1. Seaweeds

Seaweeds are marine algae: saltwater-dwelling, simple multicellular, macrothallic organisms that fall into the rather outdated general category of "plants". They are the oldest members of the plant kingdom, extending back many hundreds of millions of years. They have little tissue differentiation, no true vascular tissue, no roots, stems, or leaves, and no flowers. They are generally attached by holdfasts that serve anchorage function. They vary in size from small to huge plants more than 100 feet long. They are one of the most important marine renewable and valuable living resources and could be termed “futuristically promising plants of the oceans”, with an immense economical and commercial value (Chapman & Chapman, 1980; Levring et al., 1969).

2. Seaweeds classification

Seaweeds are classified into three divisions - Chlorophyta (green algae, 1200 species), Phaeophyta (brown algae, 2200 species) and Rhodophyta (red algae, 6500 species) (Smith et al., 1994; Dhargalkar & Pereira, 2005; www.seaweed.ie) based on their pigments and coloration. Other features used to classify them are; cell wall composition, reproductive characteristics, and the chemical nature of their photosynthetic products (oil and starch). Within each of the three major groups of seaweeds, further classification is based on characteristics such as plant structure, form, and shape. Green seaweeds are part of a large group called Division Chlorophyta, which include all green seaweeds. Only about 10% of green algae are marine species, and the rest mostly live in freshwater. The green seaweeds are most commonly found in the shallow intertidal zone. More species of green seaweeds are found in warm tropical oceans than in cold temperate seas. Some common species of green seaweeds are Ulva (sea lettuce), and Enteromorpha (green string lettuce). Brown seaweeds are found in a variety of physical forms including crusts, filaments, and large elaborate kelps. Like all photosynthetic organisms, brown algae contain the green pigment chlorophyll. They also contain other gold and brown pigments, which mask the green color of chlorophyll. The dominant pigment found in brown algae is called fucoxanthin, and it reflects yellow light. Because of the combination of pigments, the coloration of brown algae ranges from light
olive green or golden, to very dark brown. Most brown algae live in the mid intertidal or shallow/upper subtidal zone, and they are most abundant in the colder oceanic waters of the Northern hemisphere. The majority of seaweed species are part of the Division Rhodophyta, or red algae. They have a unique intercellular structure, which gives them a rubbery and springy quality. In addition to chlorophyll, red algae contain the pigments phycocyanin and phycoerythrin, which give this group red coloration. However, the colour of red algae varies, and if the pigment phycoerythrin is destroyed, they appear purple, brown, green, or yellow. The accessory pigments (phycocyanin and phycoerythrin) of red algae allow them to grow in deeper waters (subtidal) than other algae. Red algae also grow in the intertidal zone. One group of red algae, called the coralline algae, are pink in colour and contain deposits of magnesium and calcium carbonate in their cell walls. These seaweeds are hard like stones, and were once thought to be animals closely related to corals (Chapman & Chapman, 1980).

Many different uses for red seaweeds have been discovered. Two substances found in the cell walls of red algae are agar and carrageenan. These are gelling compounds, and are used in food products and scientific research. Carrageenan is an important ingredient in toothpaste and many milk products, such as ice cream and chocolate milk. Agar has many scientific applications in microbiology, biotechnology, and criminology, and is used in the packaging of canned meat. One of the most popular red seaweed food is nori (Porphyra), which is used in sushi wraps and other Japanese dishes. Nori is grown in commercial seaweed farms on the east coast of North America and in Asia (http://www.oceanlink.island.net/oinfo/seaweeds/seaweeds.html).

3. Production

Currently there are 47 countries in the world with commercial seaweed activity. China holds first rank in seaweed production, with Laminaria species, accounting for most of its production, followed by North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia, USA and India. According to FAO, between 1981 and 2000, world production of aquatic plants increased from 3.2 million tons to nearly 10.1 million tons (wet weight), upholding US$ 6 billion world trade in 2000 as compared to US$ 250 million trade in 1990. The contribution of cultured seaweeds is 15% of total global aquaculture (45,715,559 tons).
The seaweeds that are most exploited for culture are the brown algae with 4,906,280 tons (71% of total production) followed by the red algae (1,927,917 tons) and green algae (33,700 tons) (Khan and Satam, 2003). Every year about 7.5–8 million tons of wet seaweeds are being produced along the coastal regions worldwide (McHugh, 2003, 2004). The data for worldwide seaweed production is summarized in Table 1 (FAO, 2001). Since 1984, the production of seaweeds worldwide has grown by 119%. The world seaweeds production of 1,338,597 (MT) has been reported for the year 2005 by Bureau of Agricultural Statistics. Seaweeds find their applications as fertilizers and soil conditioners, animal feed, fish feed, biomass for fuel and also in cosmetics, pharmaceuticals, integrated aquaculture and wastewater treatment-phycoremediation (for reduction of nitrogen- and phosphorus-containing compounds and removal of toxic metal).

4. Trends in seaweed research

A timeline highlighting important areas in seaweed research is shown in Figure 1. Since the 1940s, when the potential of agar production from seaweeds was recognized, taxonomy, physiology and biochemistry have been the main research focus. Physiological aspects related to the production of hydrocolloids and pigments, and mass cultivation of seaweeds have been of particular interest. These areas of research laid the foundation of our understanding of seaweed biology. Despite the development and progress of functional genomics in terrestrial plants, seaweeds had received little attention worldwide and were not included in efforts to elucidate gene functions. It was not until the 1990s that studies on the molecular genetics of seaweeds were initiated. These studies were pioneered with the development of genetic transformation techniques on seaweeds and the characterization of genes involved in carbohydrate synthesis. The adaptation of molecular techniques enabled a more reliable and systematic way of inferring evolutionary relationships among different strains or species. Genetic research in seaweeds entered a new phase following the first use of the expressed sequence tag (EST) approach to study seaweed genomics– a relatively inexpensive and quick approach for novel gene discoveries. However, the number of reported seaweed ESTs to date (of which >80% are from the red seaweeds Porphyra and Gracilaria) accounts for only 0.11% of all publicly available ESTs (Cheong-Xin et al., 2006).
5. Phycocolloids

There are about 9900 species of seaweeds are known and of these only 221 species are used. 101 species (i.e. 68.33 lakh tons) are utilized for phycocolloid production (agar, algin and carrageenan), whereas 145 species are used as food (Zemke-White & Ohno, 1999). The phycocolloid content varies between different species (Black et al., 1951), seasonally (Bird & Hinson, 1992), and among species at different locations (Ohno, Quang, & Hirase 1996; Rebello et al., 1997; Freile-Pelegrin et al., 1996).

6. Agar

Agar is composed of two similar fractions, agarose and agaropectin, in which the basic unit is galactose, linked alternately with $\alpha$-1,3-(D-galactose) and $\beta$-1,4-(\alpha-L-galactose) (Figure 2). In 1945, Dr. Tseng defined agar as “the dried amorphous, gelatin-like, non-nitrogenous extract from *Gelidium* and other agarophytes, being the sulfuric acid ester of a linear galactan, insoluble in cold but soluble in hot water, a one per cent neutral solution of which sets at 35°C to 50°C to a firm gel, melting at 80°C to 100°C.”

7. Agarophytes

Nearly 33 agarophytic species have been reported till date (Zemke-White, & Ohna, 1999). These include *Gelidium pacificum, Gracilaria* species, *Pterocladia capillace, Ahnpheltia plicata, Acanthopheltis japonica, Ceramiun hypnaeordes* and *Ceranium boydenii*.

8. Applications of agar

About 90% of the agar produced is used in frozen foods, pastry fillings, syrups, bakery, icings, dry mixes, meringues, frozen desserts, instant pudding, cooked pudding, chiffons, pie and pastry filling, dessert gels; fabricated foods, salad dressings, meat and flavour sauces, food applications, textiles, pharmaceuticals, brewing and cosmetics (McLean
et al., 2001). Agar functions as stabilizer, thickener, pre-packing material (for cakes and buns, to reduce the quantity of water), smoothening, non-sticking icing material and in canned products like "scatola" meat (beef blocks in gelatine), as well as in confectionery (jellies, marshmallows and candies as a thickening and gelling agent). In the baked goods industry, the ability of agar gels to withstand high temperatures means agar can be used as a stabilizer and thickener in pie fillings, icings and meringues. Cakes, buns, etc., are often pre-packed in various kinds of modern wrapping materials which often stick to them, especially in hot weather; by reducing the quantity of water and adding some agar, a more stable, smoother, non-stick icing is obtained. In the pharmaceutical industry agar has been used for many years as a smooth laxative. The remaining 10 % is used for bacteriological and other biotechnological uses (Levring et al., 1969).

9. Alginate

Alginate is a polyuronide made up of a sequence of two hexuronic acid residues: β-D-mannuronic acid unit and α-L-guluronic acid (Figure 3).

10. Alginophytes

Most of the large brown seaweeds are potential sources of alginate. There are 41 species of alginophytic seaweeds reported so far. The main commercial sources are species of *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis, Sargassum* and *Turbinaria*. Of these the most important are *Macrocystis pyrifera, Laminaria japonica, Laminaria hyperborea, Laminaria digitata, Ascophyllum nodosum, Durvillaea lessonia, Durvillaea antarctica, Durvillaea potatorum, Ecklonia cava* and *Eisenia bicyclis* (Zemke-White & Ohno, 1999). *Macrocystis* gives a medium-viscosity alginate, or a high viscosity with a careful extraction procedure (lower temperature for the extraction). *Sargassum* usually gives a low viscosity product. *Laminaria digitata* gives a soft to medium strength gel, while *Laminaria hyperborea* and *Durvillaea* give strong gels. These are some of the reasons why alginate producers like to have a variety of seaweed sources, to produce the desired alginate to the needs of particular applications.
11. Applications of alginate

As a thickener alginate is used in various ways: in ices and ice creams, in products for pastries, in toothpastes, cosmetics, textile printing, paper printing and in water flocculation. On the other hand it is also used as a gelling agent and in entrapment and immobilization of enzymes and microorganisms and also as absorbent products. Alginites improve the texture, body and sheen of yoghurt. It is also used as a suspending agent in chocolate milk and cocoa. Alginites have some applications that are not related to either their viscosity or gel properties. Besides it is also used in the beer and wine industry, in edible dessert jellies, to preserve frozen fish, in meat juices, in pharmaceutical and medical uses, in paper for surface sizing and coating welding rods, as binders for fish feed, as release agents originally for plaster moulds and later in the forming of fibre glass plastics. The Indian alginate industry is based on Sargassum species collected from the coasts of Gujarat, Kerala and Tamilnadu. Sargassum species obtained from the western coast of India gives a low viscosity alginate, unsuitable for the main Indian market of textile printing. Turbinaria is used only when supplies of Sargassum are unavailable. The Philippines has large resources of Sargassum but this is exported mainly to Japan for use in animal feeds and fertilizers (Chapman & Chapman, 1980; Levring et al., 1969).

12. Carrageenan

Carrageenans are sulfated polymers made up of galactose units (Figure 4). Several fractions have been determined, but a common backbone can be defined. Carrageenan consists of a main chain of D-galactose residues linked alternately with α - (1 → 3) and β - (1 → 4). The differences between the fractions are due to the number and the position of the sulfate groups and to the possible presence of a 3,6 anhydro-bridge on the galactose linked through the 1 - and 4 -positions. Different carrageenans are named by Greek letter prefixes: μ, ν, λ, κ, θ, nu and xi- carrageenans (Mueller & Rees, 1968). Three basic types of carrageenans are available: κ-carrageenan from Chondrus crispus, Eucheuma and Gigartina species, τ-carrageenan from Eucheuma species and λ- carrageenan from Chondrus crispus and Gigartina species, which differ in the number and location of sulfate ester substitution.
The original classification of carrageenan is determined by the fractionation of the polysaccharide with KCl. The fraction soluble in KCl is called $\lambda$-carrageenan and the one insoluble is called $\kappa$-carrageenan (Yaphe & Baxter, 1955; Jhonston & McCandless, 1973).

### 13. Carrageenophytes

The original source of carrageenan is the red seaweed *Chondrus crispus* (common name: Irish Moss), collected from natural resources in France, Ireland, Portugal, Spain and the east coast provinces of Canada. As the carrageenan industry expanded, the demand for raw material had strained the supply from natural resources, although by this time (early 1970s) *Chondrus* was being supplemented by species of *Iridaea* from Chile and *Gigartina* from Spain. The introduction of cultivation of species of *Eucheuma* in the Philippines during the 1970s provided the carrageenan industry with a much enhanced supply of raw material (Doty, 1978).

There are 27 carrageenophytic seaweeds has been reported so far. The most important harvested carrageenophytes are *Sarcothalia crispata*, *Mazaella laminarioides*, *Gigartina skottsbergii*, *Chondracanthus chamisso* (Norambuena, 1996). Further, carrageenan is also extracted from *Kappaphycus alvarezii* (mainly kappa) and *Eucheuma denticulatum* (mainly iota) (Trono, 1993). *Gigartina skottsbergii* (mainly kappa, some lambda), *Sarcothalia* crispate (mixture of kappa and lambda) and *Mazaella laminaroides* are currently the most valuable species, all collected from natural resources in Chile. Small quantities of *Gigartina canaliculata* are harvested in Mexico. *Hypnea musciformis* has been used in Brazil (Bravin and Yoneshigue-Valentin, 2002). In India, *Hypnea musciformis* and *H. valentiae* are commercially exploited carrageenophytes (Ramalingam et al., 2003). A few of the carrageenophytic seaweeds are shown in figure 5.

The estimated amount of carrageenophytes being produced yearly has crossed 81,858 t (d. wt) with carrageenan production of about 28,650 t. This resource costs 25 kg$^{-1}$ US $ and has an approximate annual value of US $ 2.6 billion (Chopin et al., 1995; Ohno et al., 1996).
14. Applications of carrageenan

Carrageenan is mainly employed in food industry especially in the manufacture of sausages, corned beef, meat balls, ham, preparations of poultry and fish, chocolate, dessert gel, ice cream, juice concentrates, marmalade, sardine sauce fruit. It is also used in gelation, fat stabilization, thickening, suspending gelation, suspension, bodying, pulping effects, emulsion stabilization, binder, emollient. Its non-food applications include its use in manufacturing beer, air fresheners, textiles, toothpastes, hair shampoo, sanitary napkins, tissue, culture media, fungicide, etc. (Moirano, 1977).

15. Taxonomy of Kappaphycus alvarezii

Kingdom: Plantae

Subkingdom: Biliphyta

Phylum: Rhodophyta

Subphylum: Macrorhodophytina

Class: Rhodophyceae

Subclass: Florideophyceae

Order: Gigartinales

Family: Areschougiaeae

Tribe: Eucheumatoideae

Genus: Kappaphycus

Species: alvarezii (Doty) Doty ex P.C. Silva

Botanical Name: Kappaphycus alvarezii (Doty) Doty ex P.C. Silva

Three commercial species of Eucheuma are being used for their carrageenans. The annual production of carrageenans of each species is: Eucheuma cottonii, 30,000 tons producing kappa carrageenan; Eucheuma spinosum, over 6,000 tons producing iota carrageenan; and Eucheuma gelatinae, about 100 tons producing a mixture of gamma, beta and kappa carrageenans. Over 95% of the annual commercial Eucheuma crop is from farms in the tropical far western Pacific. In addition to foreign exchange earnings for those
countries exporting the seaweed, the labor-intensive farming of *Eucheuma* is of great socio-economic value to the often nearly indigent shore dwelling families who grow it.

The two species originally cultivated in the Philippines were *Eucheuma cottonii* and *Eucheuma spinosum*, and often referred to as "cottonii" and "spinosum" respectively. However, phycologists have since renamed both species: *Eucheuma cottonii* as *Kappaphycus alvarezii*, and *Eucheuma spinosum* as *Eucheuma denticulatum*. Unfortunately all the names are still in use and so an awareness of them is necessary while reading about carrageenophytes (FAO, 2003). *Kappaphycus* species are among the largest tropical red seaweed, with a high growth rate. *Kappaphycus alvarezii* is amongst the most widely studied edible seaweed (Trono, 1993). Its taxonomy has been studied by Doty (1988).

16. Life History

The reproduction of *Eucheuma* species from a classical point of view is reported by Kylin (1956), Gabrielson (1983) and Gabrielson and Kraft (1984). The nature of its triphasic life history results in their relation to the family Solieriaceae of the order Gigartinales. A diploid vegetative phase (asexual) produces haploid non-motile spores called tetra-spores. The tetraspores produce haploid gametophytes (sexual) which in turn produce diploid carposporophytes that are parasitic in the female thalli. The carposporophytes release diploid carpospores which initiate the diploid tetrasporic stage again. Superficially spinosum, cottonii and gelatinae all appear to be triphasic though the strains being farmed may not be. There is no detailed study of the life history of any member of this genus. Male thalli are as yet unknown for the commercial forms. Santos & Doty (1978) found both cystocarpic and tetrasporic thalli in quantity in only 6 of the 15 species in which they were sought. They did not find them common in the commercial species and found no male thalli. *Eucheuma alvarezii* (=*Kappaphycus alvarezii*) carpospores produce morula-like tetra-sporophytic embryos that attach by rhizoids and differentiate into an erect pyriform followed by cylindrical form as the typical cluster of apical cells develops opposite the substratum. The younger embryos have a thick gel coat and a large number of very fine hairs, each ten or
twenty times longer than the diameter of the embryo. Perhaps they may represent the microform in which some species of the genus persist in "off" seasons (Santos & Doty, 1978).

17. Morphology and Anatomy

*Kappaphycus alvarezii* plants are tough, fleshy, firm; and their length can extend even up to 2 m. The thalli are coarse, with axes and branches 1 - 2 cm diameter; heavy, with major axes relatively straight, lacking secondary branches near apices. It is frequently and irregularly branched, most branches primary, secondary branches intercalated between primary branches or mostly lacking. The plants contain a few small branches in shallow areas and in deeper waters they are large, intricately tangled as fleshy mats.

The thalli of commercial *Kappaphycus alvarezii* are often grow up to a kilogram in mass. Basically, the thallus is a multiaxial filamentous red algal genus which becomes strongly pseudoparenchymatous. The species vary greatly in form as a result of the environments in which they grow. This is particularly true of those in the section of the genus to which the cottonii forms belong. Commercial spinosum and cottonii are composed of cylindrical branches that are rigid. Mature gelatinae branches are apically flat, somewhat flexible, have marginal teeth and arise from a cushion of strongly compressed branches. Gabrielson (1983) and Gabrielson & Kraft (1984) provided much of the structural details which Kylin (1956) did not include. None of these authors treated the sections of the genus, Anaxiferae, Cottoniformia or Gelatiformia, from which the commercial cottonii and gelatinae crops arise. Weber-van Bosse (1928) and Doty & Norris (1985) provided some of the structural details of the section Cottoniformia but they were still largely undefined anatomically. The different color forms of *Kappaphycus alvarezii* such as brown, green and pale yellow forms show typical variation in their morphological features. The brown color form are dark brown in color, branches are thick and robust, profusely and irregularly branched, the branches are tapering towards the tips. The basal part of the branch is very thick; plant normally measures up to 20–25 cm. Green color forms are dark green in color, branches are thick and profusely branched with fragile tips; plants are smaller than the brown form and measure up to 20 cm. Pale yellow form are light yellowish in color, smaller than
the other two color forms, less branched. Branches very fragile and break even at moderate water current; the plant measures up to 14–16 cm. (Suresh Kumar et al., 2007)

18. Habitat

The plants occur in reef flat and reef edge, 1 to 17 m deep. Loosely attached to broken coral, or unattached fragments floating in shallow and deep waters. Can form large, moving mats of unattached thalli (Trono, 1993).

19. Geographical distribution

The two major commercial forms, *spinosum* and *cottonii*, are native to the Old World Tropics (Weber-van Bosse 1928; Laite & Ricohermoso 1981) westward to East Africa (Anderson 1953; Mshigeni 1982, 1984). A very small amount of the third, *gelatinae*, comes from the Philippines and China (Hainan Island and Taiwan). These three species of the genus are almost entirely restricted to the same brightly lit waters in which coral reefs form. *Eucheuma* is now being commercially produced in the far western Pacific. *Cottonii* has been introduced eastward through Micronesia into Kiribati and Tonga to the Society and Hawaiian Islands. *Kappaphycus alvarezii* has been introduced into Fiji and Hawaiian Islands, and also in Indonesia and China. *K. cottonii* is harvested along the coasts of Indonesia, Kenya, Tanzania, India, China, Japan, Vietnam, Malaysia, Guam and Fiji. On the other hand *K. striatum* is reported from the waters of Fiji and Hawai‘ian Islands, India, Kenya, Tanzania, Madagascar, Japan, China, Singapore and Indonesia (Guiry & Dhonncha, 2005).

20. Study of *Kappaphycus alvarezii*: Status today

Until recently in Hawaii, *Kappaphycus alvarezii* is found to reproduce sexually. Since its introduction to other localities, observations and studies have reported the propogation by vegetative fragmentation. At the tip of each branch is a cluster of apical cells potentially high in regenerative capabilities that are able to regenerate a new thallus after breaking off. A broken tip can grow into full-sized thalli in a short period of time. This
species has been highly successful at Kaneohe Bay, dominating the sandy spur and grooves on the reef flat. It inhabits barren sandy grooves where it does not appear to compete with native algal species. The red alga’s dispersal is thought to be constrained by size and weight, as it appears to become trapped in depressions and channels. The species is also constrained by high herbivory. *K. alvarezi* has managed to spread to neighboring reefs with supportive physical factors and little grazing, where it is dominating the changing marine ecology (www.hawaii.edu/reefalgae/invasive_algae/rhodo/kappaphycus_alvarezi.htm). It has been introduced throughout the warm tropics for commercial cultivation (c.f. Hurtado et al., 2001). It is a major producer of kappa-carrageenan, besides being a source of human food (Trono, 1993).

Apart from being used in various commercial products such as toothpaste, ice-cream, pet food and soft capsules, Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar has been instrumental in obtaining multiple products from *Kappaphycus alvarezi*. This process includes the following steps

1) Obtaining liquid seaweed fertilizer by crushing the fresh algae, Moreover, the granules obtained after extraction of LSF can be utilized for preparation of both refined and semi refined carrageenan (US Patent No. 6893479)

2) Alternatively, the fresh algae when dried yields a low sodium salt - Saloni K (US Patent No. 7,208,189).

3) Health drink prepared from the seaweed sap (Indian Patent No. 134 NF/2006).

4) Dried seaweed powder used as human food (Indian Patent No. 143 NF/2005).

On 11 May 2006, the President of India addressed the nation on the occasion of Technology Day 2006 at New Delhi, wherein he mentioned about this integrated technology. He also mentioned about employment generated for over 2000 families with an average income of over Rs. 6000 per month, as 40,000 rafts will be placed in the sea at Mandapam, Tamilnadu for cultivation. Its estimated number will go up to 1.5 lakhs by the end of 2007.
The present investigation focuses on the following objectives

- Cultivation of *Kappaphycus alvarezii*.
- Seasonal variation in biochemical and mineral composition.
- Analysis of biochemical and mineral composition of its color forms.
- Functional properties of protein concentrate.
- Antioxidant potential of solvent extracts.
- Phycoremediation using living and non-living biomass.

Table 1. Seaweed production (t fresh weight) in different countries during 1999 (FAO 2001).

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SEAWEED RESEARCH TIMELINE

2000-2007 Utilization of seaweeds for multiple product technologies

2000s Search for a model plant for a large-scale genomic study; shift to multi-disciplinary research

1997 Introduction of an EST approach in a large-scale study on molecular genetics.

1990s Genetic transformation and tissue culture; molecular phylogenetics; small-scale gene cloning and characterization.

1980s - early 1990s Mutant studies; seaweed cultivation and biotechnology; properties of hydrocolloids and their applications in industries; drug discovery and bioactive compounds from seaweeds; classical genetics.

1952 First International Seaweed Symposium in Scotland, UK


1940 Discovery of hydrocolloids from seaweeds; seaweed biology; research on seaweed as food.

Figure 1. A timeline highlighting important areas in seaweed research (Cheong-Xin et al., 2006)
Figure 2. Structure of agar

Figure 3. Structure of alginate

Figure 4. Types of carrageenans (structure).
Figure 5. A few carrageenophytes
References


