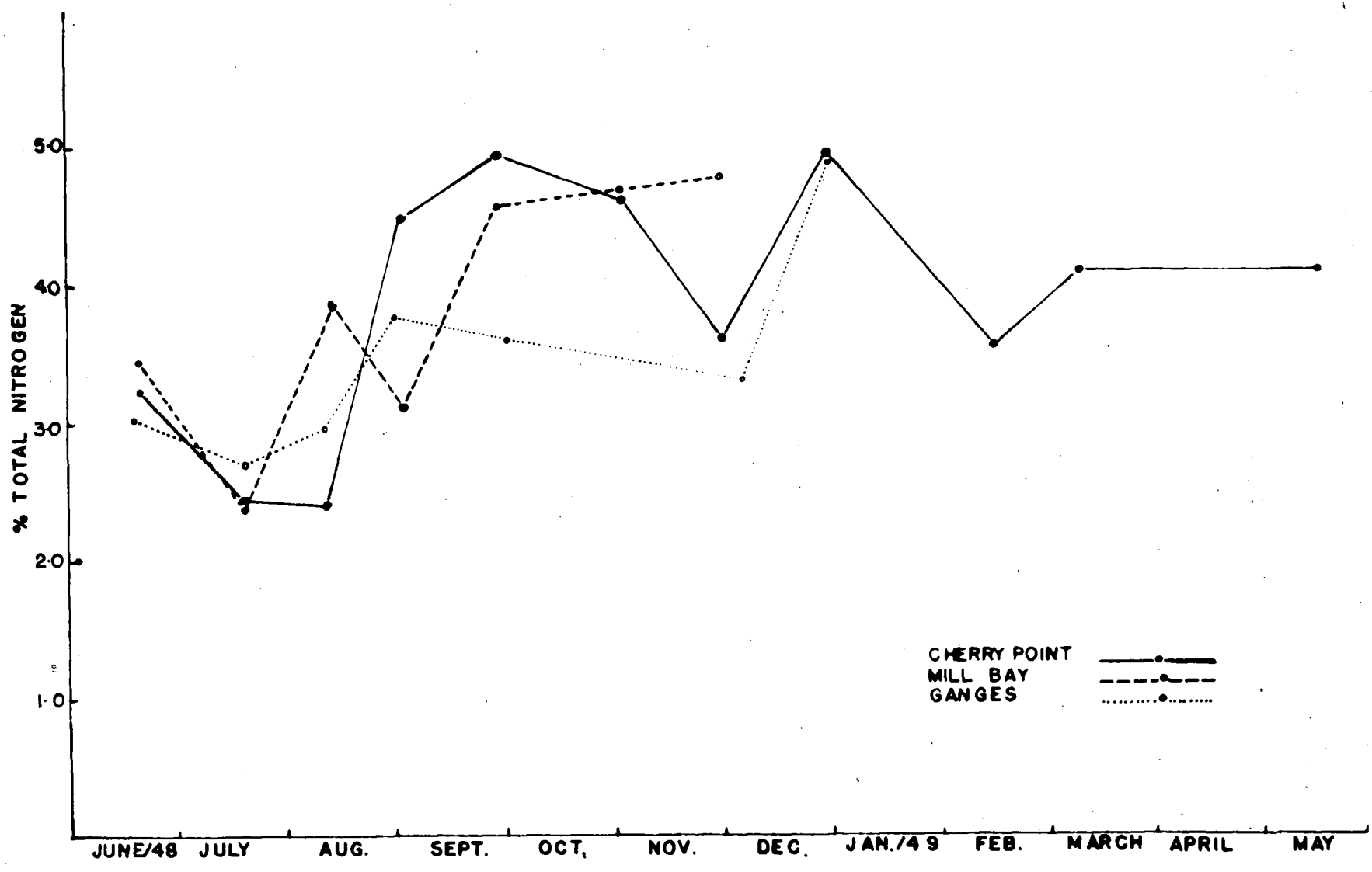


FIG. 5. SEASONAL VARIATION IN % TOTAL NITROGEN IN GRACILARIA CONFERVOIDES.

collection. There had been a severe storm during the month of November. The Gracilaria bed was uncovered again when he made the 29.12.48 collection. The foregoing may account for the sudden nitrogen decrease in the 1.12.48 collection. The nitrogen decreased again at the middle of February, rose slightly in March and remained nearly constant until June at which time it decreased again.

At Mill Bay, minimum total nitrogen was also obtained during July and maximum total nitrogen was found at the beginning of December. Minimum total nitrogen was found during July at Ganges and then it increased slightly during August and remained practically constant until the beginning of December. This was followed by a sharp increase to a maximum at the end of December.

E. Optimum conditions for the preparation of agar

Unless otherwise stated, distilled water was used in all extractions, extractions were carried out in a 2½ litre enamel pot, the seaweed solution was always heated to boiling before placing in the autoclave and all agar extracts were frozen at -5 to -7°C.

1. Temperature and time of heating in extraction

Two litres of water were added to 50 grams of Stock A Gracilaria. Extractions were carried out at 90, 100, 110 and 121°C at heating times of 2, 4, 6, 8, 10 and 12 hours. Extractions were also made at 121°C at heating times of ½, 1 and 1½ hours.

At temperatures of 90° and 100°C the seaweed was heated

on an electric hot plate with frequent stirring. In spite of stirring, at 100°C it was found impossible to prevent the seaweed from scorching on the bottom of the container. The amount of water was kept constant by dripping water into the container at a rate equal to the rate of evaporation. Extractions at 110° and 121°C were carried out in an autoclave. After the seaweed was heated for the required length of time at the different temperatures, the residue was separated from the liquor by filtering successively through (a) fine mesh cheese cloth (b) silk and (c) glass wool. The liquor was frozen for 18 hours, placed on wire screening and thawed at room temperature.

The thawed gel was then heated to dissolve the agar, cooled to 50°C and stirred for 5 minutes with a stirrer driven by a vacuum cleaner motor. The resulting porous gel was placed on glass plates and oven dried at 50°C. This part of the procedure was found necessary because oven drying space was limited. The thawed gel was very mushy and spread out thinly when poured on the glass plates and 4 to 5 glass plates were necessary to dry the agar from each extraction. The porous gel which resulted after "whipping" with the stirrer was firm enough to pile up on the plates so that only 2 were required. When the agar was dried, it was scraped off the glass plates, weighed, ground in the corn mill and stored in glass bottles.

Results. Tables 15, 16, 17 and 18 show the percent yield, gel strength (1½% gel), % total nitrogen and % ash respectively for agar prepared after heating at 90, 100, 110 and 121°C for the lengths of time given in the tables. The results are graphed in Fig. 6, 7, 8 and 9. The % total nitrogen in Stock A Gracilaria was 3.62% and the % ash was 6.39%.

TABLE 15. Temperature and time of heating in extraction: percent agar yield

Heating Time (Hours)	Temperature 90°C	100°C	110°C	121°C
½				23
1				34
1½				37
2	17	25	28	40
4	19	31	38	38
6	24	33	41	34
8	25	41	38	35
10	26	42	37	34
12	28	35	35	29

TABLE 16. Temperature and time of heating in extraction: gel strength (grams, 1½ gel)

Heating Time (Hours)	Temperature 90°C	100°C	110°C	121°C
½				59
1				58
1½				50
2	64	41	49	45
4	61	32	36	28
6	63	27	22	24
8	55	17	20	-
10	49	-	-	-
12	39	-	-	-

TABLE 17. Temperature and time of heating in extraction: percent total nitrogen

Heating Time (Hours)	Temperature 90°C	100°C	110°C	121°C
½				0.88
1				0.89
1½				0.98
2	0.91	0.78	0.95	0.96
4	0.88	0.81	0.97	0.98
6	0.75	0.88	0.97	1.03
8	0.86	0.86	1.06	.97
10	0.80	1.30	1.13	1.00
12	0.94	1.04	1.02	1.25

TABLE 18. Temperature and time of heating in extraction: percent ash

Heating time (Hours)	Temperature 90°C	100°C	110°C	121°C
½				5.79
1				5.50
1½				5.47
2	5.96	5.38	5.94	5.37
4	5.74	5.30	5.39	5.08
6	5.56	5.09	5.18	5.73
8	5.73	4.82	5.59	4.66
10	5.43	4.85	4.99	4.27
12	5.88	5.26	4.94	4.06

Agar yield. The maximum yield obtained was 40-42%. This was obtained at temperatures of 100°, 110° and 121°C and heating times of 8 to 10, 6 and 1½ hours, respectively. At 90°C the maximum yield was not obtained even up to 12 hours heating time. During the heating, there was an initial period of high viscosity of the liquor, followed by a rather sharp drop in viscosity. The maximum yields were obtained after the drop in viscosity had occurred. There was no marked decrease in viscosity at 90°C even up to 12 hours heating.

The decrease in yield, after the maximum was reached, was probably due to hydrolysis of the agar. The results showed that the duration of heating required to obtain maximum yield was dependent upon the temperature of extraction. The results also indicated that, as far as yield was concerned, it was desirable to heat at any particular temperature until the marked decrease in viscosity occurred.

Gel strength. At 90°C, the gel strength was maximum over a heating period of 2 to 6 hours and then it decreased gradually up to 12 hours heating time. The results obtained at

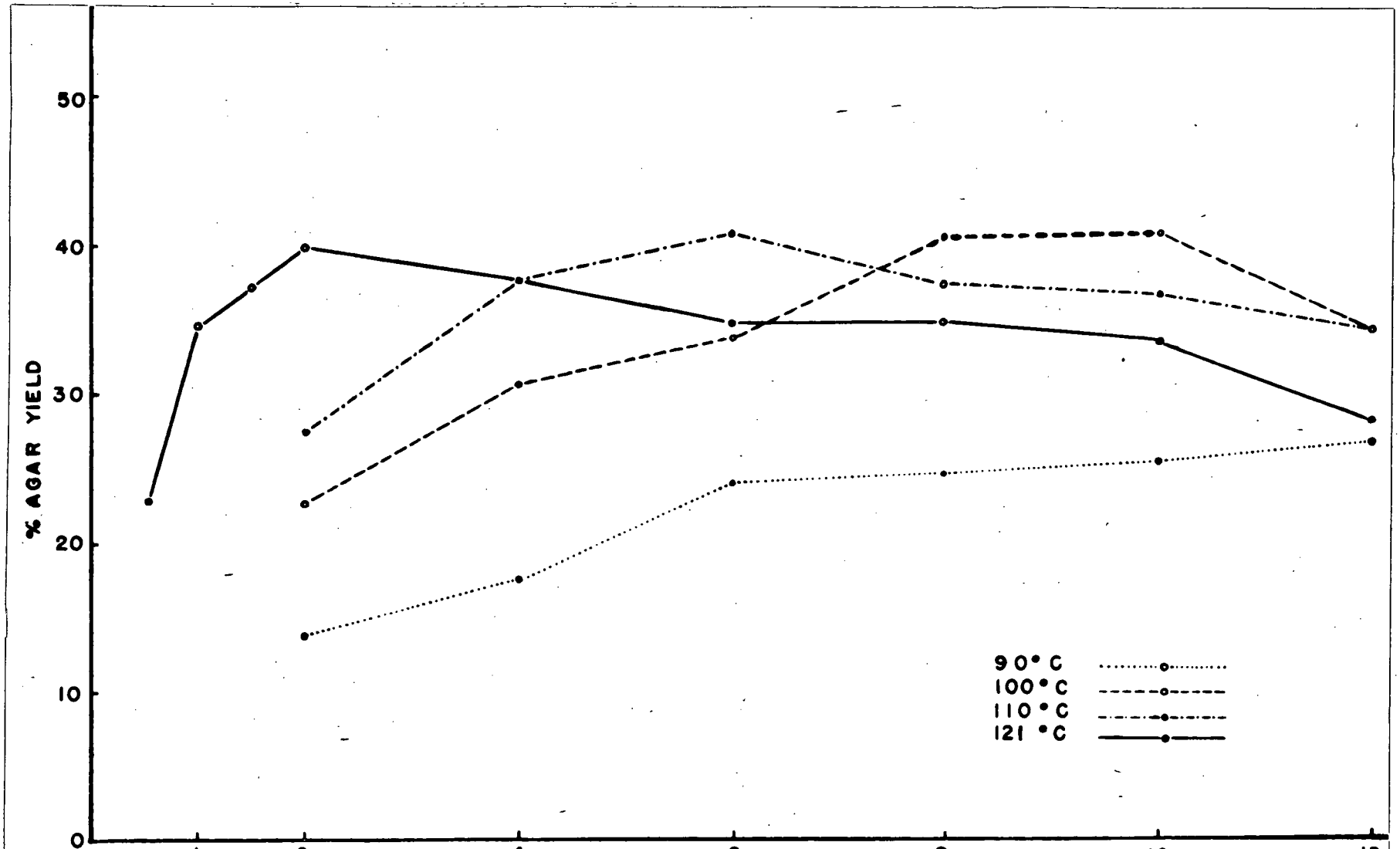
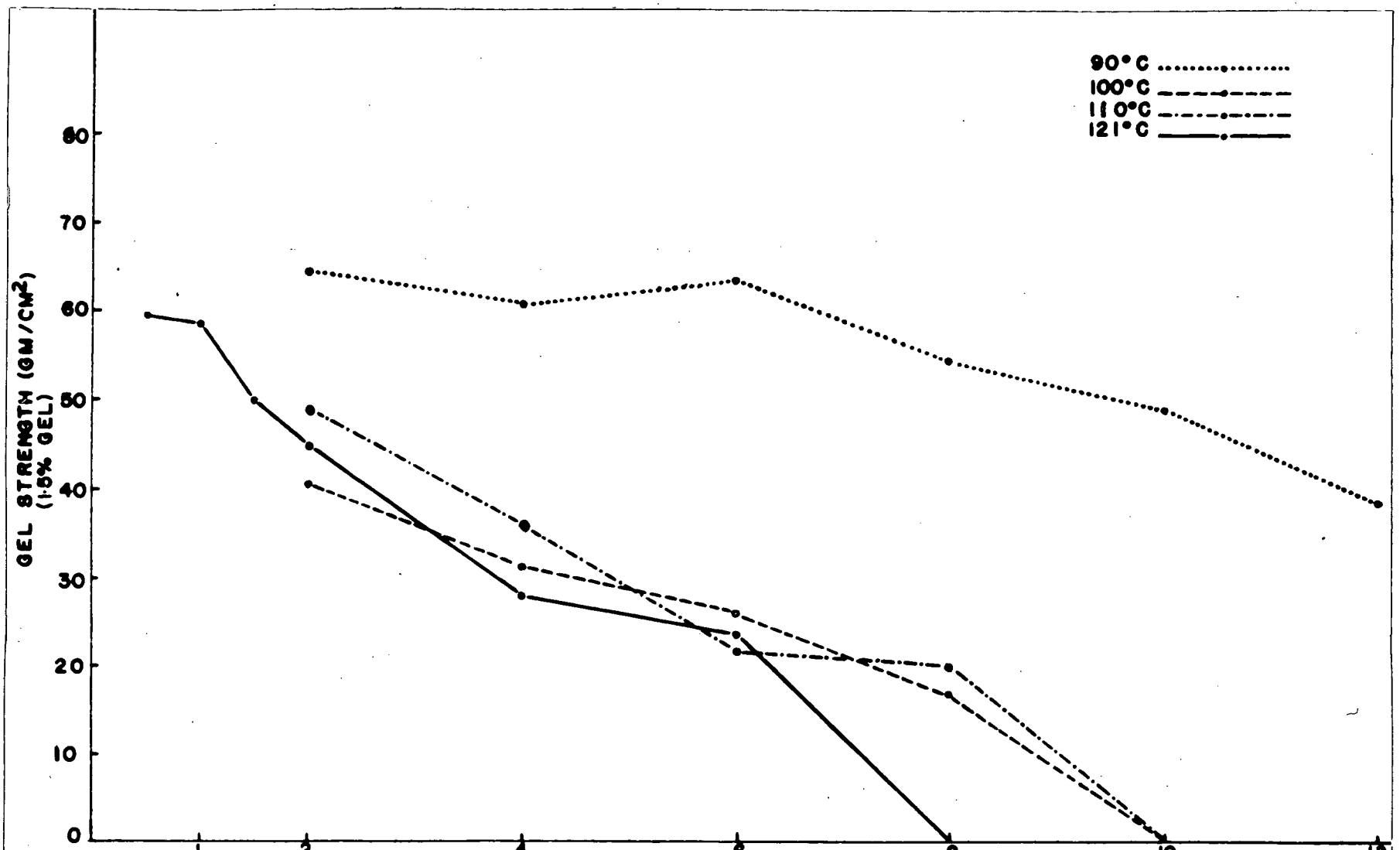
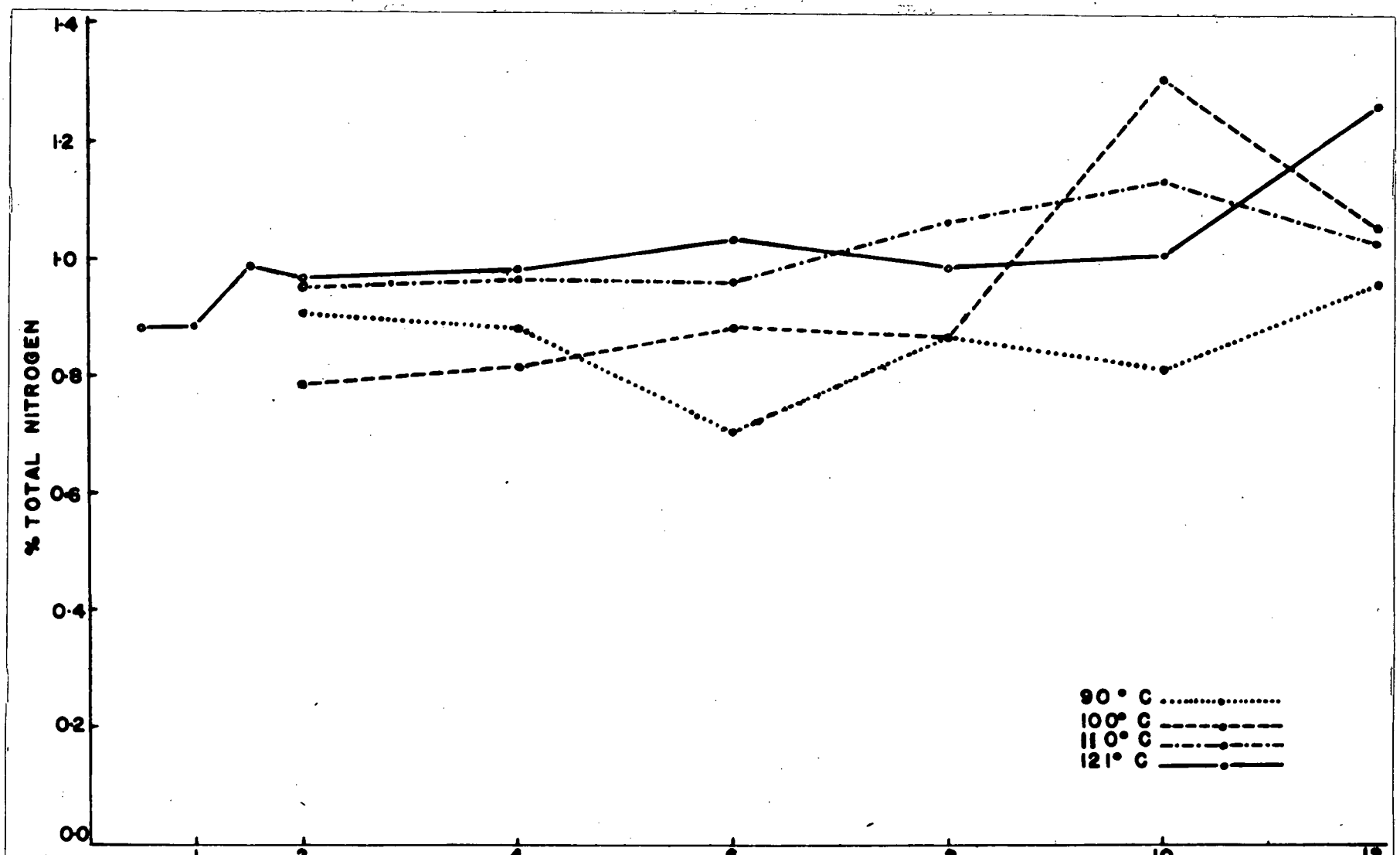


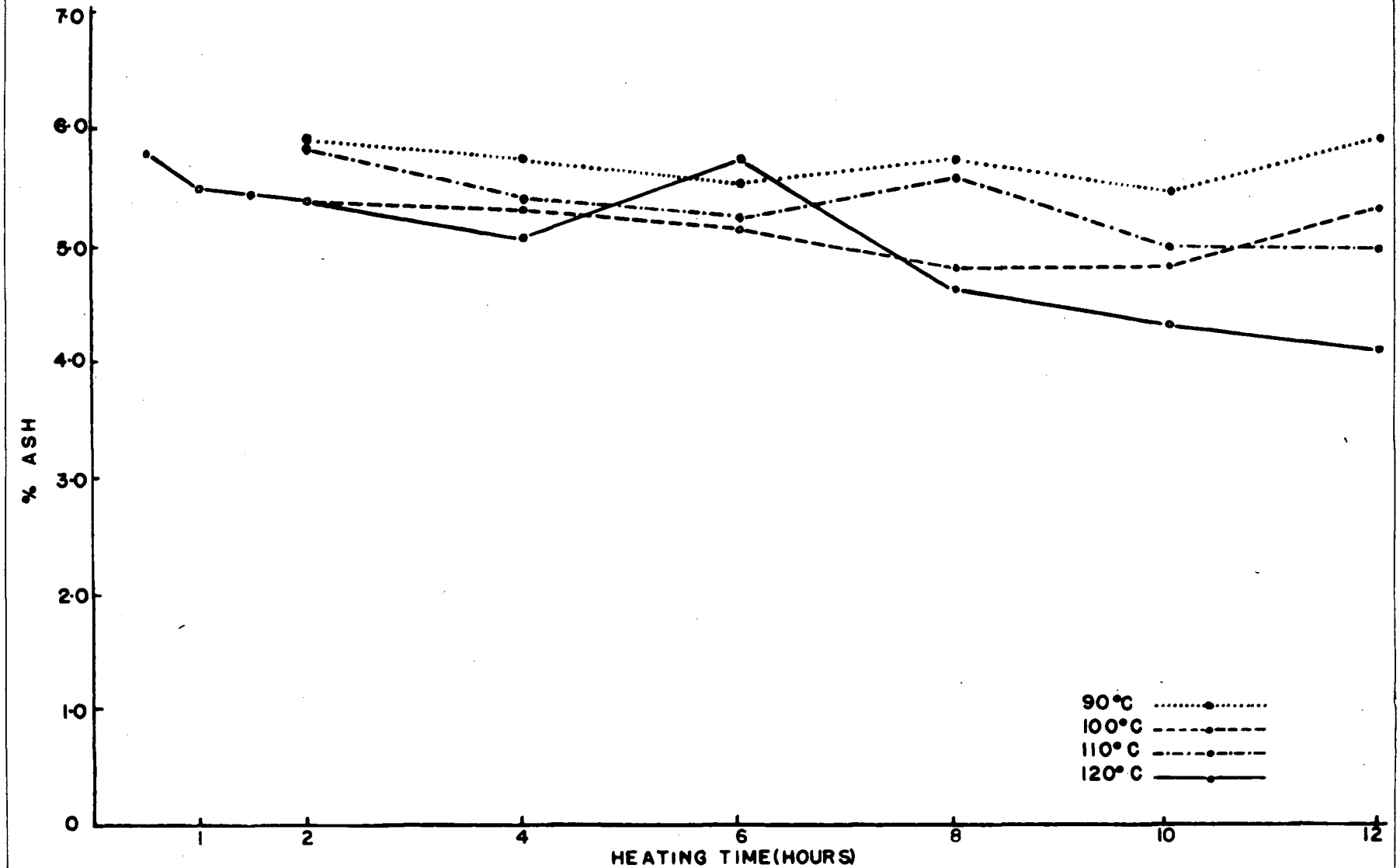
FIG. 6. % AGAR YIELDS OBTAINED WITH VARIATION IN TIME AND TEMPERATURE OF EXTRACTION.



FIG·7· AGAR GEL STRENGTHS OBTAINED WITH VARIATION IN TIME AND TEMPERATURE OF EXTRACTION·



FIG· 8· VARIATION IN % TOTAL NITROGEN WITH VARIATION IN TIME AND TEMPERATURE OF EXTRACTION·



FIG·9· VARIATION IN % ASH IN AGAR WITH VARIATION IN TIME AND TEMPERATURE OF EXTRACTION·

100°C were at variance with those at the other temperatures. This was probably due to scorching of the seaweed in the enamel container at this temperature. The gel strength value obtained after heating 2 hours at 110°C was significantly lower than that obtained at 90°C for the same time period. This suggested that a heating period of 2 hours was too long for this seaweed at 110°C. At periods of heating longer than 2 hours at 110°C, the gel strength decreased quite rapidly until at 10 and 12 hours the agar did not form a gel firm enough to test at 1½% concentration. At 121°C maximum gel strength was obtained at heating times of ½ hour and 1 hour. After 1 hour heating the gel strength dropped off quite rapidly and at 8, 10, and 12 hours the agar did not form gels firm enough to test at 1½% concentration. At the three highest temperatures the gel strength decreased at times of heating considerably shorter than the time at which maximum yield was obtained.

Percent total nitrogen and ash

The results obtained for percent total nitrogen and ash were not too consistent. There was, however, a general trend towards increased nitrogen at all temperatures as the time of heating was increased. The reverse was true with respect to percent ash. This suggested that the impurities which contributed to the ash content were different from those which contributed to the total nitrogen content. The values obtained for % total nitrogen content (0.75 to 1.3%) were considerably higher than a total nitrogen content of 0.14% in Difco Bacto agar. The % ash in Difco Bacto was found to be 2.50% as com-

pared to values between 5.94 and 4.06% for the Gracilaria agars.

Selection of optimum time and temperature for extraction

Although the gel strenth values were recorded in this section they were not determined until some time later in the investigation because the gelometer was not available. Since it was necessary to continue the work planned, a decision had to be made concerning the optimum temperature and time of heating for extraction even though complete analytical data were not available.

In view of the fact that facilities were not available to handle high viscosity extracts it was considered necessary to heat the seaweed until the marked decrease in viscosity occurred. Further, a large number of extractions were still to be carried out, thus the time factor was important. Therefore, a heating time of 2 hours at a temperature of 121°C was chosen. The yield factor was taken into consideration only insofar as it indicated that, with an extraction time of 2 hours at 121°C, little or no hydrolysis had taken place.

2. Ratio of seaweed to water in extraction

Wood (58) found that the yield of agar from Gracilaria tends to increase as the weed/water ratio decreases. He found, however, that unless the percentage agar was high enough to give a firm continuous gel after freezing, it was impossible to retain all the extracted agar after thawing. He found that a weed/water ratio of 1/25 was satisfactory from the point of view of agar yield.

Extractions were carried out at weed/water ratios of 1/30,

1/35, 1/40 and 1/50. It was found that extracts below the ratio of 1/30 were too viscous to handle. The amount of water required to give the above weed/water ratios was added to 40 grams of Stock B Gracilaria and autoclaved for 2 hours at 121°C. The liquor was filtered successively through (a) fine mesh cheese cloth, (b) silk cloth and (c) glass wool. The filtrate was left at room temperature for 24 hours, frozen for 24 hours and thawed on wire screening at room temperature. The gel remaining after thawing was firmer than that prepared from Stock A Gracilaria so it was spread directly onto the glass plates and oven dried at 50°C. The agar was then scraped off the plates, ground and stored.

The results obtained for yield, gel strength, percent total nitrogen and percent ash are given in Table 19.

TABLE 19. Ratio of seaweed to water in extraction, % yield, gel strength (1½% gel), % total nitrogen and % ash

Weed/Water Ratio	% Yield	Gel Strength (grams)	% Total Nitrogen	% Ash
1/30	22	38	1.38	6.10
1/35	30	37	1.39	6.08
1/40	34	40	1.51	6.10
1/50	36	44	1.59	7.19

An increase in agar yield was obtained as the amount of water used for extraction was increased. The gel strength remained nearly constant for all weed/water ratio extractions. The percent total nitrogen increased in the 1/40 and 1/50 extractions whereas it remained constant in the 1/30 and 1/35 extractions. The percent ash in the 1/50 agar increased consid-

erably over the constant values obtained at the three other weed/water ratio extractions.

A 1/40 weed/water ratio was chosen as the most satisfactory. At this ratio the seaweed extract filtered very readily and the thawed gel was firm enough to handle with ease. The 1/50 ratio extract filtered very readily but the thawed gel was quite mushy and difficult to recover from the wire screening. The extracts from the 1/30 and 1/35 ratio extractions were too viscous to permit easy filtration.

3. pH of extracting liquor

a. Adjustment of pH by the addition of acid or base.

Commercially, agar is extracted in neutral or acid medium. DeLoach et al (18) found, with Gracilaria confervoides, that the extraction liquor should be adjusted to pH 6.0 in order to obtain the best yields. Wood (59) found that there was no significant difference in yield when Gracilaria confervoides was boiled at pH 6.5 and pH 10. However, there was a lower yield between pH 7 and pH 8. He also found that below pH 5 the yield decreased rapidly to zero owing to acid hydrolysis. Further, extraction between pH 5 to 6.5 was considered satisfactory because filtration was the easiest over this range. Wood (59) also stated that Russian Anhfeltia was best extracted at pH 12 with lime used to adjust the pH.

As far as the writer is aware, no mention was made as to the effect on the quality of the agar when different pH's were used, except of course where the pH was acid enough to cause hydrolysis. The emphasis has been placed mainly on the fact that extraction was more rapid and complete and filtration

was easier in the acid range.

Extractions were carried out on Stock B Gracilaria, using 1/40 weed/water ratio, for 2 hours at 121°C. To 40 grams weed, 1600 ml of water was added, and after soaking 10 minutes the pH was determined. This was found to be 6.7. The pH was lowered or raised by adding glacial acetic acid or 50% sodium hydroxide and extractions were carried out at pH 4.0, 5.0, 6.0, 6.5, 7.0 and 7.5. The extraction liquor was filtered successively through (a) fine mesh cheese cloth, (b) silk cloth and (c) glass wool. The filtrate was left at room temperature for 24 hours, frozen for 24 hours and thawed on wire screening at room temperature. The thawed gel was then spread on glass plates, oven dried at 50°C; the agar was then scraped off the plates, weighed, ground and stored.

The results obtained for % yield, gel strength, percent total nitrogen and percent ash and pH of the agar are given in Table 20.

TABLE 20. pH of extracting liquor: results obtained when pH was adjusted by the addition of acid or base

pH	% Yield	Gel Strength (1½% gel)(gms)	% Total Nitrogen	% Ash	pH of Agar
4.0	12		0.99	0.85	4.2
5.0	33	18	0.34	4.30	5.4
5.5	38	20	1.42	5.14	5.3
6.0	38	28	1.39	5.17	5.6
6.5	38	33	1.30	6.77	5.6
7.0	33	53	1.33	7.29	6.1
7.5	30	68	1.16	7.56	7.5

Maximum yield was obtained between pH 5.5 and pH 6.5 and both below and above this range the yield progressively dropped. The agar extracted at pH 4.0 did not dissolve completely and what did dissolve would not gel. From pH 5.0 to 7.5 there was a consistent increase in gel strength to 68 grams at pH 7.5. The percent total nitrogen was mihimum at pH 4.0 extraction, quite constant from pH 5.0 to pH 7.0 and dropping again at pH 7.5. The percent ash was minimum at pH 4.0 and increased consistently to a maximum at pH 7.5.

The results showed a significant increase in gel strength of the agar extracted at pH 7.5, in spite of the marked increase in percent ash. This indicated that impurities which contributed to high ash content in the agar were not undesirable so far as gel strength was concerned. The results obtained indicated that extraction in alkali was a promising lead to follow. However, when an extraction was carried out at pH 8.0 the extract was so viscous that it could not be filtered. It was not known at this time, that Wood (58) had reported the fact that Australian Gracilaria agar extract was easy to filter when the extracting liquor was adjusted to pH 10 or above. Therefore, further work was not continued on alkali extraction at this point in the investigation.

b. Adjustment of pH by the addition of buffers

It was decided to carry out a series of extractions parallel to those given in the preceding section, in order to determine if there was any advantage in adjusting the pH by buffers rather than by addition of acid or alkali. A series of extractions was carried out on Stock B Gracilaria at the same

pH's used in the preceding experiment. pH adjustments were made using McIlvaines citric acid, disodium hydrogen phosphate buffer and the procedure used in the preceding experiment was followed in all other details.

The results obtained for percent yield, gel strength, percent total nitrogen, percent ash and pH of the agar are shown in Table 21.

TABLE 21. pH of extracting liquor: adjustment of pH by buffers

pH	% Yield	Gel Strength (1½%Gel)(grams)	% Total Nitrogen	% Ash	pH of Agar
4.0	25	20	1.16	4.31	5.9
5.0	32	28	1.19	4.82	5.9
5.5	30	29	1.12	4.67	5.9
6.0	34	34	1.15	6.14	6.4
6.5	33	35	1.17	6.67	6.2
7.0	32	34	1.17	5.98	5.9
7.5	27	44	1.04	7.31	6.3

The results were somewhat different from those obtained when acid or alkali alone was added. The reason this should be so was not apparent and time was not available to investigate the matter further. The percent yield was maximum at pH 6.0 extraction and fell off progressively at pH's above and below 6.0. The gel strength reached a maximum of 44 grams at pH 7.5, was constant between pH 6.0 and 7.0 and dropped to a minimum at pH 4.0. The percent total nitrogen remained practically constant from pH 4.0 to pH 7.0 and dropped slightly at pH 7.5. The percent ash values did not show a consistent increase from low to high pH as in the preceding experiment. The max-

imum percent ash was obtained at pH 7.5 and the minimum at pH 4.0. Because of the nature of the results obtained when a buffer solution was used, it was decided that if adjustment of pH was to be used in subsequent extractions it would be done by the addition of either acid or base alone.

4. Time of Freezing

The freezing and thawing process for purification of agar has proven to be the most satisfactory method of obtaining high quality agar. Agar can be precipitated from solution by the addition of alcohol or of electroylytes. Wood (58) stated that, by any of the three methods the agar is purified but in the case of the latter two methods the agar does not increase in gel strength to nearly the same extent as when freezing is used. Because of this, he concluded that there probably was an alteration of structure during the freezing-thawing process.

Extractions were made on Stock B Gracilaria, using 1/40 weed/water ratio, for 2 hours at 121°C. After heating, the extraction liquor was filtered successively through (a) fine mesh cheese cloth (b) silk cloth and (c) glass wool. The filtrate was left at room temperature for 24 hours. The variations in freezing times were, 24, 48 and 72 hours. After freezing the desired length of time the gel was thawed on wire screening at room temperature, spread on glass plates, oven dried at 50°C, and then the agar was scraped off the plates, weighed, ground and stored. Another extraction was carried out omitting the freezing: after letting the filtrate stand at room temperature for 24 hours, the gel was then transferred directly to

the glass plates, oven dried at 50°C, scraped off the glass, plates, weighed, ground and stored.

The results obtained for percent yield, gel strength, percent total nitrogen, percent ash and pH of the agar are given in Table 22.

TABLE 22. Comparison of frozen and unfrozen agar and results obtained when time of freezing was varied

Time (Hours)	% Yield	Gel Strength (1½% Gel) (grams)	% Total Nitrogen	% Ash	pH of Agar
0	51	*	1.76	11.16	5.1
24	34	40	1.51	6.10	6.1
48	32	40	1.12	4.95	6.3
72	30	38	1.14	5.11	6.5

* Did not gel

The results indicated that the yield and gel strength remained practically constant at freezing times of 24, 48 and 72 hours. The yield of course was high when freezing was not used and the agar did not gel at 1½% concentration. At times of freezing of 48 and 72 hours there was a considerable decrease in both percent total nitrogen and percent ash. When freezing was not used to prepare the agar the percent total nitrogen was about 14% higher than when a freezing time of 24 hours was used. Also, under the same conditions there was an increase in percent ash of about 46%.

This experiment established that freezing and thawing did increase the purity of the agar. It also seemed quite significant that at freezing times of 48 and 72 hours there was quite a marked decrease in nitrogen and ash and yet

no increase in gel strength. The differences in nitrogen and ash obtained for the frozen and unfrozen agar suggested that the impurities removed in the freezing-thawing process consisted of at least two distinct fractions, one an inorganic impurity and the other a nitrogenous organic impurity. Furthermore, since about 50-80% of the water is removed by thawing, the 46% reduction in ash indicated that most of the inorganic impurity was not bound to the agar whereas a 14% reduction in the nitrogenous impurity suggested that it was quite firmly bound to the agar.

5. Soaking seaweed in water before extraction

For the commercial production of agar it is usual to wash the seaweed by soaking in water for about 24 hours before carrying out the extraction. Wood (58) found that both agar yield and gel strength decreased if Gracilaria was washed for any length prior to extraction. This and the next experiment were carried out in order to determine the effect of soaking B.C. Gracilaria prior to extraction.

To 40 gram samples of Stock B Gracilaria, 1600 ml. of water was added, and soaked at room teperature for the following periods of time: 0, 4, 8, 16, 24, 36, and 48 hours. Extractions were then made for 2 hours at 121°C and the extraction liquor was filtered successively through (a) fine mesh cheese cloth (b) silk cloth and (c) glass wool. The filtrate was left at room temperature for 24 hours, frozen 24 hours and thawed over wire screening. The thawed gel was spread on glass plates, oven dried at 50°C and the agar was scraped off the glass plates, wieghed, ground and stored.

The results obtained for the analysis of the agar samples are shown in Table 23.

TABLE 23. Effect of soaking seaweed in water prior to extraction

Time of Soaking(Hrs)	% Yield	Gel Strength (1½%gel)(gms.)	% Total Nitrogen	% Ash	% of Agar
0	34	40	1.51	6.10	6.3
4	35	16	1.05	4.77	5.3
8	34	24	1.15	4.43	6.0
16	34	23	1.16	4.65	6.2
24	31	40	1.12	5.62	6.3
36	28	30	1.13	5.18	6.0
48	26	18	1.00	4.90	5.5

For periods of soaking up to 16 hours the agar yield remained practically constant and after 16 hours there was a decrease in yield with an increase in time of soaking. Maximum gel strength was obtained at 0 and 24 hours soaking. The lower results obtained at other times of soaking did not follow any particular trend and seem to be at variance. The percent total nitrogen values decreased to a nearly constant percent at all the times of soaking. The percent ash also decreased at all times of soaking although, like gel strength, there was no consistent trend.

In the next experiment the seaweed was soaked in water and prior to the extractions it was removed from the soaking water and extracted in a fresh sample of water. The seaweed used, the procedure and the periods of time of soaking were the same as in the previous experiment. The seaweed was

soaked in water for the required time and the water was then removed from the seaweed by filtration through cheese cloth. The volume of water removed was obtained and an equal volume of water was added to the seaweed. The extractions were carried out as above.

The results obtained for the analysis of the agar samples are shown in Table 24. Percent ash determinations were not obtained for soaking periods of 24, 36 and 48 hours.

TABLE 24. Effect of soaking seaweed in water: extracted in fresh water, after removing the water in which the seaweed was soaked.

Time of Soaking (Hrs.)	% Yield	Gel Strength (1½% gel) (gms)	% Total Nitrogen	% Ash	% of Agar
0	34	40	1.51	6.10	6.3
4	31	35	0.70	2.60	5.5
8	30	52	0.88	2.67	5.6
16	22	42	1.01	2.92	6.2
24	17	17	0.63	-	4.8
36	17	25	0.54	-	5.3
48	22	33	0.76	-	5.2

Again, the results did not seem to follow any specific trend. There was a considerable drop in yield with increased time of soaking. The gel strength values were variable but a significant increase was obtained at a soaking period of 8 hours. The percent total nitrogen and percent ash values were decidely reduced at all times of soaking used in the experiment. However, as in the other experiments there was no direct correlation between percent nitrogen and gel strength or between percent ash and gel strength.

6. Preliminary selection of an optimum procedure for agar preparation

The original plan in this investigation was to carry out the analytical work completely in each phase and then proceed to the next phase introducing into the procedure the optimum condition as determined in the previous phase of the investgation. This, however, was not possible because the Bloom gelometer was not available. At this point in the work, the instrument became available and the analytical data recorded to date was completed. Then a preliminary selection of an optimum procedure for agar preparation was made.

The results of this investigation so far indicated that only three of the factors studied were of any particular significance as far as increasing gel strength was concerned. The first was the time and temperature of extraction, the second was the pH of the extracting liquor and the third was soaking the seaweed and then removing the water and extracting with fresh water. Before slecting an optimum procedure it was necessary to decide what factor or factors should be given the preference. Were percent yield, percent total nitrogen and percent ash to be considered of more importance than gel strength? In many instances an increase in gel strength was paralleled by an increase in percent hitrogen and ash, and a decrease in yield. There was evidence in the literature that might explain the apparent inconsistency of the nitrogen and ash content of the agar. Wood (58) found that the amount of water frozen out of a given gel did not seem to be constant for any set of conditions, but was usually

60 to 80% of the original water present in the gel. The writer also observed that there was a marked variation in the nature of the thawed agar gel. Some gels were a continuous, firm, spongy mass whereas others were less firm and still others were discontinuous masses of solvated agar particles. No consistent correlation was observed between the amount of water lost in thawing and the nature of the gel, but the observation was made that if the extraction liquor was viscous the thawed gel was a firm spongy mass which retained 50-60% of the original water. Furthermore, the firm spongy masses when dried gave the highest gel strength. Although the nature of the thawed gel does not account for the high gel strengths it does give the explanation for the presence of high nitrogen and ash in such agar samples. Another point that could not be overlooked was the possibility that the water soluble fractions of the seaweed were indeed very complex, and perhaps the nitrogen and ash values obtained actually illustrated a complex interaction between the soluble seaweed fractions with the nature of the interaction varying with minor changes in procedure.

It was decided, for the time being at least, that percent nitrogen, ash and yield should be considered as of minor importance and to concentrate on gel strength as the most important factor upon which to base the optimum procedure. This was done with the hope that if high gel strength agar was obtained from B.C. Gracilaria, a method could be found to reduce the nitrogen and ash content without seriously affecting the gel strength of the agar.

The gel strength results obtained for the time and temperature of extraction agars indicated that a 2 hour extraction period at 121°C was too long and that a ½ hour extraction at this temperature was the most suitable. An improved method of filtering made it possible to handle more viscous extract, so a ½ hour extraction time at 121°C was chosen for the remainder of the investigation. The results obtained in the experiments to date did not indicate that other major changes in the procedure should be introduced at this time so the weed/water ratio of 1/40, the freezing time of 24 hours and the drying temperature of 50°C were retained for the subsequent agar preparations.

7. Potassium chloride extraction

The results of the investigation so far had proved to be quite disappointing. The highest gel strength obtained to date was 68 grams per cm^2 for a 1½% gel whereas the gel strength of a Difco Bacto agar gel at the same concentration was 458 grams per cm^2. It is known that a number of red seaweeds contain water soluble carbohydrates, such as carrageenin, which seem to be similar, in some respects at least, to agar. These carbohydrates do not set to firm gels if extracted in pure water; they form very viscous solutions and they have a high percent ash, (approx. 20-25%).

The agars prepared to date from B.C. Gracilaria were similar to the foregoing type of carbohydrate only with respect to the gelling property. Therefore, it had been assumed that the B.C. Gracilaria agar was not similar to the carrageenin type red seaweed extract. However, to furnish proof of this,

it was decided to use an extraction procedure which would greatly increase the gelling property of the extract from the carrageenin type seaweed.

In 1921, Sauvageau (47) showed that the gelling power of extracts from such seaweeds as Chondrus and Gigartina could be increased by the addition of certain salts, in particular potassium chloride. Haas and Hill (20) also in 1921, found that a small quantity of Rochelle salt added to the hot water extract from Chondrus greatly increased the gelling power and potassium chloride resulted in a still greater increase. Since 1921, a great deal of work has been carried out on this group of seaweeds and many valuable commercial products have been prepared by adding electrolytes during the extraction process.

In 1946, De Loach et al (17) reported the results of an investigation on Hypnea musciformis, collected along the coast of North Carolina. They stated that Hypnea extracted in distilled water would not gel in the absence of salts. Further, they stated that almost any salt would cause the Hypnea extractive to form a gel, the nature of which was dependent upon the nature and amount of solute. Also, almost any salt caused a marked decrease in viscosity of the extractive. Because of this marked decrease in viscosity, they stated that the salt should be added to the extraction water and the amount was dependent upon the temperature used during extraction. They found that a range of from 0.2% to 0.5% potassium chloride was the most suitable concentration for extraction. And they also reported that a 1.0% solution of Hypnea musciformis extractive containing 0.5% potassium chloride formed a gel of

greater strength than gels of equal concentration prepared from Gelidium cartilagineum or Gracilaria confervoides.

In 1948, Humm and Williams (27) investigated the properties of Brazilian Hypnea musciformis and found it to be similar to that from North Carolina. However, in their extraction procedure they added potassium chloride (0.2%) to the seaweed solution just before filtration.

The following extractions were made on a sample of Cherry Point Gracilaria confervoides which had been collected in the summer of 1946. The seaweed had been washed, dried and stored previously. Thus it was used for the extractions without further washing. It was known that the agar from this seaweed sample would not gel at 1½% concentration when it was extracted without pH adjustment at 121°C for ½ hour. Extractions were made with KCl concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0%.

To 50 grams of seaweed, 1500 ml of the KCl solution was added and extraction was carried out at 121°C for ½ hour. The extraction liquor was filtered successively through (a) muslin and (b) glass wool. The filtrate was left standing for 24 hours, frozen 24 hours and thawed on wire screening. The thawed gel was spread on glass plates, oven dried at 50°C, and the agar was scraped off the plates and stored. A second series of extractions was made at the same concentrations as above only the KCl was added just before filtration. To 50 grams of seaweed, 1500 ml of water was added and extraction was carried out at 121°C for ½ hour. Sufficient potassium chloride was added to give the required concentration and the

and the extraction liquor was filtered. The remainder of the procedure was the same as that given above. In addition to the foregoing an extraction was carried out adding sufficient NaOH to the extracting water to bring the pH to 9.6. No KCl was added. Otherwise, the procedure followed for preparation of the agar was the same as the above procedure.

All the KCl extraction agars set to very mushy gels at 1½% concentration. None of the gels was strong enough to support the weight of the gelometer plunger. The control agar did not gel at all. The KCl, therefore, did not have a marked effect upon the gelling property of the agar although it did improve this property very slightly. The agar prepared from the NaOH extraction gave a gel strength of 27 grams/cm^2.

The results showed quite clearly that the Gracilaria extract was not similar to the extracts from Hypnea, Chondrus, etc. The results indicated that the gelling property of the extract could be improved by the addition of NaOH to the extracting water.

8. Alkali Extraction

As stated earlier, if the alkalinity of the extracting liquor was increased beyond pH 7.5 to pH 8.0 the extract could not be filtered. An extraction was made at pH 12 and a marked viscosity decrease resulted thus permitting ready filtration. The following extractions were then carried out as a continuation of the earlier experiment on pH adjustment.

To 40 grams of Stock B Gracilaria, 1600 ml of water were added and the pH was adjusted to 12 with 50% by weight NaOH (7.5 ml). The seaweed was extracted for ½ hour at 121°C and

filtered through (a) fine mesh muslin and (b) glass wool. The filtrate was left at room temperature for 24 hours, frozen 24 hours and thawed over wire screening. The thawed gel was spread on glass plates, oven dried at 50°C, scraped off the plates, weighed, ground and stored.

The agar had a gel strength of 87 grams/cm^2 at 1½% concentration and the pH was 8.9. The high pH indicated that the extract should be neutralized at some stage in the procedure. The following modification of the above procedure was then introduced. Prior to filtration, the seaweed was neutralized with concentrated H_2SO_4. The gel strength of the resulting agar was 108 gm/cm^2 at 1½% concentration and the pH was 7.1. Although the foregoing procedure gave a higher gel strength agar, it was not considered satisfactory. At pH 12, the seaweed pigments were soluble and the method of thawing did not permit satisfactory removal of them. It was decided, therefore, to thaw the frozen extract in a large volume of water and remove the soluble impurities by filtration. This procedure obviated the necessity for neutralization.

To 40 grams of Stock B Gracilaria, 1600 ml of water were added and the pH was adjusted to 12.0 with 50% NaOH and the extraction was carried out for ½ hour at 121°C. The extraction liquor was then filtered successively through (a) fine mesh muslin and (b) glass wool. The filtrate was left at room temperature for 24 hours and frozen for 24 hours. The frozen gel was then crushed and put into 4 litres of cold tap water containing about 250 grams of ice. After thawing, the solution was filtered through muslin and the agar residue was dried

and recovered in the usual manner.

The gel strength of the agar obtained was 116 grams for 1½% gel and the pH was 8.1. Again, there was a slight increase in gel strength but the pH was rather high. The agar still retained a considerable quantity of the pigments; therefore it was decided to repeat the above procedure and give the agar residue two more washings. The above procedure was used, only after thawing in 4 litres of water the agar residue was soaked in 3 litres of cold tap water for about 15 minutes. The solution was filtered through muslin and the agar residue was again soaked for approximately 15 minutes in 2 litres of cold tap water, then filtered, dried and recovered in the usual manner. The gel strength of this agar was 116 grams for a 1½% gel and the pH was 7.3. The agar still retained some of the pigment but it seemed to be firmly bound to the agar.

Two more extractions were carried out on Stock B Gracilaria, one at pH 10.0 and the other at pH 11.0. The agar was prepared by the preceding procedure. The results obtained for percent yield, gel strength and pH of the agar are shown in Table 25 along with the results for the agar obtained by the same procedure at pH 12.0.

TABLE 25. Results obtained with alkali extraction

pH	Percent Yield	Gel Strength (1½%gel) (grams)	pH of Agar
10.0	16	108	7.4
11.0	17	110	7.2
12.0	19	116	7.3

The results showed that extraction at pH 12.0 gave a slightly

higher yield and gel strength than at the other pH's.

It will be recalled that a heating time of 2 hours was used in the earlier work on pH adjustment whereas for extractions above pH 7.5 a heating time of ½ hour was used. It was possible, therefore, that the increased gel strength resulted primarily from a reduction in time of heating. An extraction carried out at a heating time of 2 hours at pH 12.0 showed that the increased gel strength was due to the presence of alkali during extraction and not due to the reduction of heating time.

The extraction was carried out using Stock B Gracilaria. The procedure used to prepare the agar was identical to the last mentioned procedure only a heating time of 2 hours was used. The gel strength of this agar was 216 grams/cm^2 for a 1½% gel. This was an increase of nearly 100% over the agar obtained when the ½ hour extraction time was used and approximately 300% higher than the 2 hour extraction at pH 7.5. The advatange of extracting at pH 12.0 was then established. However, the above results showed another problem still remained. Why was the gel strength so much higher when a 2 hour extraction was carried out in place of an extraction time of ½ hour? Since no further work was carried out at this time the answer to the problem was not ascertained.

9. Final selection of an optimum procedure for agar preparation

It was now necessary to reconsider the time of heating which was to be used for agar extractions from Gracilaria samples collected at monthly intervals. Wood (58) found that the optimum time of extraction varied with the sample of

Gracilaria under test. Further, he found that samples from the same area dried by different people gave optimum extraction times of ½ hour to 4 hours. These facts, along with the information obtained in the preceding experiment indicated that it would be necessary to determine an optimum time of extraction for each Gracilaria sample. This however was impossible. Therefore it was decided to use a ½ hour extraction time, because there was not satisfactory evidence to suggest that any other time would be more suitable for all the monthly collection samples. The results obtained using alkali extraction were significant enough to warrant introducing pH adjustment into the agar preparation procedure. It was decided accordingly to adjust the pH of the extracting water to 12.0 for agar preparation from the montly Gracilaria collections. No other changes were introduced into the agar preparation procedure.

E. Water soluble fractions of Gracilaria confervoides

In the preceding portion of this investigation some indications were found which suggested that the water soluble fraction of Gracilaria was not a single compound, but rather, two or more compounds. Although chemical analyses had not been made, it was obvious that the agars prepared to date contained the red pigment, phycoerythrin, and those agars which were prepared by alkali extraction contained chlorophyll as well as phycoerythrin. Phycoerythrin is soluble in cold water and can be removed quite readily by soaking the seaweed in water. It was decided to extract some phycoerythrin and add it to Difco

Bacto agar in order to determine its effect upon the gelling property of the Difco agar. A solution of phycoerythrin was recovered from the seaweed and filtered through filter paper to remove sand, etc. The apparently homogeneous phycoerythrin filtrate was left at room temperature and after about 3 hours it was noticed that a precipitate was settling to the bottom of the container. This observation led to the following investigation on the water soluble fractions of Gracilaria confervoides, with the intention of isolating a factor which might have an inhibitory effect on the gelling property of the agar.

Eighty grams of ground Stock B Gracilaria was added to to 1900 ml of water in a 2 litre erlenmeyer. The solution was layered with toluene (toluene was used as an antiseptic throughout the experiment), the flask was corked and placed at 2°C for 24 hours. At the end of 24 hours the water was quite clear so the seaweed solution was put through a Waring blendor for 5 minutes and then returned to 2°C for 48 hours. The solution was then filtered successively through muslin and silk. The seaweed residue (I) was recovered and was added to 1300 ml of water. The filtrate was centrifuged at 2000 R.P.M. for 20 minutes. A reddish brown residue was thrown down. The supernatant (II) was decanted and the residue was washed from the centrifuge flasks. The residue plus wash water was frozen and thawed. The solution was centrifuged and the residue was recovered and dried in a dessicator over H_2SO_4. This was Fraction 1. The supernatant was very light pink in color indicating that very little of Fraction 1 had gone into solution. Therefore it was discarded.

The supernatant (II) solution was milky red in color but no fluorescence was observed. It (II) was frozen and thawed and a residue precipitated in the thawed solution. The solution was centrifuged at 2500 R.P.M. for 10 minutes. The supernatant (III) was decanted and the residue was washed from the centrifuge tubes, put into a cellophane dialysis sac and dialysed against cold running tap water for 24 hours. The residue was then dried by suspending the sac in an open window. Rapid evaporation followed and the dried residue was easily recovered by, first rolling the dialysis sac between the fingers to break the residue away from the sac and then tearing the sac to remove the residue. This was Fraction 2. It was dark reddish-brown in color. The preceding drying procedure was adopted because it had been found that if such colloidal materials are dried at too high a temperature the dried residue was very difficult to dissolve. The above drying technique was used throughout the experiment.

The supernatant (III) was still milky red in color. Therefore it was frozen again and thawed. A further residue was recovered by the same procedure as Fraction 2. This was Fraction 3. It was dark reddish-brown in color. The supernatant was again frozen and thawed and the residue obtained was recovered in the same manner as Fractions 2 and 3. This was Fraction 4. It was also dark reddish-brown in color. Since, Fractions 2, 3 and 4 appeared the same it was assumed that the three fractions were identical. The final supernatant (IV) from Fraction 4 exhibited the typical fluorescence of phycoerythrin. The

supernatant (IV) was concentrated to 60 ml by vacuum distillation at 50 to 60°C. The concentrated solution was not fluorescent. It was dried in a dialysis sac in the usual manner. The dried residue was Fraction 5. It was a gum, dark red in color.

The seaweed residue (I) in 1300 ml of water was soaked at room temperature for 72 hours. The solution was then filtered through muslin and silk and the brown seaweed residue (V) was added to 1000 ml of water. The filtrate (VI) was centrifuged at 2000 R.P.M. for 20 minutes and the residue was dialysed against cold running tap water for 24 hours and dried in the usual manner. This was Fraction 6. The dried residue appeared to be the same as Fractions 2, 3 and 4. The supernatant (VII) from VI was frozen and thawed and the thawed solution was centrifuged at 2500 R.P.M. for 10 minutes. The residue was dialysed for 24 hours against cold running tap water and dried in the usual manner. This was Fraction 7. This dried residue also appeared to be the same as Fractions 2, 3, 4 and 6. The supernatant from VII was intense red by transmitted light and orange by reflected light. The solution was concentrated to 50 ml by vacuum distillation at 50 to 60°C. The concentrated solution was not fluorescent. It was dialysed for 24 hours against cold running tap water and dried as usual. This was Fraction 8. It was the same color as Fraction 5 but it was brittle and not at all gum-like.

The seaweed residue (V) in 1000 ml of water was soaked at 50°C for 48 hours. The solution was then filtered through muslin, silk and glass wool. The green residue (VIII) was

added to 1000 ml of water. The filtrate was centrifuged at 2000 R.P.M. for 10 minutes but a residue was not obtained. Therefore the filtrate was frozen and thawed. The thawed solution contained a white precipitate. The solution was centrifuged at 2000 R.P.M. for ½ hour but the residue remained suspended in the solution. The solution was then filtered through filter paper. The white residde was dialysed for 24 hours against cold running tap water and dried as usual. This was Fraction 9. This dried residue was quite brown although it resembled agar in other respects. The reddish-brown filtrate was concentrated by vacuum distillation at 50 to 60°C, and the concentrated solution was dialysed for 24 hours against cold running tap water and dried as usual. The resulting dark brown residue was Fraction 10.

The residue (VIII) in 1000 ml of water was soaked again at 50°C for 72 hours. The solution was then filtered through muslin, silk and glass wool. The green residue (IX) was added to 1000 ml of water. The brownish-green filtrate was frozen and thawed and an agar-like precipitate was obtained. This was removed by filtration through filter paper and the residue was dialiysed for 24 hours against cold running tap water and dried in the usual manner. This was Fraction 11. It was light green in color. The light brown filtrate was concentrated by vacuum distillation at 50 to 60°C and the concentrated solution was dialysed against cold running tap water for 24 hours and dried as usual. This was Fraction 12. It was light brown in color and it could be powdered readily. The residue (IX), in 1000 ml of water was soaked again at 50°C for 96 hours.

The solution was then filtered through muslin, silk and glass wool. The residue (X), light green in color, was added to 1000 ml of water. The light green filtrate was frozen and thawed and a green agar-like precipitate was obtained. The solution was filtered through filter paper and the agar-like residue was dried at 50°C on a glass plate. This was Fraction 13. The filtrate was evaporated to dryness and a very small residue was obtained. This was discarded.

The residue (X), in 1000 ml of water, was soaked again at 50°C for 72 hours. The solution was filtered through muslin, silk and glass wol. The seaweed residue was discarded and the filtrate was frozen and thawed. A colorless agar-like precipitate was present in the thawed solution. This was recovered by filtration and dried on a glass plate at 50°C. This was Fraction 14. The light yellowish green filtrate was discarded. With the exception of the slight difference in color, Fractions 11, 13 and 14 seemed to be the same.

Very little analytical work was carried out on the 14 fractions. All fractions gave a positive Molisch carbohydrate test and they all gave a positive Ninhydrin protein test. The three agar-like fractions, (9, 11, and 14) did not form gels at 1.0% concentration. Fraction 13, at 1% concentration had a gel strength of 18 grams/cm^2. In addition to the above, some of the fractions were added to Difco Bacto agar in order to determine the effect of them on the gelling property of Difco agar. The effect of some of the fractions on the gelling property of Difco agar is shown in Table 26.

TABLE 26. Results obtained when the water soluble Gracilaria fractions were added to Difco Bacto agar

1.0% Difco agar plus 0.00013% of Fraction:	Gel Strength (grams/cm^2)
No fraction added	186
3	213
4	216
5	204
8	211
10	206
12	198

A significant increase in the gel strength of Difco Bacto agar was obtained upon addition of Gracilaria Fractions 3,4, 5,8,10,and 12.

Earlier in the investigation, several thawed solutions from the frozen agar gels were recovered and evaporated to dryness. Some of these dried residues were added to 1.0% Difco Bacto agar and the results obtained were similar to those in Table 26.

Since insufficient analytical work was carried out on these fractions it was not possible to say how many essentially different fractions were obtained. From their appearance, the writer considered that there were nine different fractions. It was possible, however, that the different appearances of the nine fractions resulted from variable admixtures of a fewer number of fractions.

The possible relationship which existed between Fractions 2, 3 and 4, and Fraction 5 was that the first three fractions were the protein carrier of the red pigment phycoerythrin and

Fraction 5 was the chromophoric group of the chromoproteid. Whatever the real situation the writer was certain that there were enough chemically different compounds recovered to support the concept that agar is a mixture of several complex compounds which have different solubilities in water. With normal agar extraction procedures, at higher temperatures, it is quite possible that all these compounds would be recovered as agar.

G. Seasonal Variation in some of the properties of Gracilaria agar prepared from the monthly seaweed collections.

Most investigators who have worked with Gracilaria confervoides have found that the agar recovered from it was comparable to the best Japanese agar with respect to gel strength, but that the temperature of gelation was higher than that of Japanese agar. From the point of view of use as culture media, this higher temperature of gelation was an undesirable feature. Moore (39) reported that New Zealand Gracilaria confervoides agar had a gel strength at 1.2% concentration equivalent to a 1% concentration of Japanese agar. The % ash was 3.17, melting point of 1% gel was 76°C and the setting pdnt of 1% sol was 42°C. Wood ( ) reported that Australian Gracilaria confervoides agar was of excellent quality and better in some respects than Japanese agar. He stated that the better quality agar had a gel strength up to 100% greater than Kobe No. 1 agar. The Australian agar, however, had higher setting points and viscosity. He stated that the viscosity could be reduced by dialysis of the frozen agar and by bacterial maceration, but all attempts to lower the setting temperature had failed.

De Loach et al (18) stated that North Caroline Gracilaria confervoides, agar compared favorably with that of Gelidium agar, with respect to gel strength but that the elasticity of Gracilaria agar was greater. The temperature of gelation varied with the time of year of harvesting. During July, the temperature of gelation was 42-43°C and during November it was 63°C for 1½% gels. Stoloff (48) made a comparison of several Gracilaria confervoides agars from different parts of the world. He found that South African Gracilaria confervoides agar was the only one suitable, from the point of view of physical characteristics, for bacteriological purposes. The gel strength was almost as high as commercial Bacto-agar and the temperature of gelation was 37°C at 1½% concentration. He reported that California Gracilaria confervoides agar had a gel strength approximately half that of Bacto-agar and the temperature of gelation was 47°C at 1½% concentration. In India, Bal et al (3) found that Gracilaria confervoides agar was suitable for bacteriological purposes. Marshall et al (36) reported that Gracilaria confervoides samples collected near Plymouth, England gave an agar which was inferior to Gelidium agar but suitable for bacteriological work.

The next phase of this investigation was to carry out extractions on the Gracilaria samples which had been collected at regular intervals over a period of one year. Agar was prepared from all the air dried and oven dried seaweed samples by the same procedure.

Procedure A. Forty grams of seaweed was added to 1600 ml

water. After soaking for 10 minutes the pH of the solution was obtained and then adjusted to pH 12.0 with 50% NaOH (7.5 ml). The seaweed was heated to boiling and placed in a hot autoclave, autoclaved at 121°C for ½ hour, filtered through muslin and glass wool, and the filtrate was allowed to set for 24 hours. It was then frozen for 24 hours, crushed, thawed in 4 litres of ice cold tap water, and then recovered by filtering through muslin. It was then washed twice more in cold tap water, using 3 litres and 2 litres water respectively for the washes. The agar gel retained in the muslin filter was spread on glass plates, oven dried at 50°C, removed by scraping, weighed, ground in the corn mill and stored. This agar preparation procedure was used with slight variations in later work. The designation, Procedure A, has been given to this procedure in order to avoid repetition in subsequent recording of procedure.

Tables 27, 28 and 29 show the results and observations obtained for the air dried seaweed samples collected from Cherry Point, Mill Bay and Ganges, respectively. Tables 30, 31 and 32 show the results and observations obtained for the oven dried seaweed samples collected from Cherry Point, Mill Bay and Ganges. Fig. 10, 11 and 12 show the seasonal variation in gel strengths obtained for the air dried and oven dried samples from Cherry Point, Mill Bay and Ganges, respectively.

TABLE 27. Results and observations obtained concerning seaweed extracts from Cherry Point Gracilaria (Air dried samples)

Collection Date	% Yield	Gel Strength (1½%) (gms/cm²)	pH of Agar	Viscosity of Extract	Appearance of agar gel
22.6.48	18	149	7.4	quite viscous	green
19.7.48	19	151	7.6	quite viscous	light brown
7.8.48	17	332	7.3	not viscous	light green
2.9.48	11	881	7.4	not viscous	green
27.9.48	22	337	7.8	quite viscous	dark green
2.11.48	18	176	7.1	quite viscous	colorless
1.12.48	18	139	6.5	quite viscous	green
29.12.48	22	114	7.7	not viscous	dirty green
15.2.49	20	48	7.4	not viscous	light brown
11.3.49	14	90	7.6	not viscous	brown
16.5.49	16	103	7.9	not viscous	light green

TABLE 28. Results and observations obtained concerning agar extracts from Mill Bay Gracilaria (air dried samples)

Collection Date	% Yield	Gel Strength (1½%) (gms/cm²)	pH of Agar	Viscosity of Extract	Appearance of agar gel
22.6.48	10	170	7.6	not viscous	dirty green
19.7.48	19	154	7.2	not viscous	light green
7.8.48	20	237	7.2	not viscous	light green
2.9.48	10	185	7.7	not viscous	light brown
27.9.48	15	321	7.3	not viscous	light green
2.11.48	16	111	6.9	not viscous	light green
1.12.48	14	79	7.0	not viscous	light green

TABLE 29. Results and observations obtained concerning agar extracts from Ganges Gracilaria (air dried samples)

Collection Date	% Yield	Gel Strength (1½%) (gms/cm²)	pH of Agar	Viscosity of Extract	Appearance of Agar Gel
21.6.48	25	381	7.2	not viscous	dirty green
21.7.48	22	230	7.6	not viscous	light green
6.8.48	21	311	7.6	not viscous	light green
1.9.48	16	143	7.2	not viscous	light green
30.9.48	23	153	7.3	not viscous	green
4.12.48	18	114	7.5	not viscous	green
30.12.48	20	172	7.6	very viscous	dirty green

TABLE 30. Results and observations obtained concerning seaweed extracts from Cherry Point Gracilaria (oven dried samples)

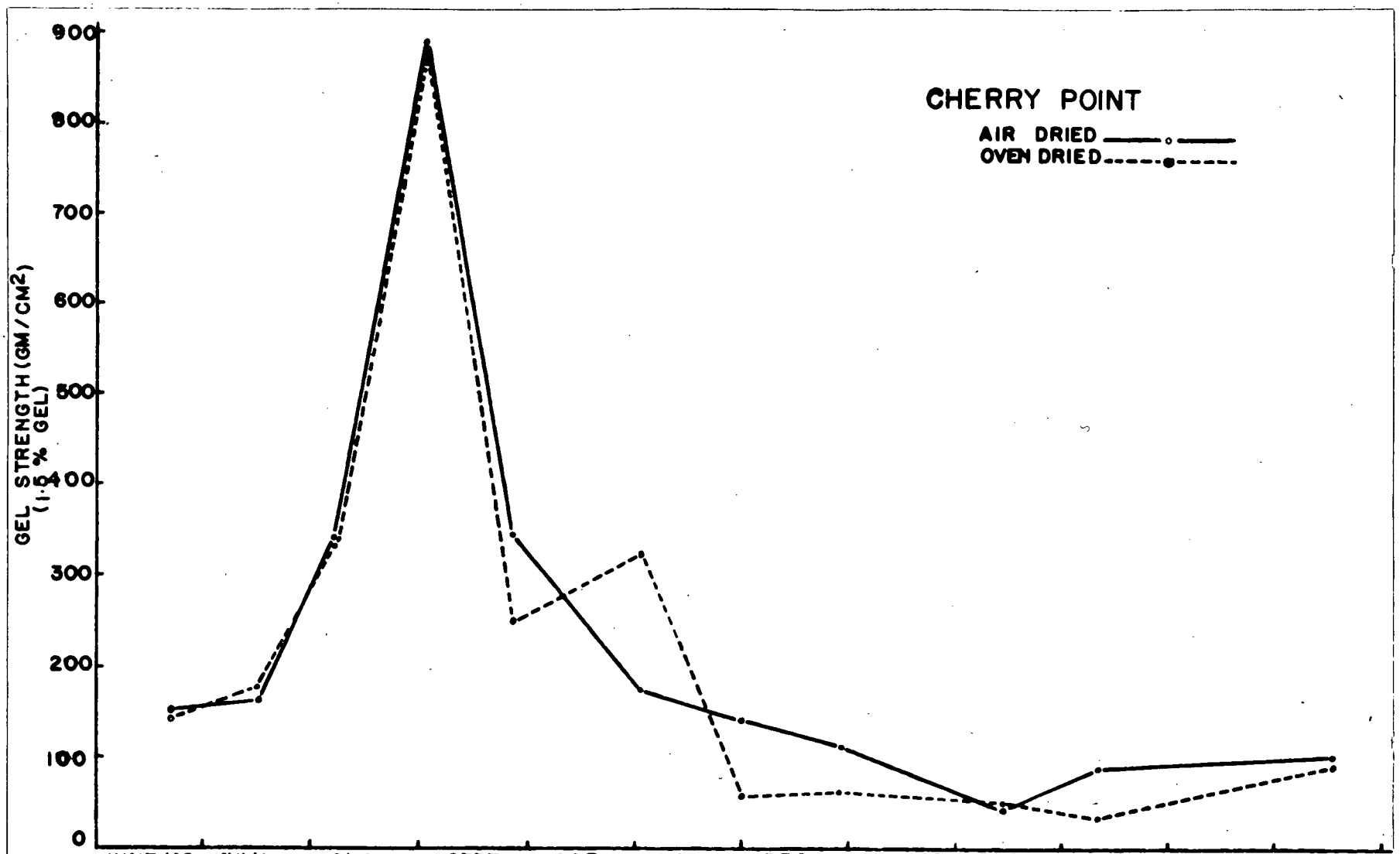
Collection Date	% Yield	Gel Strength (1½%) (gms/cm²)	pH of Agar	Viscosity of Extract	Appearance of Agar Gel
22.6.48	20	134	7.6	not viscous	light brown
19.7.48	19	271	7.5	quite viscous	light green
7.8.48	24	319	7.4	quite viscous	light green
2.9.48	12	862	7.2	not viscous	green
27.9.48	16	237	7.3	quite viscous	light green
2.11.48	12	330	7.5	not viscous	brown
1.12.48	12	55	7.6	not viscous	brown
29.12.48	13	65	7.1	quite viscous	light green
15.2.49	4	54	6.8	not viscous	brown
11.3.49	4	32	6.4	not viscous	brown
16.5.49	16	93	7.5	very viscous	green

TABLE 31. Results and observations obtained concerning agar extracts from Mill Bay Gracilaria (oven dried samples)

Collection Date	% Yield	Gel Strength (1½%) (Gms/cm²)	pH of Agar	Viscosity of Extract	Appearance of Agar Gel
22.6.48	(Oven dried sample not obtained)				
19.7.48	23	235	7.9	quite viscous	light green
7.8.48	15	242	7.3	quite viscous	dirty green
2.9.48	20	107	6.8	not viscous	light green
27.9.48	20	378	7.3	quite viscous	light green
2.11.48	15	113	7.8	quite viscous	green
1.12.48	13	109	7.0	quite viscous	brown

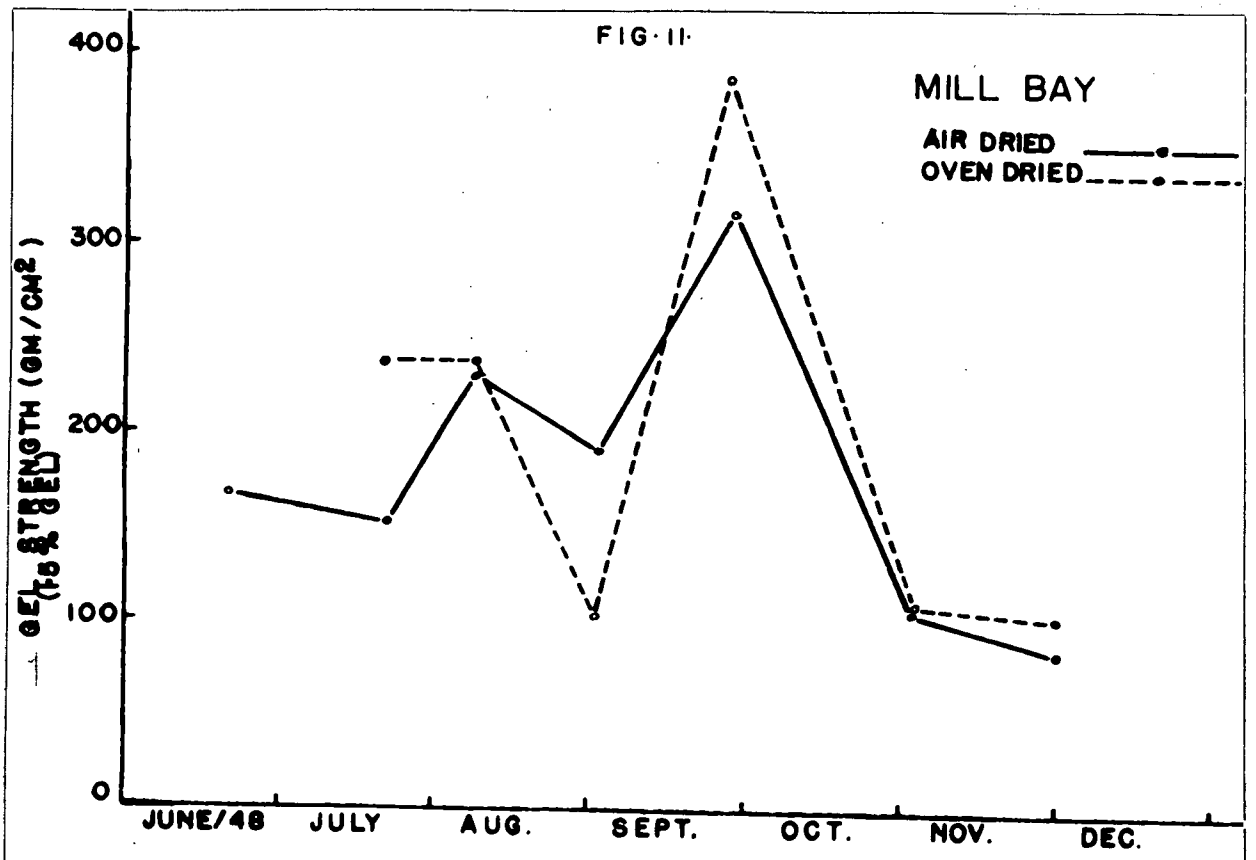
TABLE 32. Results and observations obtained concerning agar extracts from Ganges Gracilaria (oven dried samples)

Collection Date	% Yield	Gel Strength (1½%) (gms/cm²)	pH of Agar	Viscosity of Extract	Appearance of Agar Gel
21.6.48	(oven dried sample not obtained)				
21.7.48	24	253	7.5	quite viscous	light green
6.8.48	21	318	7.5	not viscous	light green
1.9.48	17	82	7.5	quite viscous	light green
30.9.48	21	150	7.4	quite viscous	green
4.12.48	18	142	7.5	quite viscous	green
3 0.12.48	(oven dried sample not obtained)				

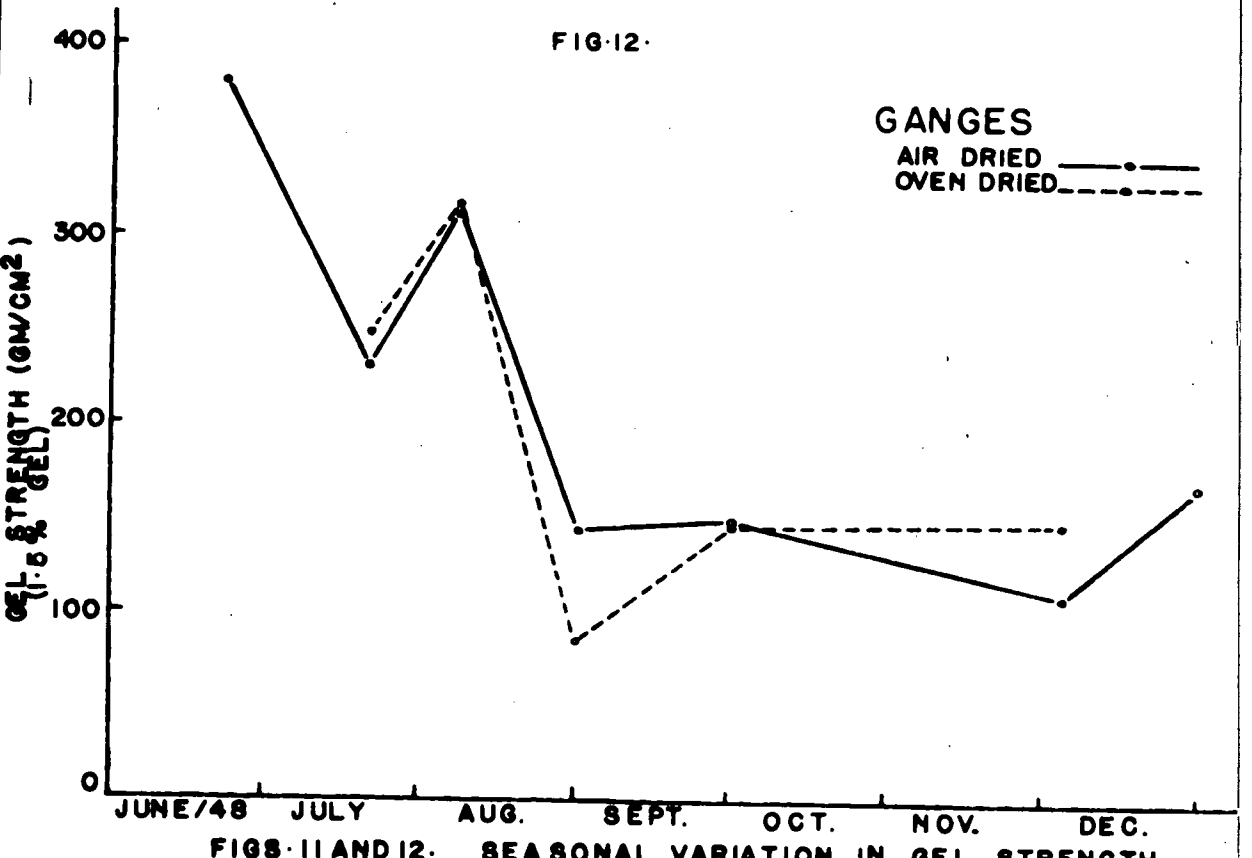


FIG·10· SEASONAL VARIATION IN GEL STRENGTH OF AGAR PREPARED FROM AIR AND OVEN DRIED GRACILARIA CONFERVOIDES.

FIG·11·



FIG·12·



FIGS·11 AND 12. SEASONAL VARIATION IN GEL STRENGTH OF AGAR PREPARED FROM AIR AND OVEN DRIED GRACILARIA CONFERVOIDES

Agar Yield. When the agar was extracted at pH 12.0 the yields were found to be quite low. The maximum yield obtained was 25% from Ganges, 21.6.48, air dried sample, and the minimum yield was 4% from Cherry Point, 15.2.49 and 11.3.49, oven dried samples. There was no consistent similarity between the yields obtained from the air dried samples and those from the oven dried samples collected from the three localities. The agar yield from the air dried Cherry Point samples collected during June, July, August and early September were lower than the corresponding oven dried samples from the same collection. But the yields obtained from the air dried Cherry Point samples collected from the end of September to the middle of May were higher than the corresponding oven dried samples from the same collections. The agar yields from Mill Bay air dried and oven dried samples did not follow any significant trend. The yields from Ganges air dried and oven dried samples from the same collections were almost the same.

Gel Strength. The maximum gel strength of 881 gm/cm^2 was obtained for the agar prepared from the Cherry Point, 2.9.48 air dried sample. The value, 862 gms/cm^2 obtained for the agar from the corresponding oven dried sample was not significantly lower. The minimum gel strength value, 32 gms/cm^2, was obtained for the agar from the Cherry Point 11.3.49 oven dried sample. Significant differences in gel strength were obtained between most of the corresponding agars from the air dried and oven dried Cherry Point samples. However, there was no consistent trend in these differences.

With Cherry Point agars the gel strength increased greatly from early August to early September and then decreased at the end of September to approximately the same value as for early August. Then the gel strength values decreased further to minima during February and March and were on the increase again by the middle of May.

At Mill Bay, the maximum gel strength, 378 gms/cm^2, was obtained for the agar from the oven dried sample collected at the end of September and the minimum gel strength was obtained with the agar from the air dried 1.12.48 sample. Again, there was no close parallel in gel strengths between the corresponding air and oven dried samples. A maximum gel strength of 381 gms/cm^2 was obtained for the agar from the air dried 21.6.48 Ganges collection and the minimum gel strength, 82 gms/cm^2, was obtained for the early September collection. The gel strength decreased irregularly from late June to early September and remained fairly constant until the end of December. The gel strength values obtained for the agar from the corresponding air and oven dried Ganges samples were, with the exception of the 1.9.48 collection, nearly constant.

pH. The pH values have been recorded primarily to show that the differences obtained for the gel strength of the various agars were not due to an excessively acid or alkaline agar.

Viscosity. There was considerable variation in the viscosity of the seaweed extracts and since the procedure was constant for all extractions, the variation in viscosity indicated

that, physically at least, the extracts were different. An examination of the results shows that there were marked viscosity differences between corresponding air and oven dried samples as well as between the different monthly samples. There was no consistent relationship between the corresponding air and oven dried Cherry Point extracts but there was between the Mill Bay and Ganges, with the corresponding air and oven dried samples. In all instances, except Ganges 30.12.48, and Mill Bay 2.9.48, the extracts from the air dried samples were not viscous whereas the oven dried sample extracts were quite viscous. It is also of interest to note that the seaweed samples, which gave the highest gel strength agars, had low viscosity extracts.

Color of the agar gels. The color of the agar gels indicated the presence of degraded phycoerythrin or chlorophyll. Most of the degraded phycoerythrin could be removed by repeated washing but the chlorophyll could not be removed by washing. The results show that agars which gave the highest gel strengths were green in color. There was no consistent relationship between color and gel strength of the agars for gels other than those which gave the maximum gel strength. It should also be mention that all those agar gels which were reported as being colorless were ~~opaque~~ TRANSLUCENT in the sol state. The colored gels were also opaque in the sol state. Thus in this respect the B.C. Gracilaria agar sols were unlike the transparent Difco Bacto agar.

A number of miscellaneous experiments were also carried out and these are recorded below.

Washing versus unwashing. As mentioned earlier, portions of some of the monthly seaweed collections were not washed after harvesting but dried directly. Other portions were washed to remove sand, etc., but not seaweed contaminants. Wood (58) found that if Gracilaria confervoides was washed before extraction the agar yields and gel strengths were lower than those obtained from unwashed seaweed. The agars were prepared by Procedure A from air dried seaweed and the results obtained for percent agar yield and gel strength of 1½% gels are given in Tables 33 and 34.

TABLE 33. Washed versus unwashed seaweed

Sample		Washed		Unwashed	
		Gel Strength (gms/cm²)	% Yield	Gel Strength	% Yield
Cherry Point	2.11.48	176	18	227	15
Ganges	4.12.48	114	18	140	8

TABLE 34. Washed with seaweed contaminants removed versus washed without removing seaweed contaminants

Sample		Contaminants Removed		Contaminants not Removed	
		Gel Strength (gms/cm²)	% Yield	Gel Strength (gms/cm²)	% Yield
Cherry Point	1.12.48	139	18	209	17
Cherry Point	16.5.49	103	16	109	20

When the seaweed was not washed a significant increase in gel strength and a decrease in yield were obtained for both Cherry Point and Ganges agars. A significant increase in gel strength was also obtained when the seaweed contaminants were not re-

moved, and a higher yield was obtained from the 16.5.49 sample but not from the 1.12.48 sample. The actual percent seaweed contamination in the Gracilaria samples was not determined. Contamination was slight and probably did not exceed 1%. The contaminants were principally Zostera and the green algae Enteromorpha and Ulva.

Grinding versus not grinding. Wood (58) found that if Gracilaria confervoides was ground before heating, the agar yield decreased and the relative viscosity increased but the gel strength was not affected. The following is an experiment to determine the behaviour of B.C. Gracilaria confervoides when it was ground prior to heating. Cherry Point 2.9.48 oven dried seaweed was ground in the corn mill and the agar was prepared using Procedure A. The results obtained for gel strength and percent agar yield are shown in Table 35.

TABLE 35. Ground versus not ground seaweed

Sample (Cherry Point 2.9.48)	Gel Strength (1½%) (gms/cm^2)	% Yield
Not ground	862	12
Ground	745	6

There was a significant reduction in gel strength and percent agar yield. Also, the extraction liquor from the ground seaweed sample was very viscous.

Once the seasonal variation in gel strength of the monthly collection seaweed samples was known, it was possible to carry out further work on some of these samples. This was done in order to determine whether or not the conditions chosen as

optimum for agar preparation were the most suitable for seaweed samples other than the one upon which the optimum procedure was based. It was necessary to limit this phase of the investigation almost entirely to the air dried portions of the three Cherry Point Gracilaria collections from which the highest gel strength agars were obtained.

The first experiment was carried out in order to show if alkaline extraction did improve the gelling property of the agars from these samples. Agar was prepared from the seaweed by Procedure A with the exception of pH adjustment. The results obtained are compared in Table 36 with the agars prepared from the same seaweed samples when the pH was adjusted to 12.0.

TABLE 36. pH adjustment versus no pH adjustment

Sample Cherry Point	pH adjusted to 12.0		No pH adjustment	
	Gel Strength (gms/cm^2)	% Yield	Gel Strength (gms/cm^2)	% Yield
7.8.48	332	17	61	25
2.9.48(oven dried)	862	12	726	34
27.9.48	337	22	249	26

When no pH adjustment in extraction was made the gel strength values were significantly lower than when the pH was adjusted to 12.0. Another significant feature of the results was that the degree of decrease in gel strength varied in the three agar samples. The percent agar yields were higher when the pH was not adjusted and the increase obtained for 2.9.48 seaweed sample was nearly 300%.

It was found that when alkali extraction was used the agar yield was always lower than when no pH adjustment was

made. This observation led to the second experiment which consisted of two parts. First, could the yield be increased without reducing the gel strength? The second problem was related to the possible presence of two different carbohydrate complexes. Did the low yields suggest that the two complexes were soluble in acid or neutral solution but that only one complex was soluble in alkaline solution?

In order to find whether the agar yield could be increased without reducing the gel strength, the following extractions were carried out.

1. Procedure B. Agar was prepared from seaweed sample 2.9.48 by Procedure A, except that a 2 hour extraction time was used instead of a ½ hour extraction time.

2. Procedure C. Agar was extracted from seaweed sample 2.9.48 by Procedure A, only, after filtering the extract was set on a steam bath and the residue was extracted again by Procedure A but no further pH adjustment was made. After filtering, the second extract was added to the first and the agar was recovered using the method of Procedure A.

The results obtained for gel strength and percent agar yield are shown in Table 37 along with the results which were obtained with the agar from the same seaweed sample by Procedure A.

TABLE 37. Results of tests to determine whether agar yield could be increased without decreasing gel strength

Method of Preparation of Agar	Gel Strength(1½%) (gms/cm²)	% Yield
Procedure A	881	11
Procedure B	885	21
Procedure C	824	22

The results showed that Procedures B and C gave an agar yield increase of 100%. There was no significant difference between the gel strengths of Procedures A and B agars but Procedure C resulted in a significantly lower gel strength. However, the gel strength of 824 gms/cm^2 for Procedure C was much higher than the value, 458 gms/cm^2, obtained for Difco Bacto agar at the same concentration.

The next experiment was carried out in an attempt to determine the nature of the "two possible" carbohydrate complexes. Earlier observations had shown that when some of the seaweed samples were added to water and the pH adjusted to 12, the seaweed became rigid. This phenomenon was very noticeable with the 2.9.48 Cherry Point Gracilaria. It was also noticed that, after this seaweed sample was extracted by Procedure A, the remaining seaweed residue was still quite intact. It was possible, therefore, to carry out the following examination on the 2.9.48 Cherry Point Gracilaria before and after extraction. Free-hand cross-sections of the seaweed thallus were made prior to extraction. The sections were examined between crossed nicols and the inner part of the cortical and medullary cell walls were bright. Since agar is anisotropic under polarized light it was assumed that this demonstrated the location of agar within the plant. Free-hand cross-sections of the seaweed, extracted by Procedure A, were then made and examined between crossed nicols. This time the walls of the cortical cells were dark and the walls of the medullary cells were bright. It seemed possible, therefore that there were different compounds in the cortical and medullary cell walls. Furthermore, this might explain the variable

agar yields at different pH's. Since there is a lower agar yield when alkali extraction is used than when acid or neutral extraction is used, it was assumed that the soluble cell wall components of both the medullary cells and the cortical cells are soluble in acid or neutral solution but only the cell wall component of the cortical cells is soluble in alkali. If such be the case, then the cell wall components of the cortical and medullary cells are chemically different.

In order to find further evidence to support the previous assumption it was decided to extract the residue without further addition of alkali. Unfortunately only sufficient 2.9.48 seaweed remained to carry out 2 extractions. The plan was to extract one seaweed sample at a pH between 7 and 12 and to extract the other sample at pH 12. However since the seaweed buffered very strongly at pH 12 the two extractions were carried out at pH 12 but at different NaOH concentrations. The agar samples were prepared by the following procedures.

Procedure D. Agar was prepared from 2.9.48 seaweed by Procedure A but instead of adding 7.5 ml 50% NaOH, 4.0 ml were added. This was Procedure D, Fraction I agar. Agar was again prepared from the residue by Procedure A but no NaOH was added. This was Procedure D, Fraction II.

Procedure E. This agar was to have been prepared by extracting with 7.5 ml 50% NaOH but a mishap occurred and 9.0 ml were added instead. Agar was prepared from 2.9.48 seaweed by Procedure A but instead of adding 7.5 ml 50% NaOH, 9.0 ml were added. This was Procedure E, Fraction I. Agar was again prepared from the residue by Procedure A but no NaOH was added. This was

Procedure D, Fraction II.

The results obtained are shown in Table 38 along with the results which were obtained from the same seaweed sample by Procedure A.

TABLE 37. Results of tests to find evidence to support the assumption that two chemically different compounds occur in the cell walls of Gracilaria

Sample	Gel Strength ($1\frac{1}{2}\%$) (gms/cm^2)	Viscosity of Agar
Procedure A agar	881	not viscous
Procedure D, Fraction I	615	not viscous
Procedure D, Fraction II	691	very viscous
Procedure E, Fraction I	967	not viscous
Procedure E, Fraction II	953	very viscous

The results showed that the gel strengths of Procedure D agars were significantly lower than those of Procedure A agar, whereas, the gel strengths of Procedure E agars were significantly higher. The gel strength of Procedure D, Fraction II was higher than that of Procedure D, Fraction I but there was no significant gel strength difference between Fractions I and II of Procedure E agar. Another noticeable feature of the result was the very high viscosity of Fraction II agars.

In the work on optimum conditions for preparation of agar it was found that a significant increase in gel strength was obtained when the seaweed was soaked in water for 8 hours and was than extracted in fresh extracting water. The third experiment was carried out in order to determine if the gel strength of the agar from Cherry Point 7.8.48 and 27.9.48 samples could be improved by soaking in water for 8 hours prior to extraction.

The seaweed was soaked in water for 8 hours, removed from the water and the agar was prepared by Procedure A. Other agar samples were prepared by the above method but no pH adjustment was made. The results obtained for gel strength of the agars at 1½% concentration are shown in Table 38.

TABLE 38. Results to show the effect of soaking and not soaking the seaweed prior to extraction and the effect of pH adjustment (Gel strength of 1½% agar gels)

Sample	Extraction without soaking		Extraction with soaking	
	pH not adjusted	pH adjusted to 12	pH not adjusted	pH adjusted to 12
7.8.48	61 gms/cm^2	332 gms/cm^2	83 gms/cm^2	-
27.9.48	249 "	337 "	259 "	491 gms/cm^2
27.9.48 (oven dried)	-	237 "	-	280 "

The results showed that there was an increase in gel strength when the seaweed was soaked for 8 hours and no pH adjustment was made and when the seaweed was soaked for 8 hours and the pH was adjusted to 12. The most significant gel strength increase was obtained for the agar from 27.9.48 seaweed when the seaweed was soaked for 8 hours and the pH was adjusted to 12 prior to extraction.

H. Comparison of B.C. Gracilaria confervoides agar and Difco Bacto agar

Before giving a comparison between some of the better Gracilaria agars and Difco Bacto agar, it is of interest to record the important characteristics of bacteriological agar, since most investigators working with Gracilaria confervoides have placed emphasis upon the suitability of the agar for bacterio-

logical culture media. The important characteristics have been recorded as follows. (2)

(a) The agar should dissolve in hot water, forming an almost colorless clear solution, which on cooling sets to a firm gel.

(b) The surface of the gel must be sufficiently hard so that inoculation with a platinum loop will not tear the gel.

(c) The gel should remain solid at a temperature well above the normal incubation temperature (37°C), but once melted it should remain liquid down to 35 to 40°C.

(d) The agar should not contain any inhibiting factor which will affect the growth of microorganisms.

The following comparison of B.C. Gracilaria confervoides agar and Difco Bacto agar is in part a summary of results already given and, in addition, results which have not been included elsewhere. The results are given for B.C. Gracilaria agar samples which were prepared by Procedure A from air dried seaweed unless otherwise stated. A comparison of some of the properties of B.C. Gracilaria agar and Difco Bacto agar is given in Table 39.

The table of comparison between some of the better Gracilaria agars and Difco Bacto agar shows the following:

(a) Some Gracilaria agars had a much higher gel strength than Difco Bacto agar.

(b) When Gracilaria agars were extracted in alkaline solution the pH of the prepared agar was above 7 whereas the pH of the Difco Bacto agar was 6.7.

(c) The percent ash was considerably higher in the

TABLE 39. A comparison of some of the properties of B.C. Gracilaria agars and Difco Bacto agar

Sample	Gel Strength (1½%) (gms/cm²)	pH	% Ash	% Total Nitrogen	Temperature of: Gelation (2.0%sol)	Melting (2.0% sol)
Difco Bacto agar	458	6.7	2.50	0.15	33.9	91.4
Cherry Point 2.9.48	881	7.2	4.95	0.93	42.8	90.3
Cherry Point 2.9.48 (Proc. E)	967	7.2	6.34	1.04	41.4	90.8
" " " (oven dried) (Procedure A without pH adjustment)	726	5.7	3.28	1.12	41.0	90.0
Cherry Point 2.9.48 (Proc. B)	885	7.5	6.21	0.97	41.0	90.8
Cherry Point 7.8.48	332	7.4	5.71	0.89	39.8	90.2
Cherry Point 27.9.48	337	7.8	6.90	-	40.1	89.9
Mill Bay 27.9.48	321	7.3	5.88	1.04	40.3	89.4
Ganges 21.6.48	381	7.6	5.02	1.06	43.4	89.6

Gracilaria agars than in Difco Bacto agar.

(e) There was some variation in the gelation temperature of the Gracilaria agars, but for all samples tested the values obtained were 6 to 9.5° higher than for Difco Bacto agar.

(f) The melting temperatures of the Gracilaria agars were about the same as Difco Bacto agar.

In addition to the foregoing the following observations were made:

(a) Slope holding capacity. All of the above agar samples were tested at 1½% concentration and none of them had shown any signs of slipping at the conclusion of the test.

(b) Syneresis. At the conclusion of the slope holding capacity test it was observed that a small amount of syneresis had occurred. There did not appear to be any significant difference between the B.C. Gracilaria agars and Difco Bacto agar.

(c) Viscosity. Viscosity observations have been recorded throughout the investigation and it should be stated that the agar sols which were recorded as "not viscous" were more viscous than Difco Bacto agar of the same concentration.

V. DISCUSSION

The results obtained throughout the investigation suggested that a separate optimum procedure for agar preparation would be required for each Gracilaria confervoides sample collected. A seasonal variation in the gelation property of agar obtained from the seaweed, indicated a seasonal change in the chemical nature of the agar within the plant. Evidence was obtained to suggest that this change was not due to varying

degrees of polymerisation of a homogeneous molecule, but rather to variable admixtures of two or more molecules.

The very marked seasonal variation in the gelation property of Cherry Point Gracilaria agars and the variation with locality suggested the significance of environment. Since Gracilaria confervoides is an estuarine seaweed, its natural habitat is associated with comparatively low salinity and high phosphate and nitrogen content of the water. Harvey (21) stated that phosphate and nitrogen content of inshore sea water is minimum during the summer. And as already mentioned, he stated that there was a tendency for diluted water (where rivers and streams enter the sea) to remain banked up near the coast during the winter and then run out as a surface layer during summer. An examination of the gel strength results obtained for Cherry Point Gracilaria agars showed that the gelation property was low during favorable conditions of salinity and mineral content of the water and high during unfavorable conditions.

At first this appeared to be an anomaly. However, the writer believes that probably a structural change in the cell wall took place during the unfavorable conditions. The nature of this change is not known but the following hypothesis is presented as an explanation warranting further investigation. If a plant were transferred from an environment of low salinity to one of high salinity, the cells would plasmolyze. At the same time, there would be an increased diffusion gradient for the movement of salts into the plant cells. In order to prevent plamolysis or a possible toxic accumulation of salts within the cells, the permeability of the cell wall was decreased. The

decreased permeability resulted from an increased synthesis of agar in the cell walls, accompanied by an increase in the degree of crystallization of the agar. It is known that maximum agar content of the seaweed is paralleled by maximum gel strength. There is evidence in the literature to support the concept that gel strength of agar is a function of the degree of crystallization; as the degree of crystallization increases the rigidity of a gel increases.

The short interval of the year in which high gel strength agar was obtained indicates the necessity for further investigation, if B.C. Gracilaria confervoides is to be utilized for commercial production of agar. If the preceding hypothesis is true, it is possible that control measures could be introduced at the Gracilaria beds in order to obtain conditions requisite for high gel strength agar. Before control measures could be applied, it would be necessary to study in greater detail the environmental factors prevailing at the time of year when gel strength is maximum.

As far as the writer is aware, alkali extraction has not been used for the commercial preparation of agar from Gracilaria confervoides. Although considerable increase in the gelation property of agar was obtained by extracting the seaweed at pH 12, the results indicated that further improvement could be obtained by increasing the sodium hydroxide concentration of the extraction liquor. It is suggested therefore that further work be carried out on alkali extraction.

VI. SUMMARY

Seasonal variation in some of the properties of agar from British Columbia Gracilaria confervoides have been studied. A very marked seasonal variation in gel strength for Cherry Point Gracilaria agar was obtained, the maximum occurring in early September. Maximum gel strength for Mill Bay agar was obtained at the end of September, while that for Ganges agar was found to occur at the end of June. Concurrent with these maximal values were high ash and nitrogen content.

Optimum conditions for preparation of agar from British Columbia Gracilaria confervoides were investigated. It was found that the gel strength of the agar could be increased by adjusting the pH of the extracting liquor. Alkali extraction resulted in low agar yield. This yield was increased without impairing the properties of the agar by increasing the duration of the extraction period, or by extracting twice. Agar prepared by alkaline extraction had a higher ash content than that prepared by acid or neutral extraction. The methods employed in the preparation of the seaweed for extraction were found to affect the properties of the agar.

Maximum gel strength values obtained during the investigation were found to be 100% and more, higher than that of commercial Difco Bacto agar. The other significant physical properties of the agar prepared in this investigation were found to be comparable to those reported by other investigators working with Gracilaria confervoides in other parts of the world.

Evidence has been presented to confirm the validity of

the theory of the complex nature of agar presented by De Loach et al.

An hypothesis has been presented to explain (in part at least) the variations observed in the gel strength of the agar extracted from Gracilaria confervoides.

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