IMPROVEMENT OF YIELD AND QUALITY OF AGAR FROM GRACILARIA EDULIS (GMELIN) SILVA

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MUMBAI-400 061

by

CHANDRASEKHARA RAO·A
(Mari. 55)

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
COCHIN - 682 014
INDIA.

JUNE 2001

DEDICATED
TO MY
BELOVED PARENTS

भा कृ अनु प
I C A R

Phone: (Off) : 394867/.....Ext 391407
Telegram : CADALMIN EKM
Telex : 0885-6435 MFRI IN
Fax : 91-484-394909
E-mail : mdcmfri@md2.vsnl.net.in

केन्द्रीय समुद्री मात्स्यिकी अनुसंधान संस्थान
(भारतीय कृषि अनुसंधान परिषद)
पोस्ट बॉक्स सं 1603, एरणाकुलम, कोचीन-682 014

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
POST BOX No. 1603, ERNAKULAM, COCHIN-682 014

Dated 30 June, 2001

CERTIFICATE

Certified that the thesis entitled **"IMPROVEMENT OF YIELD AND QUALITY OF AGAR FROM *Gracilaria edulis*(Gmelin)Silva"** is a record of independent bonafide research work carried out by **Mr CHANDRASEKHARA RAO A.** during the period of study from September 1999 to August, 2001 under our supervision and guidance for the degree of **Master of Fishery Science (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

Major Advisor/Chairman

(P. Kaladharan)
Scientist (Senior Scale)
Fishery Environment and
Management Division

Advisory Committee

(Reeta Jayasankar)
Scientist (Senior Scale)
Fishery Environment and
Management Division

(C. P. Gopinathan)
Senior Scientist
Fishery Environment and
Management Division

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सारांश

लाल शैवाल *ग्रेसिलेरिया इडुलिस* (जेमलिन) सिलवा से प्राप्त एगार का उत्पादन तथा गुणता बढ़ाए जाने के लिए इस अध्ययन में आलकली / आसिड के अनुकूलतम स्तरों तथा निष्कर्षण तरीकों में परिवर्तन किया गया. सूखे शैवालों को 2 घंटों तक पानी में रखकर अनुकूलन करने पर एगार का उत्पादन बढ़ गया बल्कि कोलोइड के जेल होने की क्षमता नहीं बढ़ गई. पानी में 12 घंटे रखने पर जेल होने की क्षमता द्रवण तापमान बढ़ गए और सलफेट की मात्रा कम हो गई लेकिन एगार का उत्पादन कम हो गया. 0.5 N तथा 1.0 N सोडियम हाड्रोक्साइड में रखे गए *जी. इडुलिस* में 3.0 N सोडियम हाड्रोक्साइड विल्यन जोड देने पर उत्पादन क्रमश: 44.91 % और 49.26 % तक बढ गया और एगार के सल्फेट के स्तर में घटती (1.5% w/v) भी हुई. लेकिन उबालते वक्त सोडियम हाड्रोक्साइड जोड देने पर आलकली में रखे गए *जी. इडुलिस* की जेल होने की क्षमता नहीं बढ़ गई . हाइड्रोक्लोरिक आसिड में रखे गए *जी. इडुलिस* को उबालते वक्त विभिन्न सान्द्रता में सोडियम हाड्रोक्साइड जोड देने पर उत्पादन और एगार की गुणता में बढ़ती नहीं हुई बल्कि एगार का हाइड्रोलिसिस ज्यादा बढ़ गया. निष्कर्षण के पहले 11 घंटे पानी में रखे गए *जी. इडुलिस* को 2.0 - 3.0 N सोडियम हाड्रोक्साइड में एक घंटे तक 80 ° C तापमान में उबालने का परीक्षण उच्चतम उत्पादन (14.16%) , अधिकतक जेल क्षमता (291 ग्रा / से मी 2), न्यूनतम सल्फेट (0.732%) तथा एगार का उच्चतम द्रवणांक (99° c) प्राप्त होने का अनुकूलतम निष्कर्णध माना गया.

ABSTRACT

Use of optimum levels of alkali/acid and other modifications in extraction methods were determined to increase the yield and quality of agar from red seaweed *Gracilaria edulis* (Gmelin) Silva. Presoaking of dry weeds in water for 2 hrs increase the yield but did not improve the gel strength of colloid. Presoaking in water for 12 hrs increased considerably gel strength, melting temperature and reduced sulfate content but not the yield. Addition of 3.0 N solution of NaOH to the 0.5 N and 1.0 N NaOH presoaked *G.edulis* on boiling resulted in yield of 44.91% and 49.26% respectively and also reduction of sulfate levels in agar (1.5% w/v). However addition of NaOH during boiling did not improve gel strength of agar from alkali presoaked *G.edulis* . Addition of different concentrations of NaOH during boiling of HCl presoaked *G.edulis* showed improvement neither in yield nor in the quality of agar and also resulted in hydrolysis of agar. Pretreatment of 2.0-3.0 N NaOH at 80°C for 1 hour to *G.edulis* presoaked for 11 hrs in water proved to be most ideal and optimized extraction to obtain higher yield (14.16%), maximum gel strength (291 g/cm^2), lowest sulfate (0.732%) and highest melting point (99°C) of agar.

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INTRODUCTION

Agar is the major constituent of the cell wall polysaccharide of certain red algae (Rhodophyceae), especially the members of families Gelidiaceae, Gelidiellaceae and Gracilariaceae. " Agar – Agar "is the Malay word for a gelling substance extracted from *Eucheuma* sp, now known to be carrageenan (Kaladharan & Kaliaperumal, 1999). The term agar is now generally applied to the algal galactan, which have agarose, the disaccharide agarobiose as their repeating units.

Agar is the first phycocolloid identified and isolated from red seaweeds as a purified extract. In 1658 a Japanese innkeeper Tarozaemon accidentally discovered the method of producing dry agar (FAO, 1990). The world demand for agar has increased rapidly in recent years (Buendia, 1998) and presently the supply of agar containing seaweeds are being greatly reduced. It is estimated that the current production of agar in the world is about 7500 – 10,000 tonnes/year and India's share is hardly 130 tonnes/year (Devaraj *et al.*, 1999). Studies over the past 30 years have revealed that the basic structure of agar is regularly alternating sequence of 3 linked β-D-galactopyranose and 4 linked 3, 6-anhydro – α – L-galactopyranose (Araki, 1966). This repeating pattern may be masked by the presence of L-galactose, 4,6-0-(1-carboxyethylidine) – D galactose, Sulfate hemiesters and 0 – methyl substituents in various positions on galactose units (Duckworth and Yaphe, 1971).

Gelidium sp is the most preferred commercial agarophyte due to the high quality of its agar. It has become unavailable to industries because of depleting wild stock due to over exploitation. Easy availability of both wild and cultured *Gracilaria* sps, has led to *Gracilaria* becoming the principal source of agar world wide (Critchley, 1993).

Approximately 60% of the world's present production of agar is derived from *Gracilaria* sp (Durairatnam, 1987). Chile is the largest producer of agar (FAO, 1996). The chemical and physical properties of commercial agar prepared in Japan, Newzeland, U.S.A., US.S.R., and Spain Vary greatly with the

agarophytes used. Based on the quality, agars have been classified into two types: namely,

(i) Food Grade Agar; and
(ii) Bacteriological agar.

Food grade agar contains about 10 – 15% essentially neutral agarose, 40% charged agarose and 40 – 50% non-gelling sulfated agar molecules. Bacteriological agar with about 3% sulfate and it should contain high concentrations of agarose. Agarose is responsible for gelling power (Armisen and Galatas, 1987). Bacteriological agar is expected to contain gel strength above 250 gm/cm^2. Mostly produced aga r in India is of food grade agar. Indian demand for food grade agar is met primarily by domestic production (Coppen, 1989) but 14 – 16 tonnes of higher-grade agar is imported annually. Japan is the major exporter to India. The total agar production in India ranges between 110 and 132 t, utilizing about 880 – 1100 t of dry weed per year (Kaladharan and Kaliaperumal, 1999).

Generally *Gracilaria* sp. yields low quality agar due to high sulfate concentration and therefore they are called 'agaroids' or ' gracilariagum' (Craigie, 1990). It is well known that the quantity and quality of phycocolloid varies not only among species (Cote and Hanisak, 1986), but they are also influenced by environmental factors (Craigie and Wen, 1984), seasonal variations (Oza, 1978; Lahaye and Yaphe 1988; Robledo *et al.*, 1997; Freile – Pelegrin *et al.*, 1999), and extraction methods (Craigie and Leigh, 1978; Armisen and Galatas, 1987). In *Gracilaria* sp, alkaline pre-treatment is necessary to change the L-galactose 6-sulfate into 3,6 – anhydro – L – galactose to increase the extract gel strength (Armisen and Galatas, 1987). Various workers had reported the extraction of agar in India from seaweeds by the treatment with dilute mineral acids (Bose *et al.*, 1943; Thivy, 1958; Kappanna and Rao, 1963).

At present food grade agar produced in India fetches about Rs.300 – 350 / Kg, whereas the bacteriological agar is sold at Rs.800 – 1000 / Kg. making quality of agar the sole criteria for its price value. Quality of agar is

decided by the gel strength and sulfate content. Gel strength is the capacity of gel to hold the substance on surface without breaking surface of gel. Hence any value addition to the indigenously produced agar such as sulfate content reduction and gel strength increase, not at expense of yield will definitely make one industry economically viable and can save foreign exchange by limiting the import of bacteriological agar. Here an attempt has been made to increase the gel strength and to improve the quality of agar with chemical manipulation from *Gracilaria edulis* ,the most common agarophyte in Indian agar industry.

REVIEW OF LITERATURE

2.1 SEAWEEDS AND THEIR UTILIZATION

Seaweeds are one of the commercially important renewable marine resources that belong to the primitive group of non-flowering plants which grow submerged in intertidal and shallow waters in the sea, and also in brakishwater estuaries (Umamaheswara Rao, 1969). Based on morphology, cell wall and pigment composition, seaweeds are classified into green, red and brown seaweeds (Chapman,J.V., 1970).

The red algae such as *Gelidiella, Gelidium, Pterocladia* and *Gracilaria* yield agar. Some other red algae viz. *Hypnea* sp, *Eucheuma* sp, *Chondrus* sp, and *Gigartina* sp are the major source for the production of carrageenan. Algin is obtained from brown algae like *Sargassum* sp., *Turbinaria sp., Hormophysa sp., Cystoseira sp., Laminaria sp., Undaria sp.* and *Macrocystis sp.* (Anon, 1987). These phycocolloids are used as gelling, stabilizing and thickening agents in food, confectionery, pharmaceuticals, dairy, textile, paper, paint and varnish industries etc. Apart from these, products such as mannitol, Iodine, Laminarin and Fucoidin are also obtained from marine algae (Kaliaperumal. 1993; Kaliaperumal *et al., 2000*). Many protein rich seaweeds such as *Ulva, Enteromorpha, Caulerpa, Codium, Monostroma* (Green Algae); *Sargassum, Hydroclathirus, Laminaria, Undaria* and *Macrocystis* (Brown algae); *Porphyra, Gracilaria, Euchecuma, Laurencia* and *Acanthophora* (Red algae) are used as human food (Anon, 1987). In countries like Japan, China, Korea, Malaysia, Philippines and other Southeast Asian countries in the form of soup, salad, curry etc. The food value of seaweeds depends on their protein, minerals, trace elements and Vitamins present in them (SEAFDEC. 1998).

Seaweeds are utilized in different parts of the world in diversified fields such as animal feed, fertilizer for land crops etc. (Deve et al., 1977). According to Sreenivasa Rao et al. (1980), Seaweeds are used as a source of energy. In presence of methane bacteria, biogas production from *Ulva* was accelerated.In India freshly collected cast ashore seaweeds are used as manure for coconut plantation either directly, or in the from of a compost especially in coastal areas of TamilNadu and Kerala (Umamaheswara Rao, 1972).

According to Chennubhotla (1996), seaweeds manure was superior to the conventional organic (farm yard) manure. The high amount of water soluble potash, other minerals and trace elements present in seaweeds are readily absorbed by plants and they control deficiency diseases. According to liu, sijan and the co-workers (1997), *Gracilaria* and prawn polyculture practice improve water quality to some extent and prevent bacterial disease of prawn. Use of *Gracilaria* as biofilter in polyculture practices is indeed two way beneficial, as it will not only helpful as biofilter but also useful to lift economic standards of prawn farmers through harvesting. Cultivation of *Gracilaria parvispora* in shrimp farm effluents enhanced growth of algae (Nelson et al., 2001). Seaweeds rich in iodine such as *Aspargopsis taxiformis* and *Sarconema* sp can also be used for controlling goitre disease (Cheunubhotla, 1996). The trace elements and growth hormones present (Kaladharan and Sridhar, 1999) in the liquid seaweed fertilizer act as growth promoters and increase the yield of crop plants by 20-30%.

2.2 SEAWEED DISTRIBUTION AND RESOURCES IN INDIA:

More than 10,000 species of marine algae have been reported from all over the world. Total world production of seaweeds is estimated to 6-7 million tonnes of which nearly 90% comes from Asia pacific region (Hurtado-Ponce, A., 1997). According to FAO data base (1998) out of 7 million tonnes of seaweeds, 4 million tonnes are brown seaweeds and 1.9 million tonnes are red seaweeds and rest of them are green algae. In India luxuriant growth of several species of green, brown, and red algae occur along the south east coast of TamilNadu, from Rameswaram to Kanyakumari, Gujarat, Lakshadweep and Andaman Nicobar islands. Fairly rich seaweeds beds are present in the vicinity of Bombay, Karwar, Ratnagiri, Goa, Varkala, Vizhinjam, Vishakapattinam and in coastal lakes like Pulikat and Chilka (Umamaheswara Rao, 1969; Chennubhotla, 1996; Kaliaperumal, 1993). About 700 species of marine algae have been recorded from different parts of Indian coast including Lakshadweep and Andaman-Nicobar islands, of these nearly 60 species are commercially important seaweeds (Umamaheswara Rao, 1969; Kaliaperumal et al., 2000). From the seaweed resources survey carried out in the intertidal and shallow water areas of east and

west coast and also Lakshadweep archipelago, so far by the C.M.F.R.I, and other research organisations such as C.S.M.C.R.I and N.I.O. it is estimated that total standing crop of all the seaweeds in Indian waters is more than one lakh tonnes (wet weight) consisting of 6000 tonnes (wet weight) of agar yielding seaweeds, 16000 tonnes (wet weight of algin yielding seaweeds (alginophytes), and the remaining quantity of edible and other seaweeds (Devaraj et al.,1997).

2.3 SEAWEEDS AND PHYCOCOLLOIDS:

Phycocolloids extracted from seaweeds have greater significance; phycocolloids refer to those polysaccharides extracted from both fresh water and marine algae (Armisen and Galatas, 1987). Polysaccharides extracted from marine red and brown algae, such as agar, carrgeenan and algin are economically important and have commercial significance, Since these polysaccharides exhibit high molecular weight, high viscosity and excellent gelling, stabilizing and emulsifying properties (Ji Minghou, 1990).

In India seaweeds are now used mostly as rawmaterial for the production of agar and sodium alginate (Coppen, 1991). Estimated world production of agar was 7000 – 10,000 tonnes valued at US$ 200 million in 1989 (Hartado-Ponce, 1997) Japan is the major consumer (about 2000 tonnes per year) mostly coming from domestic production. U.S.A consumes 1000 tonnes per year, which is mostly (80%) supplied by Chile, Morocco, and Spain. The European union needs 1300 tonnes per year (Coppen, 1989).

The brown seaweeds are source of algin. China, Korea and Japan cultivate the brown algae for food, and not for algin. The U.S gets its alginate from kelp harvested from the wild in California, Mexico produces some, but the *Elnino* phenomenon adversely affects production (Coppen, 1991). Agar-agar is often used where firm gel is needed and algin for soft and viscous product (Chennubhotla, 1996). The word ' alginate ' is a generic term, meaning the various derivatives of alginicacid (Coppen, 1991). Alginicacid present in seaweed mainly as calcium salt, with lesser amount as magnesium, sodium and potassium salts (Anon, 1987). The most important commerical derivative of

alginicacid is sodium alginate. Other derivatives produced include the potassium, ammonium and calcium salts, propyleneglycolalginate and alginicacid itself (Kaliaperumal *et al.*, 2000). The ability of alginates to increase the viscosity of solution to which they are added in low concentration enable their use in a wide range of applications in the food and pharmaceutical industries (Coppen, 1991).

In India algin is primarily used as a thickening agent in textile and printing industries (Kaliaperumal, 1993). The food industry alginates are used in the manufacure of a multitude of dairy, bakery, meat and other products, which take advantages of their thickening, gelling and stabilizing properties (Coppen, 1991; Kaliaperumal, 1993). Carrageenan is extracted from *Kappaphycus* and *Eucheuma*, which are cultured in the Philippines, Indonesia, Fiji, Micronesia and China (Hurtado – Ponce, 1997). Other sources of carraneenan are *Chondrus* and *Gigartina, Laurencia* (Kaliaperumal, *et. al.*, 2000*). Laurencia papillosa* can be used profitably as raw material (Doshi,*et al.*, 1987) for production of carrageenan as this contains carrageenan. *Chondrus* and *Gigartina* are abundant in Canada and Atlantic coasts of Europe (Pillay, 1990). Three types of carrageenan used commercially are:

i. Kappa carrageenan: - a hard gelling colloid extracted from *Kappaphycus.*
ii. Iota carrageenan: - a soft gelling colloid from *Eucheuma.*
iii. Lambda carrageenan: - a non-gelling colloid extracted form *Kappaphycus* (FAO, 1990.)

2.4 SEAWEED INDUSTRY IN INDIA AND WORLD:

Seaweed industry in India is totally based on the natural stock of agar yielding red seaweeds such as *Gelidiella acerosa* and *Gracilaria edulis* and algin yielding brown seaweed species such as *Sargassum* and *Trubinaria* (Coppen, 1991). Agar and sodium alginate are the phycocolloids produced in India. There are 40 seaweed-processing units, of which 22 produce agar. India produces 110 – 132 tonnes of dry agar annually utilizing about 880 – 1100 tonnes of dry agarophytes, and 360-540 tonnes of algin from 3600 – 5400 tonnes of dry alginophytes (Kaladharan and Kaliaperumal, 1999).

In India although, utilization of seaweeds for the extraction of soda ash, alginicacid and iodine started during the second world war period, production of agar started in 1966 (Kaliaperumal, 1997); seaweeds exported until 1975 when the Government of India banned the export of seaweeds (Silas, *et al.*, 1987; Devaraj *et al.*, 1997) in order to meet the requirement of local agar industry.

Import of agar in 1987 into Thailand was valued at Bht. 112.9 million mainly come from Japan, but there has been increase from 94 tonnes to 134 tonnes in the agar import from Chile in recent years. Malaysia imports of agar have been fairly consistent at around 250 tonnes per annum. Indonesia import agar from a variety of sources, with Asian and South American countries. India's demand for agar is met primarily by domestic production, but small amount of bacteriological agar is imported (Coppen, 1989).

Agar producers in India follow a simple method of agar extraction (Armisen and Galatas, 1987). In this they boil the dry weed, the hot extract is filtered, cooled, freeze thawed, bleached and dried in the sun. The agar is marketed either in strips or as powder (Coppen, 1991). *Gelidiella acerosa* yields industrial grade agar, where as *Gracilaria* yields food grade agar (Ji Minghou, 1990). All over the world agar is produced by two main processes:

i. Freeze thaw
ii. Press dehydration

The main steps in processing of agar from agarophytes may be summarised as:

i. Extraction of agar with hot water
ii. Filtration to eliminate the weed residue
iii. Concentration and purification (Freeze thaw or gel press)
iv. Drying

The main advantages of gel press / press dehydration process is that it omits the freeze-thaw cycle, and the final product is always in powder form.

In freeze–thaw method, square agar or strip agar is obtained (Ji Minghou, 1990). Algin is manufactured as sodium alginate at the cottage industry level through alkali deposition, precipitation by acidification, centrifuging, drying etc. (FAO, 1990). *Sargassum* and *Turbinaria* are the two main raw materials, used in algin industry. *Sargassum* is preferred over *Turbinaria* as the quality and quantity of algin yield are better from the former (Coppen, 1991). Most of the units in India are capable of producing 20-30 t/yr and total algin production in India is 360-540 t/yr. (Coppen, 1991; Kaladharan and Kaliaperumal, 1999). Other wellknown phycocolloid extracted from seaweeds is carrageenan, which is having commercial importance. World carrageenan production has showed a 4% yearly growth from 1978 to 1993. The five carrageenan markets are Europe (36%), North America (26%), Latin America (17%), Australia (13%) and Japan (8%) (Hartado-Ponce, 1997).

2.5 COMMERCIAL FEASIBILITY OF AGAR INDUSTRY:

Approximately 60% of world present production of agar is derived from *Gracilaria* sp (Durairatnam, 1987). Total agar production in the world touches 7000 tonnes, out of this 4200 tonnes derived from *Gracilaria* sp (Coppen, 1991). Six species of *Gracilaria* occuring in India have been reported to be potential source of agar (Desikachary, 1967). Capacity of agar production units in India varies between 500 and 800 kg dry agar / month using 4 - 5 tonnes of dryweed / month (Kaladharan and Kaliaperumal, 1999). They have also found that the material is purchased from the dealers at the price of Rs. 3000 – 4200 / ton for *Gracilaria* and Rs. 12,000 –16,000 / ton for dry *Gelidiella*. According to a recent survey on agar industry (Kaladharan and Kaliaperumal, 1999), the product of agar is sold weekly to the Bombay market for Rs. 200 – 250 / kg of food grade agar and Rs. 400 – 500 / Kg. of industrial grade agar. Approximately 10 lakh rupees may be required for the establishment of small agar plant capacity of 2 - 3 tonnes (Kaladharan and Kaliaperumal, 1999).

2.6 *YIELD*, GELSTRENGTH AND THEIR RELATIONSHIP TO QUALITY OF AGAR:

It is well known that the quantity and quality of the colloid varies not only among species (Cote and Hanisak, 1986), but they are also influenced by *environmental factor* (Craigie and Wen, 1984), *seasonal variations* (Oza, 1978), and extraction methods (Craigie and Leigh, 1978; Armisen and Galatas, 1987). Yanagawa (1936) offered the method of treating the agar solution or algae with alkali to improve the quality of agar of *Gracilaria* sp (FAO, 1990). `Araki'in 1956 showed the evidence, proving the heterogeneity of the agar by separating the agar into two different polysaccharides named agarose and agaropectin using the acetylation method (Duckworth and Yaphe, 1971).

The seaweed treatments prior to extraction are very important as they will condition to a high degree characteristics of the agar obtained (Armisen and Galatas, 1987). Cultured *Gracialaria edulis* used for extraction of agar under pressure for 2-4 hrs with addition of 0.5% KCl resulted slight increase in gel strength, gelling temperature and melting point (Thomas and Krishnamurthy, 1976). Agar studies in *Gracilaria bursapestoris* (Gmelin) Silva and *Gracilaria coronopifolia* indicated that agar yield in inversely related to the total nitrogen content of thalli and gelstength of agar (Hoyle, 1978). The fluctuations of gelstrength of the phycocolloid obtained from *Gracilaria corticata* showed a narrow range from 17-25 gm/sq.cm with a slight raise in the month of June, October, November and December when algae attain peak growth (Oza, 1978). The seasonal changes in the properties of phycocolloids in their study suggest that strong gels from *Neogardhiella baileyi* in the winter and in early summer months (Asare, 1980).

The agars extracted from *Gracilaria* are soft, non-rigid, plastic and intermediate and the strength of gelationcharacters could be improved considerably by alkali treatment (Whyte and Englar, 1980). Depending on season and life stages of algae, variations in gelstrength, yield and gelation of agars occur in *Gracilaria verrucosa* (whyte *et al.*, 1981.). *Gracilaria cylindrica* contain low sulfate content favourable for gelstrength and the species appear to thrive under condition that been conducive to the mass production (Doty and

Santos, 1983). Patwary and Vender Meer (1983) developed clones from wild *Gracilaria tikvahiae,* which are superior in both growth characterstics and agar quality to wild ones. It is reported that agar of the Micronesian species *Gracilaria edulis* contain the highest sulfate content and also a low gel strength (Nelson *et al.,* 1983). Friedlander and zelikovitch (1983) obtained the positive correlation between the specific growth rate of Gracilaria sp., *Pterocladia* sp., *Hypnea musciformis* and *Hypnea cornuta* and the phycocolloid content during the main growing season. Craigie and other (1984) established that alkali treatment modified agar from *Gracilaria sjoestedtii* to be the best agar produced from any *Gracilaria* sp. They also found that *Gracilaria* plants richest in nitrogen gave the highest gelstrength. However Christeller and Laing in 1989 noticed that significant increase in agar yield at low nitrogen concentration. Total agar content will be higher in algae grown in natural seawater than in enriched seawater (Rotem, et. al., 1986). Cote and Hanisak (1986) stated that melting temperature will have positive correlation with gelling temperature and agar yield but it will negatively correlate with the amount of sulfate present in agar. According to Miller and Furneaux (1987) desulfation of agar occurs relatively slowly in rapidly growing materials of *Gracillaria secundata.*

Seasonal changes have been observed in alginicacid content of *Cystoseira trinoidis* and *Hormophysa triquetra* (Kaliaperumal, *et. al.,* 1988). Durairatnam (1987) pointed out clearly that agar yield and gelstrength varies seasonally. Luhan (1992) observed distinct changed in agar yield & strength coinciding with changes in environmental conditions. There is a clear seasonality in the yield of agar from the *Champia nova. zealandier* with lower values in winter and higher in spring (Miller *et al.,* 1996). Calumpong *et al.,* (1999) observed a strong correlation between agar yield and nutrient levels in ambient water. Freile-Pelgrin *et al.* (1999) found correlation between agar yield and seasonal changes.

The quality of phycocolloids such as agar-agar and carrageenan is usually determined by their gelstrength. Vijayanthi and Rengasamy (1989) devised a new appartus for the determinaton of gel strength.

According to Ji Minghou (1990), *Gracilaria* contains a considerable amount of highly sulfated galactans varying with species, growing season and location. He proved that alkali pre treatment process for *Gracilaria* sp is an extremely important measure to improve the quality of agar product. Coppen(1990) observed that chemical treatment of agarophytes prior to extraction often produces a better agar in terms of quality of yield than one produced without such treatment. Alkali pre-treatment has been found to be most useful; particularly for *Gracilaria* sp. Miza mouradi-Givernand (1992) stated that sulfate content and 3,6 anhydrogalactose content in agar of *Gelidium sesquipedale* negatively correlated with gelling properties of agar. Mathew *et al.* (1993) performed alkali pre-treatment to *Gelidiella acerosa* and obtained significant increase in yield and quality compared to acid pre-treatment. According to Anong Chirapart and his team (1995) gelstrength of the crude agar extract increased with the increase in concentration of sodium hydroxide treatment. Freile-Pelegrin and Robledo (1997) found that alkali treatment dramatically improved the quality of agar in *Gracilaria cornea.*

According to Mirza Mouradi-Givernaud and co-workers (1999), agar rheological characters are influenced by reproductive stages of algae. It is established that the phycocolloid gel strength is more related to the mean polysaccharide chain length but not its chemical substitutes (Thiery-Givernaud et *al.*, 1999). Freile-Pelegrin (2000) studied extensively on the effect of storage time on gelstrength and melting temperature of agar and also reported that these are negatively correlated. However, Lian (2000) opined that the yield and properties of agar varied with extraction methods. It is learnt that lot of factors are responsible for determining the yield, quality of agar extracted from *Gracilaria spp.*

In the light of the above review the present investigation can fill the gap in information on

(i) Relationship between yield, gelstrength and sulfate content in agar extracted from the seaweed *Gracilaria edulis.*

(ii) The optimum requirement of alkali or acid as well as other post harvest manipulations to obtain maximum yield and improved quality of agar from *G. edulis.*

MATERIALS AND METHODS

3.1. Collection of seaweed:

Fresh seaweed *Gracilaria edulis* (Gmelin) Silva were collected from pamban area (78° 8' E, 9° 17' N) Mandapam (Fig. I). This marine red algae belongs to class: Rhodophyceae, Order: Gigartinales and Family: Gracilariaceae. Entire plants of *G. edulis*, growing attached to coral stones or sea grasses were hand picked during low tide period from the lagoon. They were washed with seawater and were transported to the laboratory.

3.2. Cleaning and Drying:

In the laboratory plants were washed thoroughly in running freshwater until the algae were free from sand and other dirt particles. Care was taken to remove epiphytes, other algae, shells and calcarious material from seaweed, whose presence may alter the yield and quality of agar. The washed and cleaned material was dried in sunlight for 5 – days, alternating with soaking over night until the sample become bleached and dried (Plate 1, 2, & 3).

3.3. Effect of Soaking:

To study the effect of soaking, weighed 20 gm of dry and clean *Gracilaria edulis* and 400 ml of potable water was added (1:20 w/v) and kept them soaked for 11 hr / 12 hrs as required prior to boiling. Control samples were boiled directly. Similarly weighed samples were soaked in dilute solution of HCl / NaOH at concentrations of 0.5N / 1.0N for 12 hrs prior to extraction.

In experiments with acid / alkali pretreatment, samples soaked in potable water for 11 hrs were transferred to 0.5N / 1.0N acid /alkali for 1 hr and were maintained at 80°c ± 2. Exactly after 1hr the acid / alkali solution was decanted from the beakers, washed well with fresh water and finally with distilled water to remove the traces of acid / alkali before boiling with distilled water.

1. Seaweed collection area

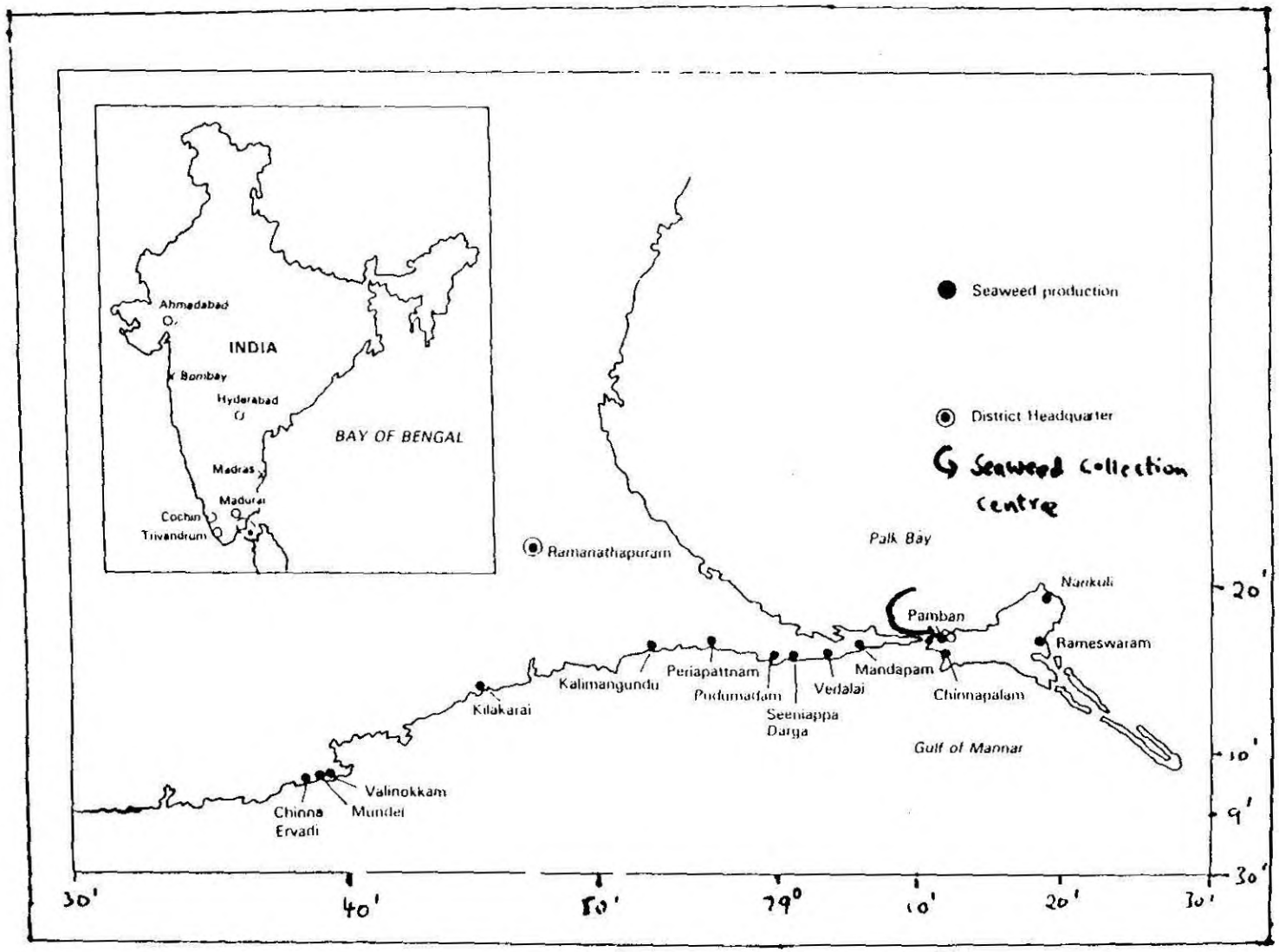

Plate 1. Washed seaweeds kept for Sun drying

Plate 2. Repeatedly washed and dried seaweeds

3.4. Extraction of Agar:

For the extraction of agar, method described by Matsuhashi (1971) was followed. Accurately weighed 20 gm of dry *G. edulis*, chopped into small bits. They were boiled separately in 1000 ml beakers after adding 400 ml distilled water and adjusting the p^H to 6.3 – 6.5 in an autoclave at 1 kg /cm^2 pressure for 2 hrs. The hot extract was recovered after filtration through muslin cloth and pressed in an expeller (Plate 4). The residue was re-extracted with 100 ml of hot distill water. The filtrates were poured into plastic trays and left to become gel at room temperature. The gel was streaked with iron scalpel. These gels were then frozen at -10°c for one day and later thawed. The thawed materials were placed on net screens kept slanting for sun drying for 3 days.

In the experiments of agar extraction from alkali / acid presoaked samples in acidic / alkaline medium, instead of distilled water. The solutions of 0.5N, 1.0N, 2.0N or 3.0N concentration of HCl / NaOH (400 ml) was added. After the extraction the p^H of filtrate was adjusted to 6.3 – 6.5 with addition of acetic acid / NaOH.

3.5. Determination of yield:

Agar samples after complete drying were weighed accurately to calculate percentage yield.

$$\text{Yield \%} = \frac{\text{wt. of agar}}{\text{Wt. of dry seaweed used}} \times 100$$

The dry materials were stored in polythene bags (Plate 5) for further physical and chemical analysis.

3.6. Determination of Gel strength:

Among physical properties of agar Gel strength and melting point were determined. To determine the gel strength and melting point 1.5% w/v agar solution was used. The solution was heated in a water bath. After complete

Plate 3. Completely bleached and dried seaweeds

Plate 4. Expeller (press)

dissolving of agar in water then the solution was allowed to form gel at room temperature for 12 hrs. Gelometer was used to determine the gel strength (*Funaki and Kojima*, 1951) of agar. Gelometer (Plate 6) was placed gently on the surface of the gel so that the cylindrical plunger (1cm^2 cross section) at bottom touched perfectly on gel. Weights were added gradually on the pan of gelometer until it broke gently the gel in 20 seconds. The weight was noted and taken as gel strength and expressed in gm/cm^2.

3.7. Determination of melting temperature:

For the determination of melting temperature, method suggested by Whyte *et al.*, (1981) was followed. The agar solution 1.5% (w/v) was heated in water bath. The solution was stirred using glass rod while boiling. After dissolution removed the solution from water bath and allowed to cool at room temperature. After the gel formation, the gel was again kept in water bath. Thermometer bulb was placed in the center of the gel and then the temperature was raised up to 50°C. Spherical glass beads (8 mm ϕ) were added one by one for every 1°C raise in temperature. The temperature was noted when the glass beads started sinking. That temperature was taken as melting temperature of the agar.

3.8. Determination of Sulfate content:

Sulfate content in agar sample was determined according to the following method (Ji Minghou, 1990).

Reagents:

A: Saturated Mg $(NO_3)_2$ in HNO_3

B: Glycerol: Ethanol (1:2)

C: Solution of NaCl – HCl [NaCl (67gm) + HCl (8ml) + H_2O (200 ml)]

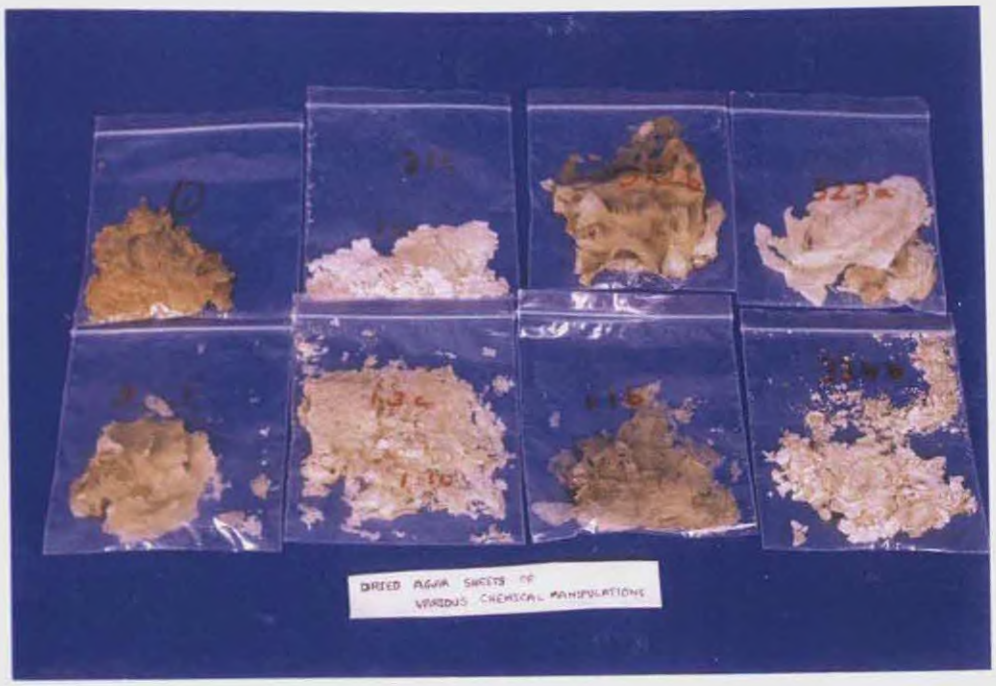

Plate 5. Dried Agar sheets of various chemical manipulation

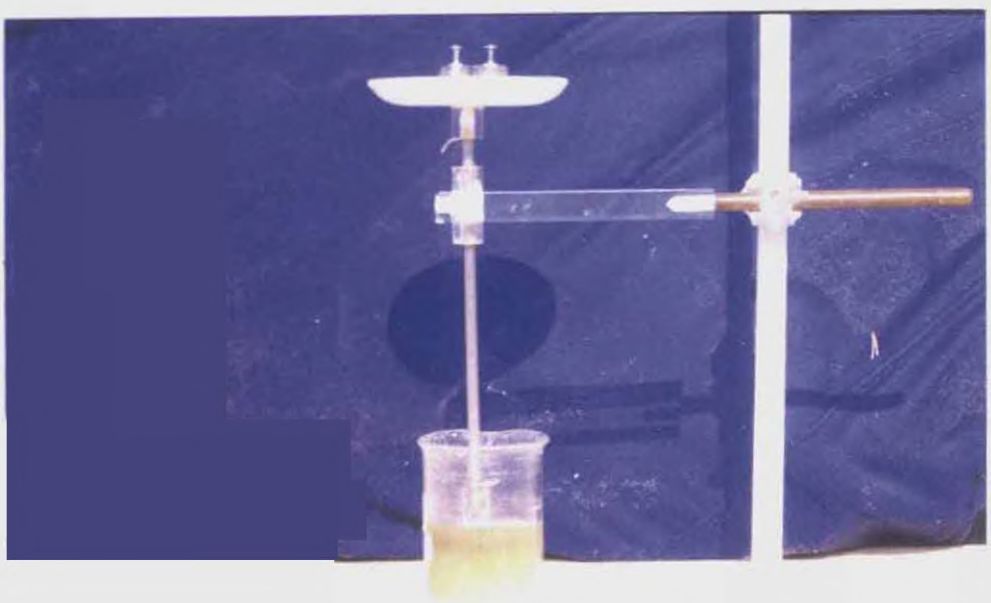

Plate 6. Gelometer

Weighed accurately 100 mg of dry agar sample using an electronic balance (Sartorius). In a crucible the sample was taken. To this 2 ml of reagent 'A' was added to the sample and it was allowed to evaporate in the hood. After complete evaporation of fumes these crucibles were placed in the muffle furnace at 400°C for 5 hrs. After 5 hrs, samples removed from muffle furnace were cooled at 80°C. After cooling of samples I.0N HCl was added to the sample. The solution was filtered through filter paper No.1 (Whatman).

The filtrate was collected in 50 ml volumetric flask. The filtrate was made up to 50 ml and transferred to 150 ml beaker. 5 ml of reagent 'C' and 10 ml of reagent 'B' were added to the filtrate. This mixture was stirred for 1 minute using a mechanical stirrer. Then to this solution 0.2 gm of $BaCl_2$ was added and stirred for 1 minute.

After settlement of solution the solution was again stirred for 15 seconds. Optical density was taken at 425 nm, using a spectrophotometer (GBC, UV/ VIS/911A). A standard sulfate solution was prepared by dissolving 0.1479 gm of Anh. Na_2SO_4 in one litre distilled water. From this standard sulfate solution, aliquots in the range of 0.5 – 7 mg/lit were prepared and the sulfate contents were determined as above.

A blank was prepared with 10 ml of reagent B as above. A standard graph was plotted and amount of sulfate content in the agar samples were calculated from graph.

3.9. Statistical analysis:

All the extractions, physical and chemical analysis were performed at least 5 times. Data were processed for statistical analysis by the one-way analysis of variance (ANOVA) and significant difference calculated using the SPSS/PC and MS EXCEL software.

RESULTS

I. EFFECTS OF PRE-SOAKING ON YIELD AND QUALITY OF AGAR:

Agar yield from *Gracilaria edulis* was varying between 10.10 – 11.61 % at different periods, of pre-soaking in potable water (Table-1). Agar yield increased slightly (11.44%) when pre-soaking period was reduced to 2 hrs. and agar yield reduced (10.10%) when pre-soaking period was prolonged to 12 hrs. Although agar yield in control (non soaked) treatment showed 11.61%, the gel strength obtained was low (54gm/sq.cm).

For different presoaking periods gel strength varied between 54-90gm/sq.cm. Gel strength of agar was the highest (90 gm/sq.cm) when presoaked in potable water for 12 hrs. (Table -I).

Agar yield and gel strength were significant at different pre-soaking periods ($P < 0.05$). ANOVA results showed that 2 hrs presoaking in potable water was preferable for agar yield, and 12 hrs treatment preferable for better gel strength. Agar yield and gel strength were negatively correlated ($r = -0.8898$). Sulfate content in agar ranged from 3.534 – 4.944% (Table-I). Sulfate was minimum (3.534%) in 12 hrs. presoaking period (Table-I).

Melting temperatures of agar were in the range of 66-77°C for different pre-soaking periods. Melting point was maximum (77°c) at 12hrs period of presoaking (Table-I). Correlation between sulfate content and melting point of agar obtained from various pre-soaking treatment were negatively ($r = -0.1828$) correlated (Table-I). Melting point and sulfate content were significant ($P<0.05$) at different presoaking periods. ANOVA showing 12 hrs treatment was the best for sulfate reduction and agar with high melting point ($P < 0.05$).

II. EFFECTS OF PRESOAKING IN DIFFERENT CONCENTRATIONS OF HCl ON YIELD AND QUALITY OF AGAR:

When *G. edulis* was presoaked in 0.5 N HCl the yield from *G. edulis* registered maximum (15.844%). When concentration of HCl was increased for presoaking, yield of agar also increased. At different concentrations of HCl pre-soaking agar yield varied in the range of 4.3761-15.844 % and the gel strength of agar was in the range of 21-60 gm/sq.cm (Table-II). At 0.5N HCl gel strength was 22 gm/sq.cm and sulfate content ranged from 4.182-4.71%. Sulfate content was minimum (4.18%) in 1.0N HCl presoaked sample (Table-II).

III. EFFECT OF PRESOAKING IN DIFFERENT CONCENTRATIONS OF NaOH SOLUTION ON THE AGAR YIELD AND QUALITY:

Presoaking of dry *G.edulis* in different concentrations of NaOH solution registered higher agar yield in 0.5N NaOH (12.4%) than in 1.0N NaOH (Table III).

Gel strength of agar ranged from 61-135 gm/sq.cm. Gel strength was maximum in 1.0N NaOH (135 gm/sq.cm). Agar yield and gel strength were negatively correlated ($r = -0.971$). ANOVA results showed that yield and gel strength were significant ($p<0.05$) in different concentrations of NaOH presoaking. Sulfate content in agar was in the range of 3.6442-4.796 %, minimum being 3.644% in 1.0N NaOH presoaked samples.

Alkali presoaking of G. edulis had improved melting point (82°C) of agar (Table-III). Sulfate content was showing significant ($p<0.05$) reduction with increasing concentrations of NaOH presoaking. ANVOA results indicated that 1.0N NaOH presoaking was ideal for sulfate reduction. Sulfate content and melting point were negatively correlated ($r = -0.09$)

IV. EFFECTS OF ADDITION OF NaOH DURING THE EXTRACTION OF AGAR FROM ALKALI PRE-SOAKED (0.5N) NaOH ON AGAR YIELD & QUALITY

Addition of different concentrations of viz. 0.5N, 1.0N, 2N NaOH or 3.0N NaOH during boiling to alkali presoaked (0.5N NaOH). *G. edulis* yielded agar in the range of 12.46 – 44.41% (Table IV). Agar yield attained higher levels (44.41%) in 3.0N NaOH addition during extraction. Agar yield was highly significant ($p<0.05$) with addition of different concentration of NaOH as mentioned above. ANOVA results revealed that addition of 3.0N NaOH during extraction of agar could increase the yield significantly.

Gel strength of agar increased when 0.5N NaOH was added during extraction (44 gm/sq.cm). Gel strength ranged from 19 gm/sq.cm in 3.0N NaOH treatment to 126 gm/sq.cm when NaOH was not added (Control) indicating addition of NaOH beyond 0.5N during extraction as unfavorable (Table-IV). Sulfate content in agar was in range of 2.28-3.756 % (Table-IV). Sulfate content remained minimum (2.28%) in 3.0N NaOH addition during the extraction of agar.

Melting point ranged from 51-65°C (Table-IV) registering the maximum (59°C) in 0.5N NaOH addition during extraction. ANOVA showed that addition of 3.0N NaOH during extraction was the ideal for sulfate reduction and addition of 0.5N NaOH during extraction favorable for gel strength enhancement (Table IV). Agar yield and gel strength were negatively correlated (r= -0.81439).

V. EFFECTS OF ADDITION OF NaOH DURING THE EXTRACTION OF AGAR FROM ALKALI PRE-SOAKED (1.0N NaOH) ON AGAR YIELD AND QUALITY:

Agar yield registered maximum (49.26%) in 3.0N NaOH addition during extraction (Table-V). Agar yield was in the range from 18.44-49.26%. Agar yield was significant ($p<0.05$) at different concentrations of NaOH addition during the extraction of agar. ANOVA also indicated that 3.0N NaOH treatment was most suitable for better agar yield. As shown in Table V, gel strength of agar was varying in the range of 18-128 gm/sq.cm. Gel strength was maximum in the

control (128 gm/sq.cm) treatment, which did not receive additional NaOH (Table-V). Gel strength and yield showed negative correlation (r= -0.77128). Sulfate content was minimum (1.578%) in 3.0N NaOH addition during the extraction of agar. Sulfate content varied in the range from 1.578 – 4.202 %, the lowest being in the 3.0N NaOH addition during extraction. Melting point was minimum (51°C) in agar extracted from 3.0N NaOH added *G. edulis* during extraction and maximum (85°C) in the control (Table-V).

VI. EFFECT OF ADDITION OF NaOH DURING THE EXTRACTION OF AGAR FROM THE ACID PRE-SOAKED (0.5N HCl) ON AGAR YIELD AND QUALITY:

Addition of varied concentrations of NaOH during extraction of agar from acid (0.5N HCl) presoaked *G. edulis* showed yield of agar could not be improved (yield ranging from 6.49-35.94%) by adding NaOH during extraction (Table VI). Addition of 3.0 N NaOH extraction showed that all the agar samples were hydrolyzed. Yield could not be determined. Gel strength in 0.5N NaOH addition showed 22 gm sq.cm but lower than the control (Table-VI). Sulfate content was minimum (4.24%) in 2.0N NaOH addition during extraction. In 3.0N NaOH addition all the samples were hydrolyzed. Similar trend was obtained when various concentrations of NaOH was added to 1.0N HCl presoaked *G. edulis* during agar extraction. Hydrolysis of agar occurred during extraction (Table-VII).

VIII. EFFECT OF ALKALI PRE-TREATMENT WITH RAISING TEMPERATURE TO 80°C FOR 1 hr ON THE AGAR YIELD AND QUALITY:

At different concentration of NaOH pre-treatment and increased temperature (80°C), the yield of agar ranged from 10.152 – 14.156%. Agar yield was maximum (14.156%) in 2.0N NaOH and minimum yield (10.152%) in 3.0N NaOH (Table-VIII). Agar yield showed significance ($p<0.05$) at different concentrations of NaOH pretreatment at 80°C. ANOVA results showed that 2.0N NaOH pre-treatment at 80°C was ideal for agar yield. Gel strength varied from 69-291 gm/sq.cm. 3.0N NaOH pre-treatment at 80°C increased gel strength to

291 gm/sq.cm (Talbe VIII) and was minimum (168 gm/sq.cm) in 0.5N NaOH pretreatment. which is higher than the gel strength achieved by control (69 gm/sq.cm). Gel strength showed significance at different (p<0.05) treatments. Agar yield and gel strength were negatively correlated (r= -0.03435). Sulfate content varied from 0.732 % - 5.548% with the least values (0.732%) in 3.0N NaOH pre-treatment at 80°c.

Melting point at different concentrations of NaOH pre-treatment at 80°C temperature ranged from 68-99°C (Table-VIII) with the maximum in 3.0N NaOH pretreatment (99°C).

ANOVA results showed that 2.0N NaOH pretreament at 80°C was highly preferable for quality (Gel strength) & yield improvement (p<0.05; Cd=-0.057 agar yield); cd = 3.305 (gel strength); cd 0.048 (sulfate %); cd = 2.075 (melting point °C)

IX. EFFECT OF ACID PRE-TREAMENT AT HIGH TEMPERATURE 80°C FOR 1 hr. ON YIELD AND QUALITY OF AGAR:

At different concentrations of HCl pretreatment 0.5N, 1.0N, 2.0N and 3.0N at 80°C temperature resulted hydrolysis of agar except in control agar could not be extracted (Table IX).

Table I – EFFECT OF PRESOAKING IN POTABLE WATER ON AGAR YIELD AND QUALITY FROM *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control	11.612 ± 0.25	54.0 ± 3.36	4.944 ± 0.05	66 ± 4.14
2 hrs.	11.44 ± 0.521	61.0 ± 7.36	4.86 ± 0.06	70 ± 4.27
4 hrs.	10.492 ± 0.32	65.0 ± 3.80	4.128 ± 0.06	75 ± 8.50
8 hrs.	10.274 ± 0.40	77.0 ± 5.26	3.846 ± 0.05	75 ± 6.80
12 hrs.	10.10 ± 0.26	90.0 ± 8.06	3.534 ± 0.08	77 ± 1.14

Table II – EFFECT OF PRESOAKING IN DIFFERENT CONCENTRATION OF HCl ON YIELD AND QUALITY OF AGAR FROM *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Soaked in Potable Water	12.364 ± 0.13	60.0 ± 4.4	4.71 ± 0.06	70
0.5 N HCl	15.844 ± 0.37	22.0 ± 1.6	4.602 ± 0.12	NIL
1.0 N HCl	4.376 ± 0.22	21.0 ± 1.09	4.18 ± 0.04	NIL

Table III – EFFECT OF PRESOAKING IN DIFFERENT CONCENTRATIONS OF SOLUTIONS ON THE AGAR YIELD AND QUALITY FROM *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT		YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control (Soaked in Potable Water)		12.5 ± 0.09	61 ± 2.58	4.796 ± 0.06	69 ± 1.92
Pre soaked in NaOH	0.5 N	12.4 ± 0.15	125 ± 2.07	3.759 ± 0.03	80 ± 2.40
	1.0 N	12.124 ± 0.04	135 ± 2.24	3.644 ± 0.02	82 ± 1.64

Table IV – EFFECT OF ADDITION OF NaOH DURING THE EXTRACTIONOF AGAR FROM ALKALI PRE-SOAKED (0.5 N NaOH) *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control	12.464 ± 0.02	126 ± 2.95	3.756 ± 0.02	65 ± 1.92
0.5 N	20.092 ± 0.14	44.0 ± 1.14	3.234 ± 0.03	59 ± 1.58
1.0 N	31.946 ± 0.19	40.0 ± 1.51	3.102 ± 0.03	57 ± 1.14
2.0 N	34.032 ± 0.11	23.0 ± 1.64	2.808 ± 0.02	55 ± 1.30
3.0 N	44.410 ± 0.42	19.0 ± 1.58	2.28 ± 0.02	51 ± 1.48

Table V – EFFECT OF ADDITION OF NAOH DURING THE EXTRACTION OF AGAR FROM ALKALI PRE-SOAKED (IN NaOH) *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control	18.446 ± 0.19	128 ± 1.78	4.202 ± 0.02	85 ± 1.30
0.5 N	28.324 ± 0.38	33.0 ± 1.14	3.90 ± 0.03	64 ± 1.58
1.0 N	34.052 ± 0.07	21.0 ± 0.89	3.85 ± 0.02	58 ± 1.14
2.0 N	36.272 ± 0.16	19.0 ± 1.14	1.72 ± 0.22	53 ± 0.87
3.0 N	49.262 ± 0.13	18.0 ± 0.70	1.578 ± 0.02	51 ± 0.83

Table VI – EFFECT OF ADDITION OF NaOH DURING THE EXTRACTION OF AGAR FROM ACID PRE-SOAKED (0.5 N HCl) SAMPLES OF *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control	35.940 ± 0.38	32.0 ± 1.09	4.658 ± 0.02	58 ± 0.70
0.5 N	16.120 ± 0.02	22.0 ± 0.90	4.33 ± 0.03	53 ± 1.14
1.0 N	9.798 ± 0.03	18.0 ± 1.14	4.296 ± 0.01	NIL
2.0 N	6.492 ± 0.02	17.0 ± 1.14	4.24 ± 0.03	NIL
3.0 N	NIL	NIL	NIL	NIL

Table VII – EFFECT OF ADDITION OF NaOH DURING EXTRACTION OF AGAR FROM ACID PRE-SOAKED (1 N HCl) SAMPLES OF *Gracilaria edulis* (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control (0.0 N NaOH)	23.6 ± 0.114	29.0 ± 1.04	4.13 ± 0.12	57 ± 1.34
0.5 N	21.56 ± 0.15	22.0 ± 0.15	3.8 ± 0.07	NIL
1.0 N	7.654 ± 0.03	18.0 ± 0.03	3.42 ± 0.03	NIL
2.0 N	NIL	NIL	NIL	NIL
3.0 N	NIL	NIL	NIL	NIL

Table VIII – EFFECT OF ALKALI PRE-TREATMENT WITH RAISING TEMPERATURE 80°C FOR ONE HOUR BEFORE EXTRACTION ON THE YIELD AND QUALITY OF AGAR (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control (0.0 N NaOH)	11.772 ± 0.03	69.0 ± 1.80	5.548 ± 0.07	68 ± 2.70
0.5 N	13.143 ± 0.05	168 ± 1.51	4.208 ± 0.01	88 ± 2.72
1.0 N	13.226 ± 0.05	191 ± 1.92	3.556 ± 0.05	95 ± 0.83
2.0 N	14.156 ± 0.06	274 ± 2.73	0.844 ± 0.03	96 ± 1.30
3.0 N	10.156 ± 0.05	291 ± 5.45	0.732 ± 0.02	99 ± 1.00

Table IX – EFFECT OF ACID PRE-TREATMENT WITH RISING TEMPERATURE TO 80°C FOR ONE HOUR ON YIELD AND QUALITY OF AGAR (MEAN ± S.D.)

TREATMENT	YIELD (%)	GEL STRENGTH gm/sq.cm 1.5% agar (w/v)	SULFATE CONTENT (%)	MELTING POINT (°C)
Control (0.0N HCl)	12.166 ± 1.14	55.0 ± 1.38	4.9 ± 0.02	72 ± 2.13
0.5N	NIL	NIL	NIL	NIL
1.0N	NIL	NIL	NIL	NIL
2.0N	NIL	NIL	NIL	NIL
3.0N	NIL	NIL	NIL	NIL

Table-X: AGAR YIELD SIGNIFICANCE AT ALKALI PRE TREATMENT AT 80 °C FOR 1 hr (2.0 N NaOH)

ANOVA Single Factor						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	50.90776	4	12.72694	4493.976	3.16E-29	2.866081
Within Groups	0.05664	20	0.002832			
Total	50.9644	24				

Table- XI: GEL STRENGTH SIGNIFICANCE AT ALKALI PRE TREATMENT AT 80 °C FOR 1 hr (2.0 N NaOH)

ANOVA Single Factor						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	161199	4	40299.76	4324.009	4.64E-29	2.866081
Within Groups	186.4	20	9.32			
Total	161385.4	24				

Table- XII: SULFATE CONTENT SIGNIFICANCE AT ALKALI PRE TREAT AT 80 °C FOR 1 hr (2.0 N NaOH)

ANOVA singlefactor

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	90.25178	4	22.56294	11258.95	3.27E-33	2.866081
Within Groups	0.04008	20	0.002004			
Total	90.29186	24				

Table-XIII: MELTING TEMPERATURE SIGNIFICANCE AT VARIOUS A NaOH PRETREATMENTAT 80°c FOR 1 hr (2.0 N NaOH)

ANOVA Single Factor

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2967.76	4	741.94	201.611	7.41E-16	2.86601
Within Groups	73.6	20	3.68			
Total	3041.36	24				

DISCUSSION

Gracilaria edulis (Gmelin) Silva was selected for the present study because this species of red seaweed is commonly used by agar industries in India (Chennubhotla *et al.*, 1987; Kaladharan and Kaliaperumal, 1998; 1999) and 60% of the world agar production is derived from *Gracilaria sp* (Durairatnam, 1987). Quality of agar is the most important criteria deciding its market value and field of its application. The current requirement in the international food market on agar is gel strength equal to or greater than 750 gm/cm^2 (1.5% gel) and sulfate content less than 4% (Armisen, 1995). where as Japanese grade-2 agar requires gel strength above 220 gm/cm^2 and sulfate content between 1-2% (Ji Minghou, 1985). Committee on Codex Specification (1981) insists melting temperature of agar to be above 85°C.

Gracilaria edulis normally yields agar of gel strength varying from 70-123 gm/cm^2 (Thomas & Krishnamurthy, 1976; Kaliaperumal *et.al.*, 1987). However agar industries in India achieved desired gel strength by blending *Gelidiella acerosa* and *Gelidium* sp with *G.edulis* in various proportions (Kaladharan & Kaliapermal, 1999).

Yield, gel strength, melting temperature and sulfate content of agar from *Gracilaria* spp are decided by a number of factors such as growing season, growth stages, location and between species (Ji Minghou, 1990; Whyte *et.al.*, 1981) temperature and salinity (Luhan, 1992; Sasikumar *et.al* 1999) and Nitrate levels in water (Christeller & Laing, 1989).

In the present study presoaking of dry weeds in water for 2 hr increased the yield to 11.44% but did not improve the gel strength of agar. On the other hand presoaking in water for 12 hrs duration increased considerably gel strength, melting temperature and reduced sulfate content (Table-I). The reason may be attributed to the removal of impurities during prolonged soaking, including some water-soluble inhibitors, which might affect adversely the agar quality.

Presoaking of dry weeds in 1.0 N NaOH solution (Table III) is preferred over presoaking with dilute HCl (Table II), to achieve the increased gel strength (135 gm/cm^2), reduced sulfate content (3.64%), higher melting temperature (82°C) in agar but not for improvement in the yield.

These findings are agreeable with earlier reports by Ji Minghou (1990) and Coppen (1990) on *Gracilaria spp.* And by Robledo and co- workers (1997) on *Gelidiella acerosa*. Addition of 3.0 N solution of NaOH to the alkali presoaked *G.edulis* during boiling resulted in remarkable increase in the yield of agar as shown in Table IV and V from 41.49%-49.26%, as well as reduced sulfate levels (1.578%). However addition of NaOH during boiling did not improve gel strength of agar. Thus pretreatment of *Gracilaria edulis* with chemicals and addition of chemicals during extraction exhibits entirely different results.

Addition of different concentrations of NaOH solution during extraction of HCl-presoaked *G.edulis* (Table VI & Table VII) showed improvements neither in yield nor in the quality of agar, resulted in hydrolysis of agar, which is agreeable to earlier studies (Mathew *et.al,* 1993). The hydrolysis of agar induced by acid pretreatment may be due to depolymerisation of long chain of polysaccharides in acid hydrolysis.

Ji Minghou (1990) stressed the need for alkali pretreatment for *Gracilaria* sp to improve quality of agar. Coppen (1990) has also viewed similarity. In the present study as shown in Table VIII, pretreatment of 2.0-3.0 N NaOH at 80°C for 1 hr to the presoaked *G.edulis* proved ideal and optimised chemical manipulation in extraction method to obtain moderately high yield (14.156%) and maximum gel strength, (291 gm/cm^2) lowest levels of sulfate (0.732 %) and highest melting temperature (99°C) of agar. These results are showing similarly to previous results obtained for *Gracilaria blodgettii* and *G.verrucosa* (220 gm/cm^2) in 2.0 N NaOH pretreatment (Sasikumar *et.al.*, 1997) and *Gelidiella acerosa,* 700 gm/cm^2 gel strength at 0.5 N NaOH treated sample (Mathew *et.al.*,1993), in *Gracilaria cornea* from Yucatan, Mexico (Freile-Pelegrin and Robledo, 1997), *Gracilaria edulis* (Durairatnam, 1987), *Gracilaria sjoestedtii*

and *G.verrucosa* (Craig *et.al.*, 1984), *Gracilariopsis heteroclada* (Baricuatro, 1997).

When the agar industry personnels in India are contacted for any requirement of technological innovations using pretested interview schedules (Kaladharan & Pillai, 1998) majority of them responded for any technology to improve quality of agar. Hence the information may be of immediate help to the agar industries in India.

From the above experiments it can be surmised that pre-treatment of presoaked *G.edulis* with 2.0 – 3.0 N NaOH solution for 1 hr at 80°C is most suitable for obtaining moderate yield of agar with desirable qualities. This information may be recommended for the agar industries after conducting few more multiseasonal trials.

SUMMARY

Price of the agar is decided by the Quality of agar such as high melting temperature, low sulfate content and high gel strength in present investigation was takenup to determine in optimum use of alkali/acid and other modification in extraction method to increase in yield and Quality of agar from Red seaweed *Gracilaria edulis*. Presoaking of dry weeds in water for 2-hrs increase the yield to 11.44% but did not improve in gel strength of colloid. On the other hand presoaking in water for prolonged durations increased considerably in gel strength, melting point and reduced sulfate content but not in Yield (Table-I). Agar yield and gel strength of agar were negatively correlated.

Presoaking of dry weeds in 1.0 N HCl resulted in low sulfate content (4.18%) and increased (60 gm/cm^2) gel strength (Table-II). Presoaking of dry *G.edulis* in 1.0 N NaOH increased gel strength (135 gm/cm^2), reduced in sulfate content (3.64%) and increased melting temperature (80°C) in agar (Table-III) but not in yield. Addition of 3.0 N solution of NaOH to the presoaked *G.edulis* during boiling resulted in remarkable increase in yield of agar to 44.41% (Table IV) and 49.26% (Table V) respectively and also reduction of sulfate levels in agar to 1.578%. However addition of NaOH during boiling did not improve the gel strength of agar from the alkaline presoaked *G.edulis* (Table IV & V).

Addition of different concentrations of NaOH solution during boiling of HCl -presoaked *G.edulis* could not improve neither the yield nor the Quality of agar. Addition of alkali levels beyond 2.0 N NaOH resulted in hydrolysis of agar (never found gel at room temperature, Table VI & VII).

Pretreatment of different concentrations of NaOH at 80°C for 1 hr proved to be in most ideal and optimized manipulations in extraction methods to obtain higher yield (14.156%, maximum gel strength 291 gm/cm^2) lowest levels of sulphate (0.732%) and highest melting temperature (99°C) of agar. At the same time pretreating *G.edulis* at different concentrations of dilute

Hcl at 80°C did not effect favorably and resulted in hydrolysis of the phycocolloid. In all the above experiments gel strength and yield of agar were always negatively correlated.

From the above study it is inferred that pretreatment of *Gracilaria edulis* with 3.0 N NaOH solution at an increased temperature of 80°C for one hour period could improve the yield of agar to 14.156%, gel strength to 291 gm/cm^2, lowest levels of sulfate content 0.732 and highest melting temperature (99°C). When the agar industry personnel's were contacted for any requirement of technological innovations using presented interview schedules, majority of them responded for immediate need of technology to increase the yield and gel strength. So that their product could be sold for high returns. The information developed during this study shall be undoubtedly beneficial to the agar industries functioning in our country.

REFERENCES

Anon, 1987. Seaweed research and utilization in India. *Bull. Cent. Mari. Fish. Res. Inst.*, **41:** 1-116.

Armisen,R.1995.World wide use and importance of *Gracilaria*. *J.Appl.Phycol.***7**: 231-243.

Armisen, R. & Galatas, F. 1987. Production and utilization of products from commercial seaweeds. *FAO Fisheries Technical Paper.* **288:** 1-57.

Baricuatro, F. L. 1997. The effect of alkali on the yield, quality and chemical composition of agar from *Gracialariopsis heteroclada* (Rhodophyta, Gracilariales*). J. The Philippine Scientist.* **34:** 5-20.

Bose, J. L, Karimullah & S. Siddique. 1943. Manufacture of agar in India. *J. Sci. Industri. Res.*, (India) **1**: 98.

Calumpong, H. P., A. Maypa, M. Magbanua & P. Saurez. 1999. Biomass and agar assessment of three species of *Gracilaria* from Negro's Island , central Phillippines . *Hydrobiologia.* **398/399**: 173-182.

Chapman, V. J. 1970. Seaweeds their uses. 2nd edn., Methuen, London.

Chennubhotla, V. S. K. 1996. Seaweeds and their importance. Bull. Cent. Mar. Fish. Res. Inst., **48:** 108-109.

Chennubhotla, V. S. K., S. Kalimuthu, M. Najmuddin, R. Panigrahy & M.Selvaraj. 1979. Seasonal variation in growth, yield of agar-agar and its physical properties in some agarophytes of Tamil Nadu Coast. Proc. *Int. Symp. Marine algae of the Indian Ocean region.* CSMCRI, Bhavnagar, India pp.41.

Chirapart, A., M. Ohno, H. Ukeda, M. Sawamura, H. Kusunose.1995. Physical and chemical properties of agar from a new member of *Gracilaria, G.lemaneiformis* (Gracilariales, Rhodophyta) in Japan. *Fisheries Science* . **61**(3): 450-454.

Chirapart, A., M. Ohno, H. Ukeda, M. Sawamura, H. Kusunose. 1997. Effects of partial acid hydrolysis on physical and chemical properties of agar from a newly reported Japanese agarophyte (*Gracilariopsis lemaneiformis). J. Appl. Phycol.* **9**(1)**:** 73-76.

Christeller, J. T. & W. A. Laing. 1989. The effect of environment on the agar yield and gel characteristics of *Gracilaria sordida* Nelson (Rhodophyta) *Bot. Mar.* **32:** 447-455.

Committee on Codex Specifications. 1981. Food Chemical Codex. National Academy Press, Washington, D.C.

Coppen, J. J. W. 1989. International trade in agar for countries in the Bay of Bengal region. *Gracilaria* Production and utilization in the Bay of Bengal *region, Songkhla, Thailand BOBP/REP/***45**. pp. 87-91.

Coppen,J.J.W.1991. Agar and alginate production from seaweed in India. *BOBP/WP/***69.** pp.1-27.

Cote, G. L. & M. D. Hanisak. 1986. Production and Properties of native agars from *Gracilaria tickvahiae* and other red algae. *Bot. Mar.* **29:** 359-366.

Craigie, J. S., Z. C. Wen and J. P. Vander meer.1984. Interspecific, Intraspecific and nutritionally determined variations in the composition of agar from *Gracilaria sp. Bot.Mar.* **27:** 55-61.

Critchley, A. T., A. J. Wilson.1998. Studies on *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine and Farnham *and Gracilaria aculeata(Hering) Papenfuss* from SouthAfrica. II. Agar production. *South African Journal of Botany,* **64**(2): 110-115.

Desikachary, T. V. 1967. Seaweed resources of India. Pro. Seminar. Sea Salt & Plants (Ed. Krishnamurthy, V.), Bhavanagar.

Devaraj, M., V. K. Pillai, K. K. Appukuttan, C. Suseelan, V. S. R. Murthy, P. Kaladharan, G. Sudhakara Rao, N. G. K. Pillai, N. N. Pillai, K. Balan, V. Chandrika, K. C. George & K. S. Shobana. 1997. Packages of practices for sustainable ecofriendly mariculture, land based saline aquaculture and seafarming. In: *Proc. 4th Ind. Fish. Forum.* (in press).

Deve, M. J., S. K. Garg & E. R. R. Iyengar. 1977. Assessment of the possibility of seaweeds utilized to be supplementary animal feed. *Salt. Res. Ind.* **13**(1&2): 33-40.

Doshi, Y. A., R. G. Parekh & V. D. Chauhan. 1987. Studies on marine *algae Laurencia papillosa* for Carrageenan. *Phykos.* **26:** 113-118.

Duckworth, M. & W. Yaphe (1971). The structure of agar part I: Fractionation of a complex mixture of polysaccharides. *Carbohydr.Res.***16:** 189-197.

Duckworth, M. & W. Yaphe.1972.The relation ship between structural and biological properties of agar. Proc.Int. *Seaweed.symp.***7**: 15-22. *Univ .of Tokyo press*, Tokyo.

Durairatnam,M.1987. Studies of the yield of agar,gel strength and quality of agar of *Gracilaria edulis*(Gmel.)Silva from Brazil.*Hydrobiologia.* **151/152**:509 – 512.

FAO, Training Manual. 6. 1990. Training Manual on *Gracilaria* culture and seaweed processing in China. pp: 1-30.

FAO report. 1996. A regional study and workshop on the taxonomy, ecology, and processing of economically important red seaweeds, Bangkok (Thailand), No. **3**: 319-325.

Freile-Pelegrin, Y. 2000. Does storage time influence yield and agar properties in the tropical agarophyta *Gracilaria cornea J..Appl.Phycol.* **12:**153-158.

Freile-Pelegrin, Y & D. Robledo.1997 Influence of alkali treatment on agar from *Gracilaria cornea* from Yucatan, Mexico. *J.Appl.Phycol* **9**:533-539.

Freile-Pelegrin,Y., D. Robledo & E.servier–Zaragoza.1999. *Gelidium robustum* agar: Quality characteristics from exploited beds and seasonality from an

unexploited bed at Souther Baja Califonia,Mexico *Hydrobiologia.* **398/399**: *501-507.*

Funaki, K. & Kojima. 1951. The studies on the properties of agar-agar from *Gracilaria confervoides. Bull. Jap. Soc. Sci. Fish.*, **16:** 159-164.

Hoyle, M. D. 1978. Agar Studies in two *Gracilaria spp. (G.bursapastoris (Gmelin)* Silva & *G. coronopifolia.J.Ag*) from Hawaii II. Seasonal aspects. *Bot. Mar.***21:** 347-352.

Hurtado-Ponce, A. Q. & I.Umezaki. 1988. Physical properties of agar gel from Gracilaria (Rhodophyta) of the Philippines. *Bot. Mar.* **31:** 171-174.

Ian J. Miller, RuthFalsaw & Richard. H. Furneaux. 1996. A polysaccharide fraction from the red seaweed *Champia novie- zealandiae* Rhodymeniales, Rhodophyta. *J. Hydrobiologia.* **326/327:** 505-509.

Ji Minghou. 1990. Laboratory methods for qualification of red seaweeds and their polysachharides. Biopolymer Research Unit, Thailand, pp. 27-50.

Ji Minghou, M. Lahaye & W.Yaphe. 1985. Structure of agar from *Gracilaria spp.* (Rhodophyta) collected in the People's Republic of China. *Bot. Mar.* **28:** 521-528.

Kaladharan, P. & Kaliaperumal, N. 1999. Seaweed industry in India. NAGA, **22**(1): 11-14.

Kaladharan, P. & Sridhar, N. 1999. Cytokinins from marine green alga, *Caulerpa racemosa* (Kuetz) Taylor, *Fishery Technology.* 36(2): 87 –89.

Kaladharan, P. Kaliaperumal, N. 1999. Seaweeds as feed for farm animals. *CMFRI Bulletin* No. 46, pp. 30 –31.

Kaliaperumal, N. & J. R. Ramalingam. 1999. Distribution, resources and uses of seaweeds. *In*: Workshop on seaweed processing and seaweed products, CMFRI, Cochin, pp 1-3

Kaliaperumal, N. & S. Kalimuthu. 1997. Seaweed potential and its exploitation in India. *Seaweed. Res. Utlin.* **19:** 33-40.

Kaliaperumal, N. 1993. Seaweed culture **In:** *Handbook on Aqua Farming entitled seaweed, Seaurchin & Seacucumber.* MPEDA. **11**:9-22.

Kaliaperumal, N., S. Kalimuthu & J. R. Ramalingam.1988. Seasonal Variations in growth, alginic acid & manitol contents in *Cystoseira trinodis* (Forsskal) *C. agardh & Hormophysa triquetra (Linnaeus) Kuetzing. Ind.J.Bot.***11**(1):17-21.

Kaliaperumal, N.,V. S. K. Chennubhotla, Reetajayasankar, S. Kalimuthu, J. R. Ramalingam, K. Muniyandi & M. Selvaraj. 2000. Seaweeds. In: Marine fisheries research and management (V. K. Pillai and N.G.Menon eds.) CMFRI . *pub. 23-27.*

Kappanna, A. N. & A. V. Rao.1963.Preparation and properties of agar-agar from Indian seaweeds.*Ind.J.Technol.***1**: 222-224.

Lewis, R. J & M. D Hanisak. 1996. Effects of phosphate and nitrate supply on productivity agar content and physical properties of agar of *Gracilaria* strain G-16S. *J. appl. Phycol.* **8**(1): 41-49.

Liu, Sijian, Jie, Zhenying, Zeng, Shufang. 1997. The commercial cultivation of Gracilaria and its polyculture with prawn in China. *J. Zhanjiang Ocean Univ./Zhanjiang Haiyang Daxue* Xueno. **2**:27-30.

Luhan Ma R J (1992). Agar yield and gel srenght of Gracilaria *heteroclada* collected from Iloilo, Central Philippines. *Bot. Mar.* **35:** 169-172.

Miller, I. J & R. H. Furneaux.1987. Chemical characteristics of galactans from the forms of *Gracilaria secundata* from New Zealand. *Bot. Mar.* ***30****: 427-435* .

Miller, I. J .,Ruth Falshaw & R. H. Furneaux.1996.A polysacharide fraction from the red seaweed *Champia novae-zealandiae* (Rhodomeniales, Rhodophyta). *Hydrobiologia.* **326/327** :505-509.

Mouradi. Givernaud, A., T. Gtivernaud, A. Morvan & J. Cosson.1992. Agar from *Gelidium latifolium* (Rhodophyceae,Gelidiales): Biochemical composition and seasonal variation. *Bot. Mar.* **35**:153-159.

*Nelson, S. G., E. P. Glenn, J. Conn, D. Moore, T. Walsh, M. Akutagawa. 2001. Cultivation of *Gracilaria pervispora* (Rhodophyta) in shrimp farm effluent ditches and floating cages in Hawaii; A two phase polyculture system. *Aquaculture* **193**(3-4)**:** 239-248.

Nelson, S. G., S. S.Yang, C. Y. Wang & Y. Mchinang.1983. Yield and quality of agar from species of Gracilaria (Rhodophyta) collectd from Taiwan and Micronesia.*Bot.Mar.***26:** 361-366.

Ohno. M., N. Hunhquang, H. Susumu. 1997. Biology and agar quality of cultivated Gracilaria from Vietnam. *Bull. Mar. Sci. Fish. Kochi Univ.* **17:**15-21.

Patwary, U. Mohsin & VanderMeer, J. P.1983. Genetics of *Gracilaria tikvahiae* (Rhodophyceae) IX; some properties of agar extracted from morphological mutants. *Bot. Mar.* **26:** 295-299.

Pillai, V. K. 1957. Chemical studies on Indian seaweeds. II. Partition of nitrogen. *Proc. Indian. Acad. Sci.,* **B.45:** 43-46.

Pillay, T. V. R. 1990. Principles and practices of aquaculture. *Fishing News Books.* Oxford Press.pp. 512-525.

Redekar, P. D. & P. C. Raje. 2000. Alginic acid and agar-agar content from the common seaweed of Ratnagiri Coast (West Coast of India). *Seaweed. Res. Utiln.* **22**(1-2)**:** 41-43.

Roleda, M. Y., E. T. Ganzon-Forter, N. E. Montano, F. N. Delos Reyes. 1997. Temporal variation in the biomass, quantity and quality of agar from *Gelidiella acerosa* (Forsskal) Feldmann of Hamel (Rhodophyta; Gelidiales) from Cape Bolinavo, NW. Philippines. *Bot. Mar.* **40** (6)**:** 487-495.

Roleda, M. Y., E. T. Ganzon-Forter, N. E. Montano, R. D. Villanueva. 1997. Acetic acid pre treatment in agar extraction of Philippine Gelidiella acerosa (Forsskal) Feldmann of Hamel (Rhodophyta; Gelidiales) *Bot. Mar.* **40**(1)**:** 63-69.

Rotem,A.,N.Roth-Bejrano & Shoshan(Malis) Arad.1986. Effect of controlled environmental conditions on starch and agar contents of *Gracilaria* sp.(Rhodophyceae).*J.Phycol.* **22**: 117-121.

Santos, G. A. & M. S. Doty. 1983. Agar from some Hawaiian Red algae. *Aqua. Bot.* **16:** 385-389.

Sasikumar, C; V. N. R Rao, R. Rengasamy.1997.Effect of alkali treatment of red algae *Gracilaria blotgetti* and *G.verrucosa* (Rhodophyta) on agar quality. *Ind.J.Mar.Sci.* **26**:191-194.

Sasikumar, C; V. N. R Rao, R. Rengasamy.1999. The effect of environmental factors on the qualitative and quantitative characteristics of agar from the marine red algae *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Indian J. Mar.Sci.* **28**(3): 270-273.

SEAFDEC Asian Aquaculture.1998. Seaweed Culture **20** (1)**:** 11-23.

Silas, E. G., P. V Ramachandran Nair & V. S. KChennubhotla. 1987. Introduction. *In*: CMFRI *Bullettin* No.41, CMFRI, Cochin, pp.1-2.

Thivy, F. 1960. *Proc. Symp. Algology*, ICAR, pp. 345-365.

Thivy, F. 1958. Economic seaweeds. In: Fisheries of West coast of India, Bangalore. pp.74-80.

Thomas, P. C and V. Krishnamurthy.1976. Agar from cultures *Gracilaria edulis* (Gmelin) Silva. *Bot . Mar.* **29:**115-117.

Umamaheshwara Rao, M. 1969. Agar and Algin Yielding sea weeds of India. *Proc.Int. Seaweed symp.* **6**:715-721.

Umamaheshwara Rao, M. 1972. On the Gracilariaceae of the seas around India. *J.Mar.Biol.Ass.India.* **14**(2): 671-696.

Vyjayanth, V & R. Rengaswamy. 1989.A new apparatus for the determination of gel stength of phycocolloids.*Curr.sci.* **58**(14): 795-796.

Whyte, J. N. C & J. R. Englar. 1980. Chemical composition and Quality of agars in the morphotypes of *Gracilaria* sp.from British Colombia. *Bot. Mar.* **23:** 277-283.

Whyte, J. N. C , J. R. Englar,R.J.Saunders & J.C.Lindsay.1981. Seasonal variations in the biomass, quantity and quality of agar, from the reproductive and vegetative stages of *Gracilaria* (verrucosa type). *Bot. Mar.***24**: 493-501.

*Not refered