Training Manual on Gracilaria Culture and Seaweed Processing in China

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Prepared for the
Gracilaria Culture and Seaweed Processing Training Course
conducted by the
Zhanjiang Fisheries College
People's Republic of China
and organized by the
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Demonstration Project (RAS/90/002)

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Training Manual 6

PREFACE

Gracilaria is a group of warm water seaweeds. There are more than one hundred species in the world, some of which have very important economic value. Gracilaria is used as food and in the preparation of food products. It is also an important raw material in agar-agar production. At present, the world's annual output of Gracilaria is about 30,000 tons, dry weight, most of which comes from natural production. For example, the natural production in Chile, Argentina, and Brazil accounts for one third of this total output. With the increasing demand for Gracilaria, greater attention has been focused on the development of its artificial culture by many countries, especially those in Southeast Asia. China is the earliest country that artificially cultured Gracilaria. Today, the culture area in South China is about 2,000 ha producing 3,000 tons dried material annually. Taiwan produces 1,000 tons dried Gracilaria yearly from 400 hectares under cultivation.

Nowadays, Gracilaria is cultured mainly using the following methods: bottom culture, raft culture, stake-rope culture and pond culture. Pond culture can be divided into two systems, monoculture and polyculture with shrimp and other species. The varieties of culture methods can be adapted for different areas.

In view of the importance of Gracilaria as a seafarming commodity, the National Coordinators of the Regional Seafarming Project recommended the dissemination of its culture and processing technology, through a regional training and demonstration activity, as a means to further increase the opportunities to develop the seafarming industry of the region.

This manual has been prepared for the training course on Gracilaria culture under the Regional Seafarming Development and Demonstration Project (RAS/90/002) to be held at Zhanjiang Fisheries College, Zhanjiang, China in August, 1990. The training course includes processing of seaweeds, thus the manual devotes a chapter on the properties, manufacture and application of agar, algin and carragenan.

The manual was edited by Professor Wu Chaoyuan who also reviewed all the manuscripts, Professor Ji Minghuo, Mrs. Li Renzhi, Associate Professor and Mr. Wang Xiaohang, Associate Professor, all of the Institute of Oceanology in Qingdao; Professor Liu Sijian of the Zhenjiang Fisheries College; and Mr. Miao Zenian, Associate Professor of the Yellow Sea Fisheries Research Institute, Qingdao. Mr. Sun Jimin of the Yellow Sea Fisheries Research Institute computer-processed the text and images. The staff of the Seafarming Development Project in Bangkok provided the final editing and prepared the manual for publication.

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Department of Aquatic Products
Ministry of Agriculture
China, May 1990

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CHAPTER I: BIOLOGY OF GRACILARIA

1. CHARACTERISTICS OF THE GENUS GRACILARIA AND FEATURES OF SEVERAL IMPORTANT SPECIES IN CHINA

The genus of *Gracilaria* is cosmopolitan in distribution, and has been reported from the arctic, temperate and tropical regions. Greville set up the genus *Gracilaria* in 1830, which comprised then only four species. Agardh reexamined the genus in 1852, and increased it to 23 species. In 1876 and 1901 he re-identified them again and reported 61 species altogether. Since then, the number of *Gracilaria* species reported from many places over the world have reached about 150, with 24 species reported in China.

a. Characteristics of Gracilaria

External appearance

Erect thalius arise from a small discoid holdfast. The thalli are generally cylindrical, depressed or blade-shaped, with lateral, alternate or subdichotomous branches. Sometimes several different branches may be found in one plant. The external appearance of thalli may be used to identify species. The style of the apex and the base of branches are different with species.

The thalli of most species are cylindrical. Several commercially important species will be discussed. Some species (such as *G. asiaticamaides*) are compressed, their thalli growing horizontally along the substratum and form secondary holdfasts from the margin of branches. A few species (such as *G. textorii*) are blade-shaped.

Anatomy of main axis

The vegetative thalli of *Gracilaria* consist of cortex and medulla. Cortical cells are smaller. The outermost 1~2 layers are pigment cells. Medulla comprise large parenchymatous cells (Fig.1). The layers of cortex, the size and number of medullary cells and the change of cells from cortex to medulla are used for identification of species.

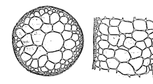

Fig. 1. Transection and longitudinal Gection of main axis of *Gracilaria*

Tetrasporangia

The tetrasporangia are densely scattered on the cortex surface. Each tetrasporangia is composed of four tetraspores arranged in a cruciate manner (Fig. 2).

Fig. 2. Surface view and transection of tetrasporangia of *Gracilaria*

Spermatangia

The spermatangia are globular or oval, scattered over the surface of thalli. The location and type of spermatangial conceptacle are three important considerations for identification of species (Fig. 3).

A. Spermatangia scattered on the surface layer of thalli continuous or interrupted by cortical cells.
B. Spermatangia in shallow depressed spermatangial conceptacles.
C. Spermatangia in deeper conceptacles ovoid to long elliptical in sectional view.

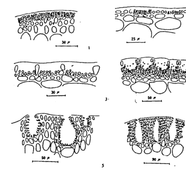

Fig. 3. Transections of spermatangia of *Gracilaria*

Cystocarp

Cystocarp is prominent, protruding, globose or hemispherical, scattered over the surface of the fronds. It may be divided into 4 portions (Fig. 4).

Fig 4 Transections of cystocarps of *Gracilaria*

A. Pericarp

Consisting of several layers of cells, outmost layer is composed of pigmented cells.

B. Gonimoblast

In the center of cystocarps, consists of parenchymatous cells.

C. Carposporangia

Formed at the top of the gonimoblast, round or ovoid in shape.

D. Absorbing filaments

Extended from the gonimoblast tissue to the pericarpic-layer. Some species possess absorbing filaments.

Introduction of several important species

Gracilaria asiatica

Thallus erect, solitary or caespitose, cylindrical throughout, arising from a small discoid holdfast; purplish brown to dark brown, sometimes greenish or yellowish; branches irregularly alternated.

G. asiatica is widespread along the China coast, and was first reported in 1866 as *Gracilaria verrucosa* Papenfuss. In 1985, Chinese phycologists Zhang and Xia compared *G. verrucosa* specimens collected from England with Chinese and Japanese specimens both identified as "*G. verrucosa*". They found that the Chinese and Japanese specimens were of the same species (Japanese phycologists were of the same opinion), but since the Chinese species differed from the English *G. verrucosa* species, they named the Chinese plant *Gracilaria asiatica*.

The *asiatica* species may be readily distinguished from English *G. verrucosa* by the deeper spermatangial concepticales, larger tetraspores, smaller carpospores and especially by the structure of the pericarp (Fig. 5).

Fig. 5. Comporicon in pericarps of *asiatica* from China and Japan with *verrucosa* from England

Gracilaria lemaneiformis

G. lemaneiformis is one of the commercially important species because of its fast growth and high quality gel. It is distributed along the coasts of north China, and is similar in appearance to *G. asiatica*. Thallus is purplish red, arises from a larger red holdfast, and usually grows in gravel in the lower intertidal zone, with the base portions of thallus often covered with sand.

Gracilaria tenuistipitata

There are two varieties in south China coasts: v. *tenuistipitata* and v. *liui*. Variety *tenuistipitata* grows on gravels and shells in the sublittoral region of lower salinity. It is characterized by its extreme slenderness near the base. The thallus arises from a small disklike holdfast, and is simply, moderately, or alternately branched near the base. The branches tend to become like the main axis.

Variety *liui* differs from the variety *tenuistipitata* by the slender thalli bearing numerous, delicate, short to long lateral branchlets, branching mostly from the percurrent axes. Its cystocarpic structure and shallow spermatangia are similar to those of v. *tenuistipitata* (Fig. 6).

This variety frequently occurs naturally in muddy substrate in fish ponds and shallow intertidal areas. When cultivated, as in Hepu, Guangxi Province and in Taiwan, the thalli are usually detached and without holdfasts, and may tumble about in fairly large masses. The Taiwanese "*G. verrucosa*" (Shang 1976 and Chiang 1981) has been examined and is being place among the variety *liui* of *G. tenuistipitata*.

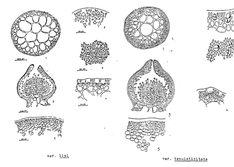

Fig. 6. Comparison between *G. tenuistipitata* var. *liui* and *G. tenuistipitata* var. *tenuistipitata* 1. Main axis. 2. Tetrasporangia. 3. Cystocarp. 4. Spermatangia. 5. Pericarp.

2. LIFE HISTORY OF GRACILARIA

Sporophytes and gametophytes of *Gracilaria* occur alternately in its life cycle (Fig. 7).

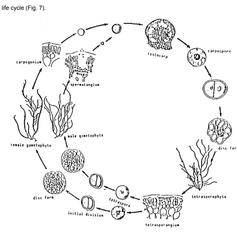

Fig. 7. Life history of *Gracilaria*

Mature tetrasporophytes (2n) produce (meioses) tetrospores, some of which develop to male gametophytes (n), while others grow to female gametophytes. The male mature gametophyte forms spermatangia, the female forms carpogonia. After fertilization and cystocarps are formed on the female plant, carpospores (2n) are released and develop to tetrasporophytes again.

The vegetative tetrasporophyte, male gametophyte and female gametophyte have no obvious difference. Wild *Gracilaria* collected consist of tetrasporophytes, spermaphytes, and carposporophytes in different stages, respectively.

Gracilaria tenuistipitata v. *tenuistipitata* is the dominant species of some natural *Gracilaria* beds. The yield and quality of phycocolloid from this species is high. Spores adhering experiments have been carried out on how to efficiently protect and enhance those resources.

A 300 m^2 protected experimental ground was set off in the natural beds. Triplicate of 1 m^2 nets were settled in the ground every half month. The experiment lasted one year. Observations and studies were made on the growth condition of the wild plants in the bed and of those adhering to the nets, the development of sporelings, the rapid growth period, and the duration of spores discharge. The results are presented below:

Except July and August, mature tetrasporophytes and mature male and female gametophytes were found the year round, and spores were adhering to the nets. No spores were found adhering to nets placed in July and August until when the temperature was falling.

The development of tetrasporophytes was similar to that of the gametophytes. Cell division occurred 15 minutes to one day after the adhesion of spores. After 2~7 days, hemispheres were formed and then, discs arose from hemispheres. The center of the discs began to arch by the end of 10 days, and one or more erect fronds occurred gradually. The whole process generally took more than 30 days.

After adhering for 40~70 days, the sporelings grew to 2 mm in length, and could be seen with the naked eye. The duration of the process differed with seasons, from January to April it took 60~70 days, in other months about 40 days.

November to April was the rapid growth season, when there were more and larger (30~60 cm long) thalli in the bed and nets. In May, when the water temperature rose to 29°C, the thalli stopped growing. In June, thalli decayed from their apex to the basic portions of plants and disappeared finally, the larger plants first and the smaller ones later. By the end of June, only small matured thalli could be found (the smallest matured fronds were only 1 cm long).

When seawater temperature reached 31°C, no old thalli could be found in the bed or the nets. In the meantime, some new sporelings yellow in color and less than 2 mm could be detected under microscope. They were almost not growing in July and August, but recovered normal color and started growing well again when temperature dropped.

3. EFFECTS OF ENVIRONMENTAL FACTORS ON THE GROWTH OF GRACILARIA

Studies on *Gracilaria* are of special interest because of its increasing market value as a source of agar, its importance in human diet and as food in the cultivation of abalone. As the supply of *Gracilaria* from the natural population has not substantially increased, *Gracilaria* is cultivated to supplement harvests from natural populations. The commercial beds of *Gracilaria* in China are so limited that the supply is unstable and can not meet the ever-increasing demands.

There are two varieties of *Gracilaria tenuistipitata* along the coast of south China: *G. var. tenuistipitata* and the faster-growing *G. var. liui* adaptable to brackish seawater. *G. tenuis.tipitata* var liui is, thus, the most common species under pond cultivation in Guangxi and Hainan Provinces. Unfortunately the growth rate sometimes fluctuates greatly. The effect of temperature, salinity, nitrogen, culture density and depth on the growth were investigated between April 1985 and March 1986 in outdoor ponds. The purpose of the study was to determine the relationship between the main environmental factors and the growth rate, so that knowledge gained could be applied to stabilize and increase pond production.

Temperature

Triplicate samples of 500 g 5~10 cm long *Gracilaria* fragments were cultured at 0.5~1 m water depth in a one square meter rectangular net cage and weighed every 15 days. Changes in the wet weight were used to calculate specific growth rates from the formula $y = \left[\left(\frac{N_t - N_0}{N_0}\right)^{\frac{1}{dt}} - 1\right] \times 100\%$ where N_0 is the initial biomass and N_t is the final biomass at time (t). Algal densities were maintained at desired levels by harvesting at the proper growth period.

Fig. 8. shows the year-long growth of *G. tenuistipitata* v. liui in the experimental field. The mean daily growth rate was 2.4%. This means the weight was doubled each month. March, April, and May and September, October, and November were months of maximum growth when mean daily growth rate was 3.3%. In winter and summer it was only 1.5%. In the course of the experiment, temperature ranged from 15~32°C. The recorded growth rates were lowest at 15°C (lowest temperature) and 32°C (highest temperature). Growth rate was over 2% from 20~30°C. Thus 20~30°C may be considered as the temperature range most favorable for growth.

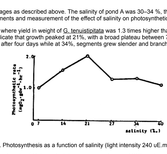

Fig. 8. Growth rate and sea water temperature

Salinity

Gracilaria were cultured in two ponds with different salinities by using similar net cages as described above. The salinity of pond A was 30~34 ‰, that of pond B was 24 ‰. Total nitrogen content of the two ponds was controlled to the same level by adding nitrogen fertilizer to pond A. Outdoor tank culture experiments and measurement of the effect of salinity on photosynthetic rate were concurrently conducted.

After one month of experimentation the best algal growth was obtained in pond B, where yield in weight of *G. tenuistipitata* was 1.3 times higher than that in pond A. This clearly shows that salinity is an especially important factor influencing the growth of this alga. Results obtained from tank culture (Table 1) indicate that growth peaked at 21‰, with a broad plateau between 7~ 27‰. Much lower or higher salinity was unfavourable for growth. At 3% salinity decolorization of apical segments occurred within two days and necrosis occurred after four days while at 34‰, segments grew slender and branches were softer, while at 47‰, segments decolored after 2 weeks.

Fig 9. Photosynthesis as a function of salinity (light intensity 240 uE.m^{-2}.sec^{-1})

Effect of salinity on the photosynthetic rate is shown in Fig. 9. Maximum photosynthetic rate of *G. tenuistipitata* v. *liui* was obtained at 21‰ and was not markedly affected when the salinity was reduced to 14% or increased to 27%.

Table 1. Growth in weight as a function of salinity.

Salinity (%)	Fresh weight (g)	
	1 week	2 weeks
3	---	---
7	28.3	29.5
14	27.1	33.2
21	29.1	35.2
27	28.2	32.1
34	25.0	28.1
40	24.2	26.6
47	23.7	24.0

CHAPTER 9: THE ARTIFICIAL CULTIVATION OF GRACILARIA

THE METHODOLOGY OF CULTIVATION

Gracilaria species are tropical seaweeds. The need for Gracilaria increased further. Experiments on artificial cultivation have been carried out in many countries in recent years. The dates of artificial cultivation of the tropical species of Gracilaria are: Gracilaria gracilis (=Gracilaria verrucosa), Gracilaria lichenoides, Gracilaria corticata, Gracilaria debilis, Gracilaria dura, Gracilaria edulis, Gracilaria sjoestedtii, Gracilaria tenuistipitata and some others. The dates of artificial cultivation of the tropical species of G. Tikvahiae in Canada, great cultivation of Gracilaria lemaneiformis (=G. bailiniae) in Taiwan were reported by Chang Kangmann, left column of G. lemaneiformis in the total zone and the column of G. lemaneiformis in the total zone, respectively. Gracilaria lemaneiformis (=G. bailiniae) is a species of the temperate zone. Gracilaria lemaneiformis (=G. bailiniae) is a species of the temperate zone.

Prior to the formation of the tropical zone, Gracilaria, Gracilaria species, Gracilaria species, Gracilaria species

CHAPTER 10 PROPERTIES, MANUFACTURE AND APPLICATION OF SEAWEEDS POLYSACCHARIDES : AGAR, CARRAGEENAN AND ALGIN

PROPRIYA BANERJEE, PRIYA SHARMA, PRABHAT KUMAR SINGH, CHANDRAKANT MAI-SOM

TO AGAR : A NEW STUDY OF SEAWEED POLYSACCHARIDES

1. INTRODUCTION

1.1 Introduction

Agar was the first phycocolloid discovered and prepared as a purified extract. According to a Japanese legend the original manufacturing method of agar was discovered in the middle part of the 17th century, precisely in 1658. In Japan, the production of agar was a cottage industry for many years. The production was carried out in the winter because the temperature was low enough for the gelling and subsequent freezing of the extracted material. Production was also carried out in other Asian countries, such as China and Korea, but the production was not as extensive as in Japan. Agar was introduced to the Western world by Dutch merchants who had been in Japan and China.

Agar is the name of a seaweed and flavoured gum that has been known in the Orient for a long time. It is known as "Kanten" in Japan, "Tchoukou" in China and "Tchoukou" in Korea (Kanten). The name "agar" is Malayan and is used in the Western world. The Malayan word "agar" means "jelly" and was used to describe the product when it was introduced to the Western world. The Latin name for agar is "Agar Agar" and is used in the Western world and in Japan. The Japanese name for agar is "Kanten" and is used in the Orient. The Chinese name for agar is "Tchoukou" and is used in the Orient. The Korean name for agar is "Tchoukou" and is used in the Orient. The Malayan name for agar is "Agar" and is used in the Western world and in Japan.

Agar was also little known when North that could be a future reaction. Nestle first succeeded in using agar as the culture medium in solidifying gelatin. The trial was commercialized in Retail North. In 1940, Kirsch announced the use of agar in the production of canned meat products. The production of agar was also carried out in the United States, but the production was not as extensive as in Japan and other Asian countries.

Agar production by modern industrial processing techniques was initiated in 1941 in California, U.S.A. Agar production in Japan was carried out in the winter because the temperature was low enough for the gelling and freezing of the extracted material. The production of agar was commenced in Portugal and Spain as well.

Agar is a hydrophilic colloid and is used in the food industry as a thickening agent, a stabilizer, a gelling agent, and a texture agent. The agar market is expanding because using the mechanical freeze-thaw process.

The agar market is expanding because the production of agar using the mechanical freeze-thaw process has allowed the production of agar to be carried out in the winter because the temperature is low enough for the gelling and freezing of the extracted material.

The agar market is expanding because the fundamental principle of extraction and purification (freeze-thaw) of agar is similar to that found by Tanaka in the Japanese patent more than three hundred years ago.

1.2 Introduction

In 1940 Dr. Tseng defined agar as "the dried precipitate, gelatin like, non-nitrogenous material from Gelidium species, Gracilaria species, and related genus of the class Rhodophyceae". The term "agar" is Malayan and is used in the Western world and in Japan. The term "agar" is used in the Orient. The term "agar"

κ and ι-carrageenan in κ/ι family give the strong gel strength in the presence of K^+ and Ca^{2+}, respectively. All carrageenans in ι family do not gel regardless the case of the presence of 2-sulfate group in ι. It binds Ca-selective receptor, which are related to atrial action.

More recently a new type of carrageenan, ω-type, not belonging to above three families, was found in the hot water extract of Reposella verrucosa (Vegetinines), its structure being confirmed by using ^{13}C-NMR spectroscopy as shown in Fig. 11 and Table 12.

As shown in Table 12 the polysaccharides from Chondrus, Euchema cottonii, E. striatum, Hypnea sp., Fucellaria sp., etc are mainly composed of K-carrageenan, and those from E. spinosum, and Gigartina spp. are composed of κ- and λ-carrageenan respectively.

Up to date 12 types of carrageenans were identified from the polysaccharides of various carrageenophytes, indicating the complexity of the carrageenans. Among them only κ-, ι- and λ-carrageenans are of commercial significance.

These dissolved carrageenans are not present in the nature, but some are present predominantly in some molecules, which are mixed together, and some are connected together in the same molecular chains, i.e. in molecular hybrid.

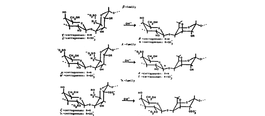

Fig. 11 The repeating (disaccharide) structures of different carrageenan types

Table 12 The repeating disaccharide residues in different carrageenan from various carrageenophytes

Family	Residue R-(1,3)-linked	Residue R'-(1,4)-linked	Type name	Source
D-galactose		D-galactose-6-sulfate	κ	Euchema spinosum
		D-galactose-2,6-disulfate	ι	Euchema striatum, Euchema farlesianum
		3,6-anhydro-D-galactose	κ	Euchema cottonii, Euchema striatum
		D-galactose-2,6-sulfate	ν	Lithotrix, Gymnogongrus
	D-galactose-4-sulfate	D-galactose	μ	Gigartina, Chondrus, Iridaea
		3,6-anhydro-D-galactose	ξ	Fucellaria, Euchema cottonii
		D-galactose-2-sulfate	λ	Gymnogongrus stolatus
		3,6-anhydro-D-galactose	ω	Reposella verrucosa
L-3,6-anhydrogalactose		D-galactose-2-sulfate	κ	Gymnogongrus, Chondrus, Euchema, Hypnea, Gigartina, Iridaea, Tichocarpus, Sarcopeltis
		D-galactose-2-sulfate	ι	Euchema, Gigartina
		D-galactose-2,6-disulfate	λ	Gymnogongrus, Gigartina
		3,6-anhydro-D-galactose-2-sulfate	ν	Tichocarpus, Gigartina
		D-galactose-2-sulfate	ξ	Euchema, Gigartina
	D-galactose-2-sulfate	D-galactose-6-sulfate	κ	Euchema, Gigartina
		D-galactose-2,6-disulfate	ι	Euchema, Gigartina
		3,6-anhydro-D-galactose-2-sulfate	ω	Reposella verrucosa
	3,6-O-1'-carboxyethylidene-D-galactose-2-sulfate	D-galactose-2-sulfate	κ	Gigartina, Reposella
	D-galactose-6-sulfate	D-galactose-2-sulfate	ω	Reposella verrucosa

4. Properties of carrageenans

The charged nature of the sugar units in carrageenan and their structural arrangement within the macromolecule render the carrageenans highly reactive chemically and account for their physical properties.

A. Solubility

The solubilities of carrageenans in various media are summarized in Table 13.

Table 13. Solubility of carrageenans

Medium	κ-carrageenan		ι-carrageenan		λ-carrageenan
Hot water	Soluble above 70°C		Soluble above 70°C		Soluble
Cold water	Na^+ salt soluble. From limited to high swelling of K^+, Ca^{2+} and NH_4 salt		Na^+ salt gives thixotropic dispersion		All salts soluble
Hot milk	Soluble		Soluble		Soluble
Cold milk	Gelling		Gelling		Soluble, even with thickening
Cold milk (Versicolan complexation)	Thickens or gels		Thickens or gels		Increased thickening or gelling
Concentrated sugar solutions	Soluble hot		Difficulty soluble		Soluble hot
Concentrated salt solutions	Soluble in cold and hot		Soluble hot		Soluble hot
Water miscible solvents	Insoluble in cold and hot		Insoluble		Insoluble
Organic solvents	Insoluble		Insoluble		Insoluble

Hot water: The carrageenan solution can be prepared with concentration up to 10% in hot water.

Cold water: Sodium salts of κ- and ι- carrageenans are soluble in cold water, while κ$^+$ and Ca^{2+} of κ-type and ι-type and salts are not soluble. Ca^{2+} salt gives thixotropic dispersion in κ-carrageenan solution system.

Cold milk: κ-carrageenan has the greatest ability to disperse in 5~10°C milk and thicken it without adding solubility salts. κ-carrageenan is insensitive to K^+ and Ca^{2+}. For κ- and ι-carrageenans, due to the higher content of 3, 6-anhydrogalactose, the gelling temperature of κ-carrageenan is higher than that of ι-carrageenan, and Ca^{2+} salts are constituents of the milk. Since κ-carrageenan is practically insoluble in cold milk, they may be used effectively for thickening and gelling if sesbaniform gum(Polygalactomannan) (TSIPH is added).

Concentrated sugar and salt solution: κ- and ι-carrageenans are soluble in hot sucrose solutions with concentrations up to 65% after heating to 70°C, while κ-carrageenan is not easily soluble in hot sucrose solution at any temperature. κ-carrageenan solution alone will tolerate high concentrations of electrolytes such as NaCl up to 20~25%, while ι-carrageenan will be salted out. In theory, κ-carrageenan is also soluble in concentrated salt solutions, but in practice, κ-carrageenan always contains some κ-component which makes them less salt-sensitive.

Water miscible solvents: κ-carrageenan is insoluble in water miscible solvents, such as alcohol, acetone, etc. The amount of solvent tolerated depends upon the molecular weight and type of carrageenan present. Pectin water miscible solvents make excellent dispersants for carrageenan. In general, these factors which lower the carrageenan dispersion more hydrophilic will increase its solvent tolerance. For example, the higher the sulfate content and the lower the molecular weight of the carrageenan, the greater the solvent tolerance.

Viscosity and molecular weight: The viscosity of carrageenan depends on concentration, temperature, the presence of other solutes and the type of carrageenan and its molecular weight. The viscosity increases almost exponentially with concentration. For the gelling carrageenan, the viscosity measurement is carried out at high temperature (e.g. 75°C) to avoid the effects of gelation, usually 1.5% of concentration being used, while for the non-gelling carrageenan, 5% solution is used at 20°C. The viscosity of κ-, ι- and λ-carrageenan at 1.5% concentration and 75°C are 5, 20 and 200 cps respectively. The viscosity of λ-carrageenan is very high. Commercial carrageenans are generally available in viscosities ranging from 5 to 800 cps (1.5% at 75°C). The salts lower the viscosity of carrageenan solutions by reducing the electrostatic repulsion among the sulfate groups. The solutions of carrageenans having viscosities >100 cp, displays the Newtonian flow, varying degrees of pseudoplasticity for sodium and lambda carrageenan, and thixotropic characteristics in the case of calcium kappa carrageenan. The latter is typified by a decrease in viscosity with increasing shear or agitation and return to normal viscosity at rest.

The viscosity of carrageenan sol increases with molecular weight (Fig. 12) in accordance with Mark-Houwink equation:

$$[\eta] = KM^a$$

where [η], the intrinsic viscosity, is defined as the limit of the ratio of specific viscosity to concentration (sp/C) on extrapolation to zero concentration. Intrinsic viscosity correlate closely with normal viscosity measured at 1.5% concentration. K and a are constants. The molecular weight of carrageenan can be calculated from the intrinsic viscosity. The molecular weight of κ-carrageenan from different seaweeds varies from 100,000 to 500,000. The molecular weight of carrageenan from food grade seaweed ranges between 150,000 to 500,000. The majority of carrageenan products have a molecular weight of about 250,000.

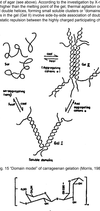

Fig. 12 Carrageenan viscosity as a function of molecular wt.

Average molecular wt.

A. Predominantly sodium salt of carrageenan
B. Predominantly calcium salt of carrageenan

Gelling and melting temperatures: Carrageenan gels like agar are thermally reversible in that they remelt when heated to a temperature higher than the gelling temperature. The gelling temperature of K-carrageenan ranges between 30°C and 65°C, and melting temperature about 35~80°C. The extent of hysteresis is dependent on the type of carrageenan: for Kappa it is the first one to gel and the last one to melt, while the gelling temperature of the ι-and λ-carrageenan, respectively, with the concentration added (Fig.13).

K-Carrageenan gel in the presence of K^+ is rigid and brittle and exhibits marked syneresis due to the higher content of 3, 6-AG and low content of sulfate and the hydrophobic nature, while κ-carrageenan gel in the presence of Ca^{2+} is elastic and gives low syneresis due to more hydrophilic.

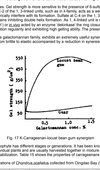

Fig.13 Carrageenan gel temperature of carrageenans

Gel strength: Carrageenans at concentration as low as 0.2% may form thermally reversible water gel in the presence of cation. Gel strength of carrageenans is determined with a gel tester (same as for agar) using 1.5% carrageenan solution. The gel strength of κ-carrageenan is much higher than that of ι-carrageenan and λ-carrageenan does not form gel. The consistency of carrageenan gels varies from hard and brittle to soft and elastic. Gels of κ-carrageenan tend to be more brittle than those of ι-carrageenan.

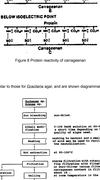

Fig. 14 Gelled effect on gel strength of carrageenan elasticity of cape jelong agar and κ-carrageenan and amount of 2-sulfated residues in the polymer

The gel strength of carrageenans from Euchema vs. varies from very weak (gel 20 g/cm²) to 800g/cm², depending upon the species of carrageenophyte and the condition of the alkali treatment (Table 14).

Table 14. The yield and properties of carrageenan extracted from Euchema vs. treated with different concentration of alkali (Shi et al, 1985)

Species	Concentration of alkali	Yield (%)	Gelling temperature (°C)	Freq. (%)	S. A.kg (g/cm²)
Euchema Gelatinae	Water	19.6	37	29.3	14.7
	0.5% NaOH	30.3	40.1	34.1	14.2
	1.0% NaOH	28.2	40.1	34.1	14.2
	2.0% NaOH	28.9	39.6	41.4	15.2
	Concentrated NaOH	29.2	40.1	41.4	15.2
Euchema cottonii	Water	25.1	40	32.1	16.8
	0.5% NaOH	28.1	40	32.1	16.8
	1.0% NaOH	28.2	40	34.1	16.4
	2.0% NaOH	28.2	39.6	32.8	17.2
	Concentrated NaOH	28.2	39.6	32.8	17.2

The gel strength and the yield of carrageenan in carrageenophyte exhibit seasonal variation. The investigation on the Indian carrageenophytes, Hypnea musciformis and G. salicornia shows that the yield varies parallel to the gel strength with the maximum in the month of December. The yield of κ-carrageenan is 30% and the gel strength is 1000g/cm² in December.

Gelation: The mechanism of gelation of κ- and ι-carrageenans is virtually similar to that of agar (see above). According to the investigation by X-ray diffraction and optical rotatory dispersion techniques it was demonstrated that the gel formation is attributed to the formation of double helices bundles. At temperatures higher than the melting point of the gel, thermal agitation overcomes the tendency to form helices and the polymer exists in solution as a random coil. As the sol cools, the polymer chains become increasingly ordered and at the gel point, the polymer chains have sufficient order to form helical segments which can cross-link to form the gel network. Further cooling increasing ordering of these 'domains' into a cohesive gel structure forming the junction zones in the gel. Cold ι-carrageenan solution forms double helices from different domains. Helix-helix association occurs only in the presence of cations (typically K^+ for κ- and Ca^{2+} for ι-carrageenan) which can suppress electrostatic repulsion between the highly charged participating chains by packing within the aggregate structure (Gel II).

Helix
Helix
Helix

CHAPTER III (Contd.)

3) ALGIN – A BROWN SEAWEED POLYSACCHARIDE

3. 1. Introductory information

Algin is a brown polysaccharide, a common constituent of cell wall in species of the brown seaweeds (Phaeophyceae). It is extracted by alkaline solution.

Algin was discovered in 1881 by an English chemist, E. C. C. Stanford, who obtained a viscous mucilage by extracting Laminaria stenophylla (Laminarazeae) with alkali. He called the product 'algic' his further found that, if a mineral acid was added to the viscous solution, a fibrous material was precipitated. Stanford's work was the first to make an 'algin product' and he then started commercial investigations on the potential and possible uses of the product. Later, he started manufacturing algin on a commercial scale.

In 1927 Thornton Kelp set up a company to produce alginate in San Diego, U.S.A. which in 1929 merged with Kelco Company, Production in the United Kingdom was initiated by Algitate Industries Ltd. during the period 1929-1931 and was taken over by Kelco Company in 1931. Production in Norway started in 1932 by A/S Alginate Fabrik. In 1934 Kelco Company produced 25% of the world's alginate. The main alginate producer in Norway in 1937 was Norsk Algin, followed by Companies in Japan and Greece.

In China algin production was initiated in 1957 in Qingdao from Sargassum species. From the late 1960's Laminaria japonica has been used for algin production in place of Sargassum, since Laminaria was cultivated on a large scale.

Algin is the term 'algin' is widely used in commerce, referring specifically to the sodium salt of alginic acid. Alginate is the common name for alginic acid and its salts, including the insoluble alginic acid and the soluble sodium, potassium, ammonium and organic amine salts. Commercial alginates including sheet materials, alginate fibers, alginate flakes, alginate powder and alginate granules are known as the alginate product alginate.

3. 2. Habitat/ecology and algal production

The algae used in commercial alginate production is extracted from the species of Macrocystis, Laminaria, Ascophyllum, Durvillaea, Sargassum, etc. The main commercial sources of alginate are shown in Fig. 22 and the main countries using them are shown in Table 10.

Table 10. Algal alginate/algin and the countries using them

Alginate/algin	Countries using algin production
Macrocystis pyrifera	North America	U.S.A
Laminaria digitata	Europe, Asia	Norway
Ascophyllum Nodosum	North Atlantic	Canada
Durvillaea antarctica	Southern Hemisphere	Chile
Laminaria hyperborea	Scottish, Ireland	England
Laminaria japonica		
Sargassum fulvellum	Yellow Sea, Korea	China
Sargassum fusiforme	Japan	Japan
Lessonia nigrescens	Japan, Korea, South Africa	
Ecklonia maxima		
Sargassum spp.	Mexico, S.E.Asia	
Table 11 indicates the estimated world production of dried alginate/algin in 1980.

Fig. 23 Distribution map of alginate

Table 11 Estimated world production of dried alginate/algin (1980)

Country	Production (Tonnes)
Canada	2,000
Chile	2,000
Denmark	1,000
France	1,000
Iceland	1,000
Ireland	1,000
Japan	4,000
Mexico	1,000
Norway	6,000
Peru	1,000
Spain	1,000
United States	6,000
Total	26,000

3. Chemistry of alginate

3. Seaweed variations in the content and structure of alginate

Fig. 24 The seasonal variation in the content and structure of Laminaria japonica

The content and viscosity of alginate in the brown seaweed varies with the seasons, usually the content increasing from little amount of young algae to the maximum at the mature stage, and its viscosity approximately changing in the opposite way to the content. The seasonal variation in the content of Laminaria japonica is shown in Fig. 24 (Kawai and Kitani, 1962). This study has been used for alginate production in the small scale and the former is using algae from Laminaria japonica.

Fig. 25 The seasonal variation in the content and structure of Sargassum spp.

Since Stanford discovered the algin and in 1881, M. Mirkil through huge amount of research work on the chemical components by ton of seaweeds, it has been verified that algins exist as a linear polymer (composed of 1, 4-linked β-D-mannuronic acid residues and α-L-guluronic acid residues) and that algins are composed of three kinds of polymeric fractions (mannuronic acid, guluronic acid and alternate). The ratio of the three fractions is different according to the species of brown seaweeds. Sodium alginate dissolves in water and forms a viscous solution. The viscosity of 1 % solution of sodium alginate is 20-1,000 cps and it is pH dependent and very differential as a function of the molecular weight. The viscosity of alginate solution is stable in the pH range of 6-9 and it is unstable in the pH range of 3-5. Alginate is a polyelectrolyte and it forms a salt with a cation such as Ca, Mg, Na, K, Fe, Al, Cu, Co, Ni, Zn, Pb, Cd, Sr, Ba, Mn, Cr, Sn, Ag, Hg