EXTRACTION AND CHARACTERIZATION OF SODIUM ALGINATE FROM BROWN SEAWEED

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DECLARATION

I hereby, declare that this dissertation contains my original research work. Sources of findings reviewed herein have been duly acknowledged.

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ABSTRACT

Sodium alginate was extracted from brown seaweed *Sargassum* sp. collected from Tanjung Simpang Mengayau, Kudat, Sabah. Extraction was carried out at different time (1-24 h) and concentration (0.05-0.50 M) of Na_2CO_3. The effect of acid (HCl 0.10 M) pretreatment on yield was also investigated. The IR spectra of all the extracts were determined and compared. The results showed that the optimum extraction time was 3 h, while percentage yield increased about five fold for ten-fold increase in Na_2CO_3 concentration. The alginate yield for 3 h extraction by 0.50 M Na_2CO_3 was 71.48 %. This yield increased to 79.84 % following acid pretreatment. All extracts exhibited identical IR spectra which showed characteristics absorption bands of sodium alginate.

PENGEKSTRAKAN DAN PENCIRIAN NATRIUM ALGINAT DARI RUMPAI LAUT PERANG

ABSTRAK

Natrium alginat diekstrak daripada rumpai laut perang *Sargassum* sp. yang diperolehi dari Tanjung Simpang Mengayau, Kudat, Sabah. Pengekstrakan dijalankan pada masa ekstraksi (1-24 jam) dan kepekatan (0.05-0.50 M) Na_2CO_3 yang berbeza. Pengaruh pra-rawatan asid (HCl 0.10 M) terhadap hasil juga dikaji. Spektra IR untuk kesemua hasil ekstrasi ditentukan dan dibandingkan. Keputusan menunjukkan bahawa masa ekstraksi yang optimum adalah 3 jam, manakala peratus hasil alginat meningkat sebanyak 5 kali apabila kepekatan Na_2CO_3 meningkat sebanyak 10 kali. Hasil alginat untuk ekstraksi 3 jam dengan 0.50 M Na_2CO_3 adalah 71.48 %. Hasil ini meningkat kepada 79.84 % berikutan pra-rawatan asid. Kesemua hasil ekstraksi memberikan spektra IR yang serupa, dan sepadan dengan ciri-ciri serapan pancaran IR natrium alginat.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	degree Celcius
%	percentage
α	alpha
β	beta
η	intrinsic viscosity
g	gram
h	hour
mL	mililitre
L	litre
M	mol/L
$Ca(Alg)_2$	calcium alginate
$CaCl_2$	calcium chloride
CDTA	cyclohexa-1,2-diaminotetraacetic acid
EDTA	ethylenediaminetetraacetic acid
EtOH	ethanol
FT-IR	Fourier transform infrared
HAlg	alginic acid
HCl	hydrochloric acid
KBr	potassium bromide
NaAlg	sodium alginate
Na_2CO_3	sodium carbonate
NaOCl	sodium hypochlorite
NMR	nuclear magnetic resonance

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CHAPTER 1

INTRODUCTION

1.1 Context and Relevance of Study

Alginate is a type of polysaccharide that possesses immense commercial value. Alginate is used widely in many industries including food, pharmaceuticals and textile printing. Their uses are based mainly on its thickening, stabilizing, and gelling as well as general colloidal properties (Barbaroux, 1990; Rajan, 2001; McHugh, 2003). The alginate of greatest industrial importance is the sodium salt (i.e sodium alginate). Alginate is derived from brown seaweeds, which can be found abundantly in cold temperate or tropical waters (Ahmad, 1995; Dawes; 1998; Rajan, 2001).

While any brown seaweed can be a source of alginate, the actual chemical structure of the alginate varies from one species to another. The alginate contents and quality of seaweeds depend on the species, season and source obtained (Laserna *et al.*, 1982; Sumera *et al.*, 1992; McHugh, 2002). The yield of alginate isolated from brown seaweeds also depends on the extraction method and conditions (Fasihuddin, 1989; Panikkar and Brasch, 1996; Calumpong *et al.*, 1999; Gillespie *et al.*, 1999; Hernández-Carmona *et al.*, 1999a; 1999b; Larsen *et al.*, 2003).

The coastal waters of Malaysia, including the state of Sabah, are noted for its abundant seaweed resources (Doty *et al.*, 1986). This is because Malaysia has an extensive coastline and coastal shelf that supports a prolific growth of seaweeds (Choo, 1990).

1.1 Objectives of Study

The objectives of this study are:

a. To extract sodium alginate from brown seaweed.

b. To determine the effect of extraction conditions on the yield of sodium alginate.

c. To characterize the sodium alginate obtained.

1.2 Scope of Study

The purpose of this study is to produce sodium alginate from brown seaweed collected from Kudat, Sabah. Sodium alginate is extracted using alkalization method with variations in extraction time and sodium carbonate concentration to determine the optimum conditions needed to produce the highest yield. Characterization of the sodium alginate is also carried out.

CHAPTER 2

LITERATURE REVIEW

2.1 Seaweeds

2.1.1 Introduction

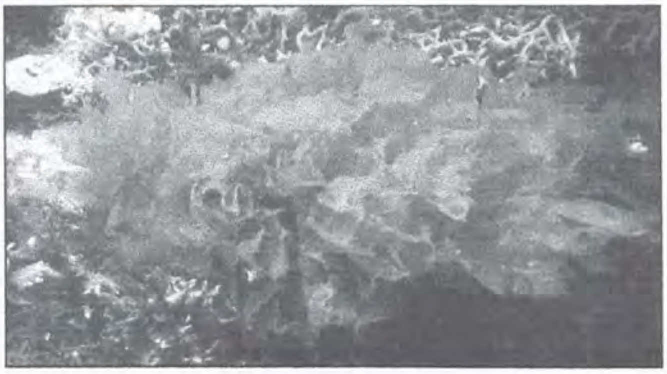
Linnaeus first introduced the term "algae" in 1754. Algae can be defined as "photosynthetic, nonvascular plants that contain chorophyll *a* and have simple reproductive structures" (Trainor, 1978). Algae are found in aquatic environments, being distributed in both fresh and salt water. There are about 30 000 species of algae known (Rajan, 2001). Seaweeds are called macroalgae, because of their macroscopic size, mutlicellular construction and attachment to firm substrata (Dawes, 1998). Therefore, the term "seaweeds" and "algae" are used interchangeably.

Traditionally, seaweeds are primarily classified into three major divisions based on the predominant or unique pigmentation. Botanists refer to these divisions as Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae) respectively (Dawes, 1998; McHugh, 2002). Nevertheless, for better classification of seaweeds, several evident characters are compared. These include photosynthetic pigments (chlorophyll, karotenoid and biloprotein), storage products or reserve food located in the cell, cell wall composition and structure, motality (number of flagellum and flagellar construction) and morphology of the thallus. Red seaweeds remains the largest division of marine algae, with 2540 Rhodophycean species listed worldwide, followed by 997 Phaeophycean species and 900 Chlorophycean species (Morris, 1988; Ahmad, 1995; Dawes, 1998).

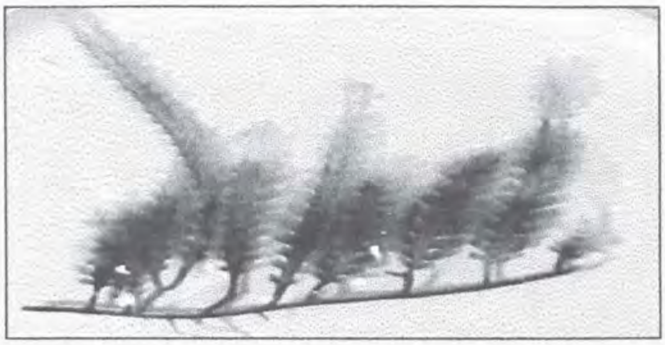
2.1.2 Division Chlorophyta (Green algae)

Green algae, though can be present in salt water, are more commonly located in freshwater and on land (Naylor, 1976; Rajan, 2001). Green seaweeds are classified under the class Chlorophyceae, which contains the majority of the species. Only 6 of the 15 orders of Chlorophyceae are marine species (Dawes, 1995). In general, green seaweeds are called so due to their deep green grass colour. This occurs because of the photosynthetic pigments, chlorophyll *a* and *b* arranged in plastids (chloroplasts). The cell wall is mainly built up of cellulose and pectin (Morris, 1988; Ahmad, 1995).

Among the common green seaweeds found is *Enteromorpha*. This tubular seaweed can be found in estuarine and oceanic waters. Species of the foliose *Ulva* is located as far as the artic to tropical regions whereas *Caulerpa*, which grows in tropical and subtropical waters, have thallus made up of multinucleated tubes (Figure 2.1). All species of *Caulerpa* possess rhizome that produces erect "blades" and rhiziods that penetrate soft sediments. Another interesting seaweed is the "coralline algae", *Halimeda*. Its thallus is impregnated with calcite calcium carbonate, thus giving it a coral-like structure (Dawes, 1998). *Enteromorpha, Ulva, Bryopsis,* *Caulerpa, Halimeda, Avrainvillea* and *Udotea* are the common green seaweeds found in Malaysia (Ahmad, 1995).



Ulva



Caulerpa

Figure 2.1 Examples of green seaweed.

2.1.3 Division Rhodophyta (Red algae)

Majority of red algae are distributed in marine environments while only about fifty species belong to freshwater. In addition to the pigments chlorophyll *a*, *c* and *d*, red seaweeds contains α and β carotene as well as phycoerythrin. Phycoerythrin, present in large quantities in plastids of red seaweeds are largely responsible for the red colouration (Ahmad, 1995; Rajan, 2001). However, red seaweeds are not always red, sometimes being purple or even brownish red, but they are still classified by botanists as Rhodophyceae because of other characteristics (McHugh, 2003).

Red seaweeds are usually small, generally ranging from a few centimetres to a metre in length (McHugh, 2003). They are widely distributed in all global oceans, including the Artic and Antartic. They grow attached to rocks and other substrata (Dawes, 1998; Rajan, 2001). The class Rhodopyhyceae is divided into two subclasses, namely Bangiophycidae and Florideophycidae (Morris, 1988). The genus *Gracilaria* records the most number of species, whilst *Gigartina* is one of the largest red seaweed found in North America (Figure 2.2). *Galaxaura, Amphiroa, Gracilaria, Hypnea, Laurencia* and *Acanthophora* are red seaweeds that can be found in Malaysian waters (Ahmad, 1995).

Gracilaria

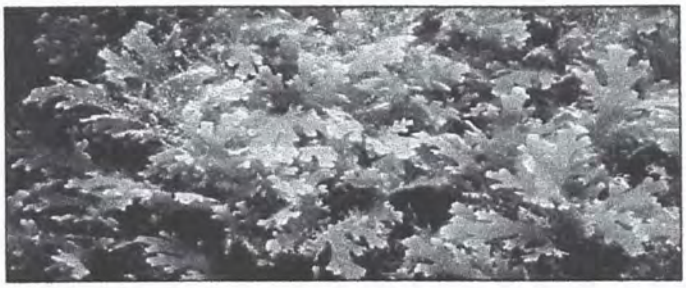
Gigartina

Figure 2.2 Examples of red seaweed.

2.1.4 Division Phaeophyta (Brown algae)

Brown algae are placed in a single class, Phaeophyceae, consisting about 1000 species that are almost exclusively marine. Brown seaweeds contain chlorophylls *a* and *c*, β carotene, and xanthophylls. The predominance of xantophyll pigments, namely fucoxanthin, neofucoxanthin and diatoxanthin in the chromatophore give off the brownish colour (Ahmad, 1995; Dawes, 1998; Rajan, 2001). Phaephyceae exhibits complex morphologies compared to other divisions of algae (Morris, 1988). The cell wall is made up of alginic acid, fucoidans and cellulose whereas reserve foods are of laminarin and mannitol (Zvyagintseva *et al.*, 1999). The plant body is multicellular whilst morphology of the thallus varies according to species. Reproduction of brown seaweeds could be sexual or asexual. Most species have air bladders, which help them keep afloat (Ahmad, 1995).

Brown seaweeds are mostly found in cold temperate waters, ranging from small filamentous forms (*Ectocarpus*) to massive intertidal rockweeds (*Ascophyllum, Fucus*) to subtidal large kelps (*Macrocystis, Nereocystis*). Many are sublittoral in distribution, up to the depth of 20-90 metres (Rajan, 2001). *Macrocystis* grows on rocky bottoms where it can be found as large underwater forests, with plants rising to and growing along the surface, at times up to 100 metres in length (McHugh, 2003). In tropical warm waters, these temperate species are replaced with the genera *Sargassum* and *Turbinaria*. *Sargassum* can occur as drift population, forming large floating masses, as such found in the Sargasso Sea (McHugh, 1987; Rajan 2001). The main genera found in Malaysian waters include *Dictyota, Lobophora, Padina, Sargassum* and *Turbinaria,* as shown in Figure 2.3 (Ahmad, 1995).



Dictyota

Lobophora

Padina

Sargassum

Turbinaria

Figure 2.3 Examples of brown seaweed found in Malaysia.

2.2 Applications of Seaweeds

Seaweeds have been known to human beings since ancient time, though their scientific study is merely recent. Seaweeds are used extensively in domestic, industrial and agricultural activities (Dawes, 1998; Trono, 1999; McHugh, 2003). In 1987, the world production of seaweeds was estimated at 324 3400 metric tons wet weight, of which brown seaweeds accounted for 67%, red seaweeds 30% and others 3% (McHugh, 1987).

2.2.1 Seaweed as a source of food

For centuries, seaweeds have been highly valued and widely consumed as a direct human food by oriental communities (Naylor, 1976; Rupérez, 2002). Presently, human consumption of seaweeds is high in Asia, particularly in Japan, China and Korea. Statistics shows that green seaweeds make up 0.4% of consumption, brown seaweeds, 66.5% and red seaweeds, 33.0% (Dawes, 1998). The three most cultivated edible seaweeds are species of *Porphyra* ("nori" or "purple laver"), *Laminaria* ("kombu") and *Undaria* ("wakame") (Ahmad, 1995; McHugh, 2002; Rupérez, 2002). Many seaweed species are also used as flavouring in soups and garnishing in food recipes (Trono, 1999).

Additionally, several species of *Caulerpa* and *Gracilaria* is eaten raw in Malaysia whilst *Euchema* is a favorite in East Malaysia and the Philippines (Ahmad, 1995; Trono, 1999). *Rhodymenia* or "dulce" is consumed in parts of North America and Ireland, where it is sold for use as a relish on meat and fish dishes (McHugh,

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