EXTRACTION OF KAPPA CARRAGEENAN FROM LOCAL EDIBLE SEAWEED

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EXTRACTION OF KAPPA CARRAGEENAN FROM LOCAL EDIBLE SEAWEED

SITI MAHIRA BINTI AHMAD

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

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2014

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

Signature :
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Date : 28 JANUARY 2014

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :
Name : SITI MAHIRA AHMAD
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Date : JANUARY 2014

Dedication

In The Name Of Allah Most Gracious, Most Merciful

To be able to thank Allah for a blessing is a blessing within itself.
~ Imam Shafie'

Love special dedicated to..

To my lovely parent, Ahmad B. Abdullah and Wan Maznah Bt. Mohd Daud for having faith in me before I learned to have faith in myself

To my brothers and sisters who had support me unconditionally in this journey

To my truly best friends who had gave me so much strength and support

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ABSTRACT

Kappa carrageenan is a polysaccharides which can be extracted from red seaweed such as *Eucheuma cottoni* species. This study report the extraction of kappa carrageenan from local edible seaweed. The effect of various concentrations of extracting solvent (KOH), time and temperatures on kappa carrageenan properties was studied. The KOH solutions (concentrations 0.03,0.05, 0.1M) were used as extracting solvent. Extraction process was carried out in oil bath with a constant ratio of seaweed weight to solvent volume (1:20 g/ml) at temperatures (80, 90, 100 ℃) for 1,2,3, and 4 hours. During extraction process the samples were stirred using magnetic bar and hot plate stirrer for 10 minutes for every half an hour. Then for the isolation process, the potassium chloride (2% w/v) was used to make the kappa carrageenan become precipitated. The precipitated carrageenan were left to dried at room temperature and were kept in desiccators until further analysis. Higher KOH concentration lead lower sulphate content and higher gel strength of extracted carrageenan. In terms of yield the 0.05M KOH, sample C2 (0.05M, 80℃ , 3h) showed the highest yield, while to get highest gel strength the best parameter is L3 (0.1M,100℃, 4h)

ABSTRAK

Kappa karagenan merupakan polisakarida yang diekstrak daripada rumpai laut merah dari spesies *Eucheuma cottoni* . Kajian ini menunjukkan kaedah pengekstrakan kappa karagenan daripada rumpai laut tempatan. Kajian ini melibatkan kesan penggunaan kepekatan larutan pengekstrakan (KOH), masa dan suhu yang berbeza terhadap ekstraks kappa karagenan yang terhasil. Larutan pengekstrakan yang digunakan ialah kalium hidroksida (kepekatan : 0.03M, 0.05M, 0.1M) . Proses pengekstrakan dilakukan menggunakan minyak dengan nisbah berat rumpai laut dan larutan pengekstrakan yang sama (1:20 g/ml) pada suhu (80, 90, 100 ℃) selama 1,2,3, dan 4 jam. Semasa proses pengekstrakan, sampel akan dikacau selama 10 minit bagi setiap setengah jam. Selepas itu, Kalium klorida (2% w/v) digunakan ketika proses pengasingan kappa karagenan daripada jenis karagenan yang lain. Mendapan kappa karagenan ini kan dibiarkan kering pada suhu bilik dan disimpan di dalam desiccators sehingga analisa seterusnya. Dari hasil kajian menunjukkan bahawa kepekatan kalium hidroksida yang tinggi membawa kepada pengurangan kandungan sulfur, dan peningkatan kekuatan gel yang terhasil. Dari segi hasil pengekstrakan, parameter 0.05M, sample C2 (0.05M, 80℃ , 3h) adalah yang tertinggi sementara untuk menghasilkan gel yang kuat parameter yang terbaik ialah L3(0.1M, 4jam, 100℃).

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LIST OF ABBREVIATIONS

W_1	weight of dried carrageenan of Equation 1
W_2	weight of white ash of Equation 1
w_0	initial weight of sampel of Equation 2
w_t	weight of swollen gels of Equation 2

LIST OF ABBREVIATIONS

KOH	Potassium hydroxide
KCL	Potassium chloride
PDA	Potato Dextrose Agar
TPC	Total Plate Count
$BaCl_2$	Barium chloride
$BaSO_4$	Barium sulphate

1 INTRODUCTION

1.1 Motivation and statement of problem

Many ingredients are added to various food systems in order to provide a wide selection of products for the consumer to choose from. Food hydrocolloids or food gums are added to food systems for numerous reasons, mainly to modify the texture, increase the stability, or reduce the fat or calories of a product. Specifically, food hydrocolloids are used to thicken, gel, control syneresis, stabilize an emulsion or suspension, function as a coating, and bind water. Use of food hydrocolloids continues to increase with recent development of low-fat and reduced-fat products as well as in the formulation of products in need of thermal or freeze-thawing stability. There are a variety of hydrocolloids on the market, including those derived from plants or seaweed, and those produced by microorganisms. Increasing numbers of products in the form of a blend of hydrocolloids are now available commercially for specific areas of applications such as reduced gelling points or increased viscosity. However, only limited information exists in the literature that fully characterized their applicability in mixture. It has been demonstrated that the structure of the hydrocolloid, including the type and number of monosaccharide backbone as well as the type, number, and distribution of side units, determines its characteristics and behavior in solutions. Moreover, the net charges on the polymeric side chains also play an important role in their functionality as well. In general, hydrocolloids have a sugar backbone that contains protruding substituents such as esters, sulphates, or additional sugars. Hydrocolloids available for food applications are either neutral or negatively charged (Sadar, 2004) .

Carrageenan, the third important hydrocolloid in the world after the starch and gelatine, occurs as matrix material in various species of red seaweeds (*Rhodophyta*). It is a negatively charged hydrocolloid derived from red seaweed plants, has been widely used in ice cream, chocolate milk, jellies, sauces and dessert gels (Sadar, 2004). Nowadays, the global market is based on three types of carrageenan, namely kappa carrageenan, lambda carrageenan and iota carrageenan. Kappa carrageenan forms strong , rigid gels when combined with potassium ions in the mixture while iota forms weak elastic gel, the lambda carrageenan is non-gelling type (Mustapha *et al*., 2011).

Common use of carrageenan is as a food additive, but the researcher now has increased attention of its value to the pharmaceutical industries, as an alternative choice of making vegetarian capsule, which is animal free.

Nowadays, gelatine is one of the widely used raw material in food (thickening agent), pharmaceutical (gelatine capsule) as well as cosmetics product (creams, lotion). In the pharmaceutical industry, gelatin is used as hard and soft capsules, sugar-coated pills, tablets, serum substitute and vitamin encapsulation. The use of gelatine an all rounder. It will increase the chance of Muslims and vegetarian to exposed to animal based gelatin (Sahilah *et al*.,2012). It can be seen that, kappa carrageenan has the same characteristic with gelatin in terms of strength and elasticity. Thus it is the most suitable substance to use as alternative gelatine.

A good source of kappa carrageenan is *Eucheuma Cottoni*, which have been harvested commercially in Sabah, Malaysia (Distantina *et al*., 2011). Hence this research problem statement is to extract kappa carragenan which is a good source to produce strong and rigid gel with high yield by modifying various parameters, as alternative to replace gelatine is high demand among Muslim and vegetarian. To enhance the gelling properties it needs to be mixed with alkaline solvent, normally potassium hydroxide (KOH) will be used as a extracting solvent., a well known method to extract the carrageenan. Furthermore, some physico-chemical of the seaweeds is also being investigated in order to evaluate their potential use for other product.

1.2 Objective

The following is the objective of this research:

- To study the operating conditions of extracting solvents (KOH) on kappa carrageenan from local seaweed.

1.3 Scope of this research

The following are the scope of this research:

i) To investigate the effect of different concentrations of extracting solvent (KOH) (concentrations: 0.03, 0.05 and 0.1 M) that will be used to extract kappa carrageenan

ii) To investigate the effect of different temperature (80, 90 and 100°C) on kappa carrageenan

iii) To investigate the effect of different extracting time (1h,2h,3h 4h) on kappa carrageenan

1.4 Main contribution of this work

This study will provide data for the best operating condition of extraction of obtaining kappa carrageenan that can be used for further research. Thus help to increase seaweed processing as there are high demand of kappa carrageenan in the market especially in food and pharmaceutical industries. Other than that, it indirectly will help people to cultivate socio-economy development in rural area, particularly in Sabah.

2 LITERATURE REVIEW

2.1 Seaweeds

Marine macroalgae, also known as seaweeds, are not true plants, as they does not have flowers or any clearly marked steam or leaves. They also does not have true roots but held fast which does not absorb food but simply attaches the plant firmly to a stone or rock. In some stage in their life cycles , all seaweeds are unicellular, as spore or zygotes, and may be temporarily plank tonic (Fard, 2009).

Seaweeds are differentiate by their pigmentation, morphology, anatomy and nutritional composition (Benjama *et al.*, 2011). There are over 9,000 species of seaweeds which can be catagorized into three major types, they are : red (*Rhodophyta*), brown (*Phaeophyta*) and green (*Chlorophyta*) (Figure 2.1). Among these three types, red seaweeds are the most rich group (6,000) followed by brown (2,000) and lastly green (1,200) (Fard, 2009).

Figure 2. 1: Three different types of seaweed (a) *Rhodophyta* (b) *Phaeophyta* (c) *Chlorophyta*

Source: CP Kelco ApS , 2001

Around 250 seaweeds species have been commercially utilized worldwide and about 150 species are favourably consumed as human food. In most Asian countries, seaweeds have been consumed since ancient times as source of human food, animal feed and fertilizer. High consumption of brown (66.5%), red (33%) and green (5%) is in Japan and China compared to other Asian countries. While in Western countries seaweed polymers are used as a source of hydrocolloids, thickening, and gelling agents in food and pharmaceutical industries (Benjama *et al.*, 2011) .

According to McHugh (2003) other countries such as Republic of Korea, the United States, South America, Ireland have showed increasing in terms of consumption, production and marketing of seaweeds. About one million tonnes of wet seaweeds were harvested in 35 countries as a source of food, polysaccharides, fertilizer, fuel and cosmetics annually. However in Malaysia, seaweeds are not common to consume as a food. Seaweeds is only consumed in certain coastal areas especially along the east coast of peninsula Malaysia, where it is used in salad dish (Fard, 2009).

Seaweeds is a good source of polysaccharides, protein , vitamin, minerals and dietry fiber (Cox *et al*.,2011). Due to that , they have been recognized to give a huge beneficial to human as well as animal health. The content of nutrients in seaweeds are greatly depending on the species, habitats, maturity and environmental conditions (Benjama *et al.,* 2011) .

2.2 Polysaccharides as stabilizing agents

Polysaccharides are well known additives in the food industries as their had the capabilities of gel forming and thickening agent. They are commonly extracted from seaweeds which yield carrageenan. In market, there are types of carrageenan namely kappa, iota and lambda. Others source of polysaccharides that used as stabilizing agents are dextran and xanthan which are produced by microbial fermentation (Glickman, 1969). There are some stabilizer that are derived by chemical modification of natural products such as low-methoxy-pectin, methylcellulose and carboxymethyl starches (Glickman, 1969).

These gums are used to stabilizer or to improved the body of such products as jams, sauce and mayonnaise is due to their thickening affect. Gel formation which is a property of relatively few polysaccharides is the basis of their application as gelling agents in products, for examples, chocolate milk, puddings and mousses. The properties and applications of gums are reviewed by Whistler and Be Miller (1959) and Glickman (1969). In this thesis special attention will be paid to the properties of kappa carrageenans and to the mechanism on which their application in the food and pharmaceutical industries.

2.3 Carrageenan

A. Origin and chemical composition

The carrageenan is a water soluble hydrocolloid which is come from seaweed. The content of carrageenan can be vary based on type of species and seasonally and its can be found in the cell wall of the seaweed from which it is derived. It is harvested from various regions of the world including the northern part of the US, Philippines, Indonesia, Chile, Argentina, Morocco and France. There are 3 species that are commonly used commercially, they are *Eucheuma*, *Chondrus Crispus* and *Gigartina.* The main species of *Rhodophycae* used in commercial production of carrageenan are *Eucheuma cottonii* and *E.spinosum*. these are spiny bushy plants, approximately 50cm in high, which grow on reefs and in shallow lagoons (Philips *et al*., 2000).

Carrageenan is polymer consists of repeating liner chains of galactans units with negative charge of numerous ionic sulphate half-ester groups. The repeat unit is a dimer of galactose and anhydogalactose linked by a beta 1, 4 glycosidic linkage (Figure 2.2) . These dimmers are then linked together through alpha 1,3 glycosidic linkage. This secondary structure expect the chair conformation to minimize steric repulsions caused by axial components (Fisher, 2009).

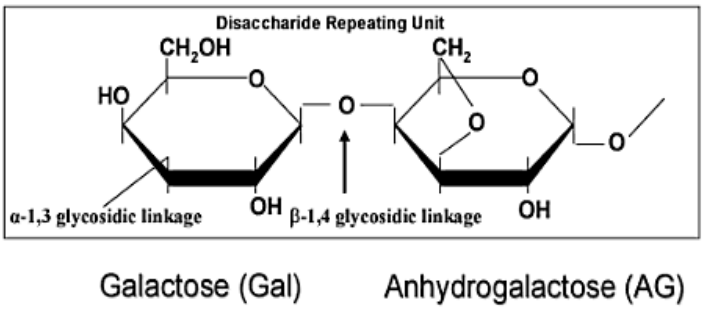

Figure 2. 2:1, 4 glycosidic linkages between galactose and anhydrogalactose monomers in carrageenan

Source: Fisher , 2009

B. Types of Carrageenan

There are three forms of carrageenan, namely kappa, iota and lambda. Each carrageenan is determined by the number and position of the sulphate groups on each sugar and the presence or absence of the 3,6 anhydro group on the B monomer. The 3,6 anhydro group promotes ∝ helix formation which is important for gelling. This due to increase flexibility that promotes a random coil structure.

Figure 2.3 shows kappa carrageenan contains one sulphate group per repeat dimer, located on the O-3 galactose ring. It has structure is right-handed double helix of parallel chains, due to this structure, kappa carrageenan forms durable thermoreversible gels by itself. In the presence potassium salts it forms even more strong and rigid gels.

A right handed double helix of parallel chains contains two sulphate groups per repeat dimer in iota carrageenan located one on each of the sugar units. It will form strong, elastic , thermoreversible gels with limited syneresis. When it mix with calcium forms ionic bridges between iota carrageenan chains, yielding gels with increased gelling and melting temperatures. While for lambda carrageenan, non-gelling carrageenan , it does not have 3,6 anhydro group necessary to form the double helix. It just consists of three sulphated groups with repeat dimer units of D-galactose-2-sulphate-D-galactose-2, 6-disulphate. The molecular structures of kappa, iota and lambda carrageenan was shown in Figure 2.3 (Fisher, 2009).

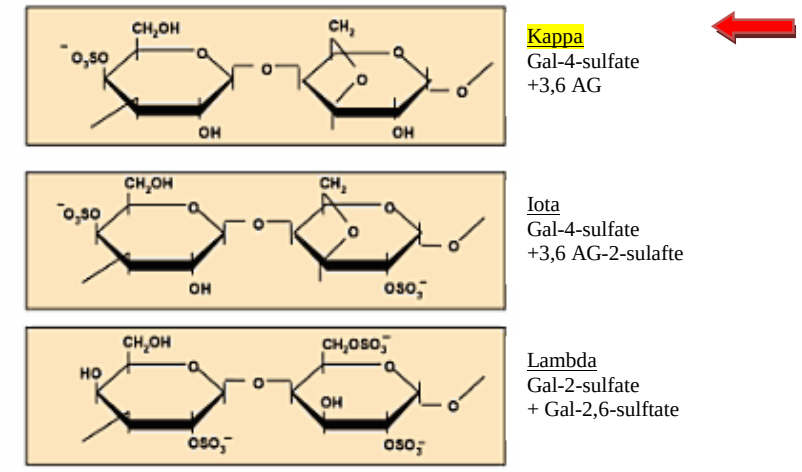

Figure 2. 3: Molecular structures of kappa, iota and lambda carrageenan.
Kappa carrageenan (red arrow) is use in this study.

Source: Fisher , 2009

C. Bioactive component in kappa carrageenan

There are several types of bioactive components of seaweed, mainly are polysaccharides, antifungal ,anti-microbial and antioxidant. One of the examples of polysaccharides is carrageenans which is considered as a nutraceutical components (Riouxn *et al*., 2009). According to Plaza *et al* (2009) sulphated polysaccharides also known as carrageenan contain bioactive component such as anti-viral, anti-tumor, antihyperlipidemia, and anti-coagulant, reduce total and LDL cholesterol.

D. Modes of molecular interaction in kappa carrageenan

1. Gelling

Gelling properties in kappa carrageenan is one of its great properties widely used in food industries. The tertiary structure of the carrageenan type is thought to dictate gelation as local regions of ordered molecular associations aggregate to form a disordered polymer network. Whether gelation can occur is highly dependent on the concentration and type of carrageenan, and generally kappa carrageenan require heat about 80℃ to compeletly solubilized. The polymer chains are released into a colloidal state once it solubilized (Figure 2.4). When the carrageenan solubilized it has the negative charged sulphate groups all along the polymer chain, which stimulate the repulsion that forbid chain folding and intermolecular associations. Intermolecular interaction occur when the cation interaction neutralize the negative sulphate groups. When the carrageenan solution get cools, intramolecular hydrogen bonds stabilize the ∝ - helix conformation in individual carrageenan chains and intermolecular hydrogen bonds stabilize the formation for the double and triple helices between carrageenan chains (Fisher ,2009) .

Gelation of solubilized carrageenan polymers

$\boldsymbol{K}^{+}$ promote ∝-helix associations and double helix formation

$\boldsymbol{Ca}^{2+}$ form ionic bridges between (-OS$\boldsymbol{O_3^-}$)

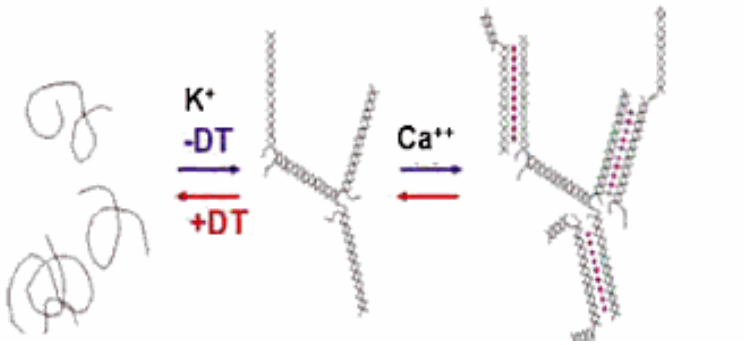

Figure 2. 4:Molecular associations involved in the gelation of carrageenan

Source: Fisher ,2009

As the kappa carrageenan solution cools, firstly the random coil will form double helices before the aggregation occurs (Figure 2.5). When the same gel is reheated, the process reverses, begin with dissociating the aggregates then by restoration

of random coil. According to Fisher (2009) extraction of kappa carrageenan at various concentration and temperatures using small angle X-ray scattering found that kappa carrageenan formed two to three double helices during gelation. Eventually this will promotes tighter and more extensive molecular aggregation and yields a rigid.

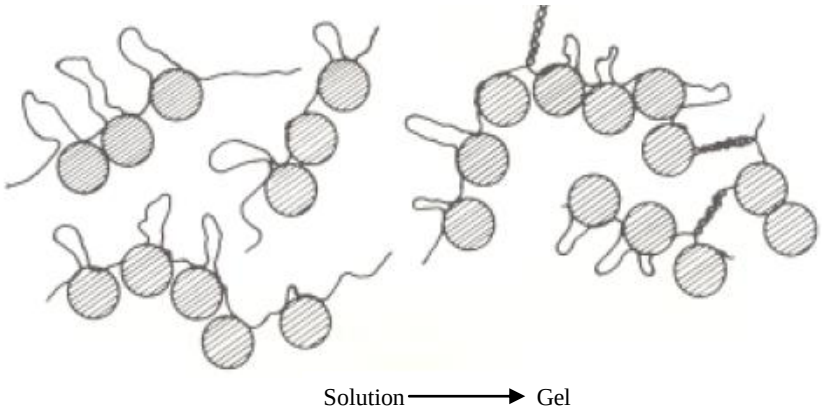

Figure 2. 5: Gelation of carrageenan

Source: Cp Kelco ApS, 2001

Gel promotion is to increase ionic interaction which increase intermolecular associations and change gel transition temperatures. Fisher (2009) found that kappa carrageenan (0.7 to 1.4 %) solution will form weak gels without addition of potassium ions. Kappa carrageenan gelation in various salt showed that $K^+ > Ca^+ >> Na^+$ in effectively increasingly gelling rate, gel melting temperature and gel strength. He also found that thermal transition of kappa carrageenan solution with and without KCl also showed that the conversion temperature from coil to helix and vice versa was higher with addition of KCl. Potassium chloride has the highest effect on gel strength per potassium unit but other potassium salts may be used for taste considerations. Potasium ions also have the effect of incresing the melting and gelling temperatures as illustrated in Figure 2.6 (Cp Kelco ApS, 2001).

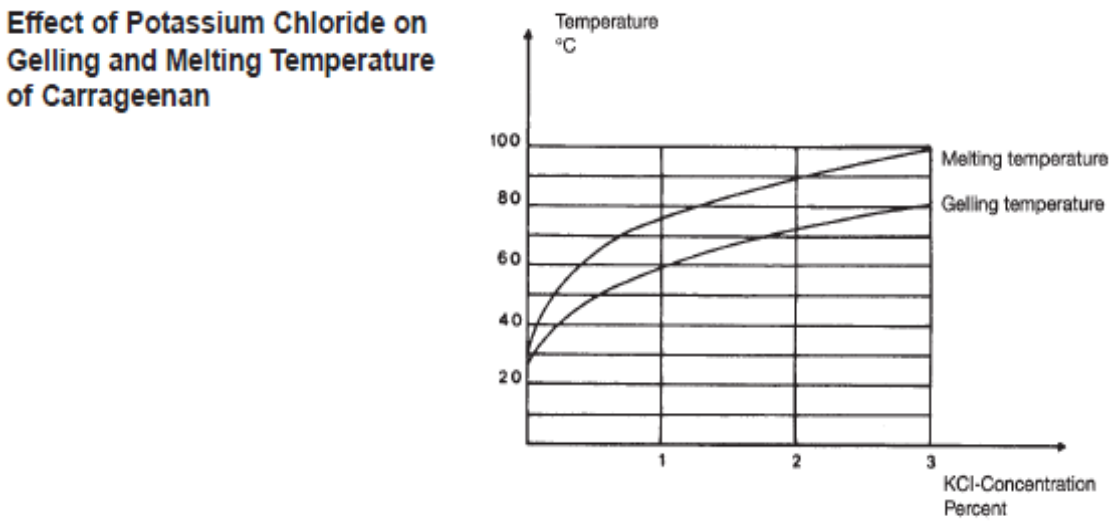

Figure 2. 6:Effect of potassium chloride on gelling and melting temperature of carrageenan

Source: Cp Kelco ApS, 2001

It is believed that barium ions form bridges between adjacent double helices through an electrostatic binding to two adjacent sulphate groups, thus stabilizing and strengthening the network (Figure 2.7). When removing cations which cause gelation of carrageenan from the medium as well as from the carrageenan, a solution of carrageenan is obtained which does not form a gel irrespective of the temperature. As soon as gelling cations are present the carrageenan solution will gel at a specific temperature, the gelling temperature. Thus, the gelling temperature of a carrageenan solution is a function of the concentration of gelling cations present in the system (Cp Kelco ApS, 2001). In this research the barium chloride is used in analysis of yield.

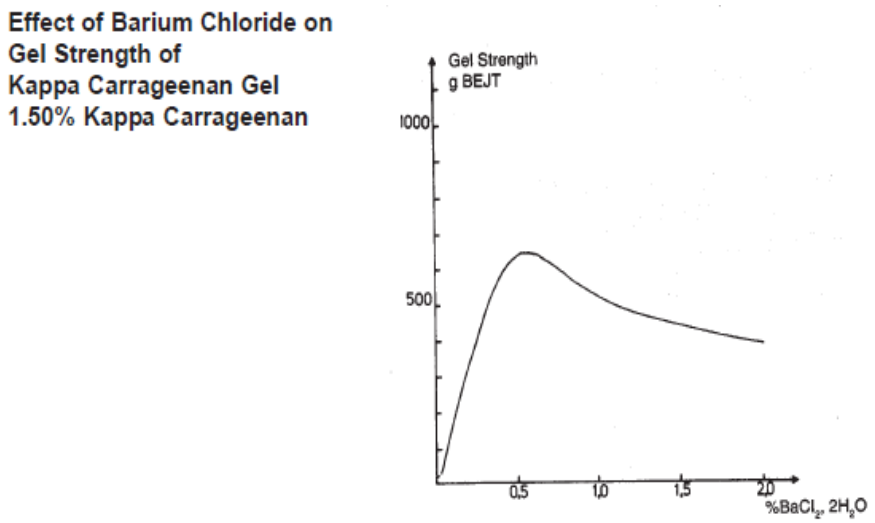

Figure 2. 7:Effect of Barium Chloride on gel strength of kappa carrageenan gel

Source: Cp Kelco ApS, 2001

E. Extraction of kappa carrageenan

Extraction in certain red seaweed of the Rhodophyceae class consists of carrageenan, a sulphated linear polysaccharide. It is used in the food industry as thickening, gelling agent and recently it has been used as excepient in pill and tablets (Distantina *et al*., 2011). Currently, global market based on three types of carrageenan namely kappa carrageenan, iota carrageenan and lambda carrageenan. Among these three, kappa carrageenan will forms strong, rigid gel after combined with potassium hydroxide solution (Mustapha *et al*.,2011). A summary of the solution and gelation properties of carrageenan and its synergy with other materials is given in Table 2.1 (Philips *et al*., 2000).

Table 2. 1:Summary of carrageenan properties

	Lambda	Iota	Kappa
Solubility			
Hot (80°C) water	Soluble	Soluble	Soluble
Cold (20°C) water	All water soluble	Na^+ salt soluble Ca^{++} salt gives thixotropic sols	Na^+ salt soluble Limited swelling of K^+, Ca^{++} salts
Hot (80°C) milk	Soluble	Soluble	Soluble
Cold (20°C) milk	Thickens	Insoluble	Insoluble
Cold milk (TSPP added)	Increased thickening or gelling	Thickens or gels	Thickens or gels
50% sugar solutions	Soluble	Insoluble	Soluble hot
10% salt solutions	Soluble hot	Soluble hot	Insoluble
Gelation			
Effect of cations	Non-gelling	Strongest gels with Ca^{++}	Strongest gels with K^+
Gel texture	–	Elastic	Brittle
Shear reversible gel	–	Yes	No
Syneresis	–	No	Yes
Hysteresis	–	5–10°C	10–20°C
Freeze-thaw stable	Yes	Yes	No
Synergy with locust bean gum	No	No	Yes
Synergy with konjac flour	No	No	Yes
Synergy with starch	No	Yes	No
Salt tolerance	Good	Good	Poor
Stability in acid	Hydrolysis	Hydrolysis of solution, accelerated by heat Gels are stable	
Protein reactivity	Strong interaction increasing at acid pH		Specific reaction with kappa-casein

Source: Philips *et al*., 2000

Figure 2.8 (a) show that kappa carrageenan selects for potassium ions to stabilize the junction zones within the characteristically firm, brittle gel. While, iota carrageenan selects for calcium ions to bridge between adjacent chains to give typically soft elastic gels as shown in Figure 2.8 (b) (Philips *et al*., 2000).

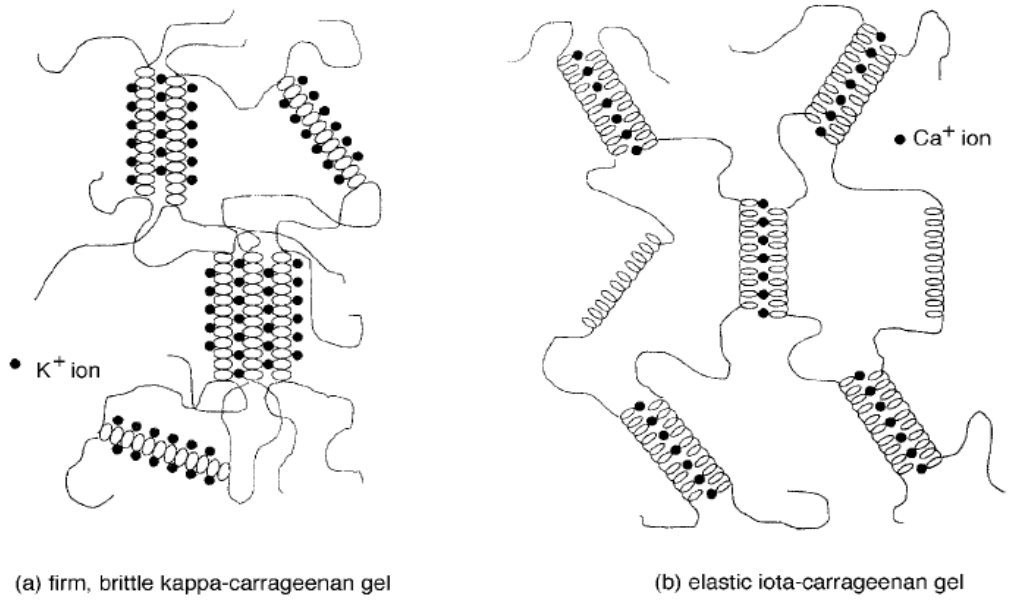

Figure 2. 8:Gelation of kappa and iota carrageenans with cations

Source: Philips et al., 2000

Hot solutions of kappa and iota carrageenans set to form a range of gel textures when cooled to between 40 and 60ºC depending on the cations present. The ionic composition of a food system is crucial for effective utilization of the carrageenan. The seaweeds are usually extracted with alkali at high temperature (Distantina *et al*., 2011). Characteristically, solution contain 1-2 % agar by weight will gel at about 35°C and melt at about 85°C, agar gel produce are strong and brittle (Laurienzo, 2010). The potassium hydroxide (KOH) is used as the extracting solvent because there is a significant relation between gel strength and KOH concentration as illustrated in Figure 2.9 (Distantina *et al*., 2011).

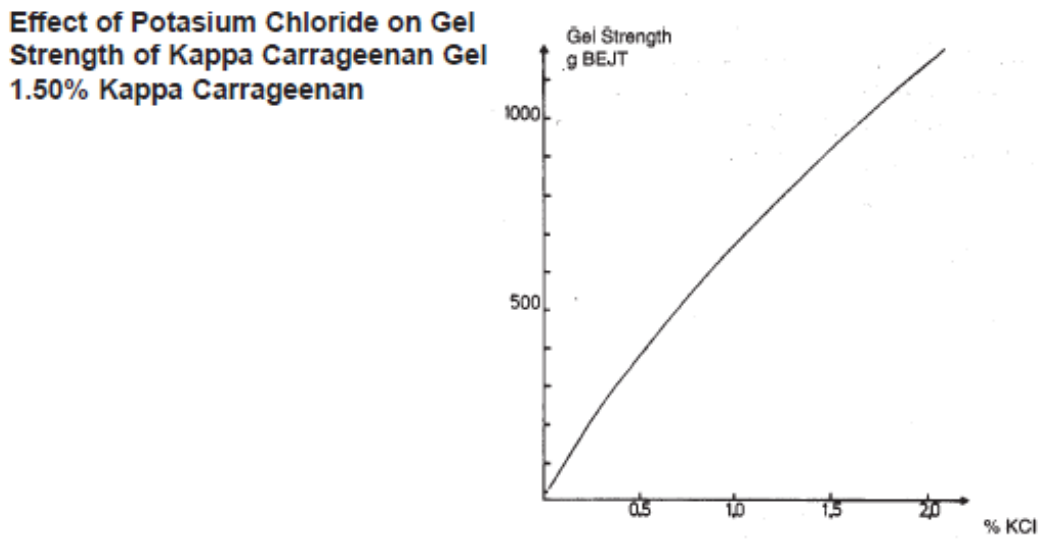

Figure 2. 9:Effect of potasium Chloride on gel strength of kappa carrageenan

Source: Cp Kelco ApS, 2001

Based on other research that comparing of using potassium hydroxide (KOH) and sodium hydroxide (NaOH) as extracting solvent showing that KOH is more preferable because of greater yield and lower losses KOH solution as extracting media compared to NaOH (Tuvikene *et al*., 2006). According to Mustapha *et al*. 2011, selection of Ca$(OH)_2$ as extracted solvent showing no gel formation in all test conditions. By utilizing this research data, it is known that selecting KOH as extracting solvent to extract kappa carrageenan will enhance the gelling properties of seaweed.

2.4 Applications of kappa carrageenan

Carrageenan has been widely used in food industry as it has various uses. The basic carrageenan types may be used individually or mixed with others to form blends. The food applications of carrageenan gum have been divided into dairy based and water based topics (Sadar, 2004). There are also non-food applications of carrageenan (CP Kelco ApS, 2001).

A. Dairy Based Application

Carrageenan is use in milk such as flans and custards that imparts a creamy mouthfeel and reduce the syneresis. In product such as pudding and pie fillings carrageenan function to reduce the amount of starch, minimizing syneresis, as well as modifying the texture of final product. Carrageenan gum is widely used in diary product especially in ice cream or ice milk. It will control the ice crystallization as well as whey separation. Other product that used carrageenan is chocolate, eggnog and fruit flavoured pasteurized milks. In cocoa, the carrageenan will suspended particles as it give a rich mouthfeel by adding thickness to the product. Another product carrageenan is added is in calorie milk, it will control calorie milk drinks in order to prevent fat particles from settling out as well as adding a rich mouthfeel.

Carrageenan is also used in creamed cottage cheese, it provide the ability of the creamy mixture to cling on the cottage cheese curd and became stable. It is used in process cheese to give the final product good mouthfeel characteristic, good grating, melting and slicing properties. Addition carrageenan into evaporated milk can prevent fat separation and infant formulas it is added to stabilize proteins and fats. While in whipped cream and yogurt the carrageenan help to stabilize and suspend (Sadar, 2004). Table 2.2 shows the typical applications of kappa carrageenan in dairy products.

Table 2. 2: The typical applications of kappa carrageenan in dairy products

Use	Function	Carregeenan type	Use level (%)
Ice cream, ice milk	Whey prevention	Kappa	0.01-0.02
Cooked flans	Gelation, mouthfeel	Kappa, kappa+ iota	0.2-0.3
Cold prepared custards	Thickening, gelation	Kappa, iota, lambda	0.2-0.3
Pudding and pie fillings	Reduced starch, lower burn-on	Kappa	0.1-0.2
Aerosol cream	Stabilise overrun, emulsion stabilisation	Kappa	0.02-0.05
Chocolate milks	Suspension and mouthfeel	Kappa	0.015-0.03
Evaporated milk	Emulsion stabilisation	Kappa	0.005-0.015
Cheese slices and blocks	Improve slicing and grating	Kappa	0.5-3.0
Soy milk	Suspension and mouthfeel	Kappa + iota	0.02-0.04

Source: Philips *et al*., 2000.

B. Water Based Applications

Carrageenan is used in various products such as dessert gels, cake glazes and low calorie jellies for the main purpose of controlling syneresis. Other than that, it can provide elastic and cohesive texture. There are many benefits of using carrageenan in food, such as it can improves water retention, cooking yields, slicing properties, mouthfeel and succulence in canned meat product, luncheon meats and pet food. In chocolate syrup and salad dressings, carrageenan help to suspend particles like cocoa in chocolate syrup and herbs and spices in salad dressings. While in artificial milk and creams, carrageenan adds body and stabilizes the emulsion. All these products are based on the firm, brittle gels properties of kappa carrageenan. Some of the typical application for kappa carrageenan in water are shown in Table 2.3.

Table 2. 3: Typical applications for kappa carrageenan in water

Use	Function	Carrageenan type	Use level (%)
Dessert gels	Gelation	Kappa + iota	0.5-1.0
Low calorie gels	Gelation	Kappa+iota	0.5-1.0
Non-dairy puddings	Emulsion stabilisation	Kappa	0.1-0.3
Syrups	Suspension, bodying	Kappa, lambda	0.3-0.5
BBQ and pizza sauces	Bodying	Kappa	0.2-0.5
Whipped toppings	Emulsion stabilisation	Kappa, iota	0.1-0.3

Source:Philips *et al*., 2000.

Newly improvement in the combinations used for these applications has produced vegetarian products which have a similar appearance and texture to traditional gelatine products with additional advantages of fast setting and stability at ambient temperatures (Sadar, 2004). An example recipe is shown in Table 2.4.

Table 2. 4:Formulation fruit-flavoured water dessert jelly

Ingredients	%
Sugar	15.00-20.00
Carrageenan (kappa-iota blend)	0.60-0.90
Potassium citrate	0.20-0.35
Citric acid	0.30-0.45
Colour	As required
Flavour	As required
Water	To 100.00
Total	100.00

Source: Philips *et al.*, 2000.

The same gels are also used aspic and gels in canned meats and petfoods and in cooked sliced meats. In these latter products the carrageenan is incorporated to improve moisture retentation, cooking yields, slicing properties and mouthfeel and succulence (Sadar, 2004). Table 2.5 shows a typical formulation for a 30% added-water sandwich ham.

Table 2. 5:Cooked ham with 30% added brine

Ingredients	**%**
Meat, lean ham muscles	62.50
Carrageenan (firm gelling kappa)	0.60
Sodium tripolyphosphate	0.50
Nitrate salt	1.67
Sodium chloride	0.53
Dextrose	1.2
Water	32.95
Total	100.00

Source: Philips *et al*., 2000.

Processed *Euchema* seaweed is principally used in cooked sliced meats. It is very cost effective material and it disperses readily without lumping in meat brines. Some differences can be seen during processing between processed *Euchema* and traditional carrageenan extract. The small particles do not swell in the brine and may reduce damage when injected into the meat. The existing of cellulose network in processed *Euchema* seaweeds lessen the rate of hydration during heating so that solutions develop viscosity after heating with high temperature and long hours. A lower rupture strength with a less cohesive and more fragile structure are due to the presence of the cellulose in the finishing gel (Philips *et al*., 2000). Even though carrageenan has many uses but it still has limitations, one of it is the carrageenan instability at low acidity. If the carrageenan is exposed to a pH at around 4.5 or below it loses gel strength (Sadar, 2004).

C. Vegetarian Capsule (A new approach)

Nowadays, gelatin is becoming one of the widely used raw materials in foods, pharmaceutical (gelatin capsules) and cosmetic products (creams, face masks, lotions), which could be extracted from bones, fat, meat waste, used cooking fats and oils of animals (Sahilah *et al.*, 2012). In general, there are two types of gelatins that available in the market, which are type A, obtained from pork skin by hydrolysis with an acid and type B comes from bones and animal skin by hydrolysis with an alkaline solution mainly deep-water fish. Guillen *et al*., (2009) claimed the most abundant sources of gelatin come from pig skin (46%), bovine hide (29.4%), pork and cattle bones (23.1%) and other which include fish gelatin (1.5%).

In the pharmaceutical industry, gelatin is used as hard and soft capsules, sugar-coated pills, tablets, serum substitute and vitamin encapsulation. The benefits of using gelatin is because it helps to protect the medicines against harmful influences, such as light and oxygen. The soft capsules are mainly used for liquid fillings, while hard capsules are used for powders. Thus, the use of gelatin is an all rounder. Chances of Muslims exposed to haram gelatin are becoming greater (Sahilah *et al.*, 2012). There are increasing needs of non-animal based forms gelatine particularly by vegetarian, ethical or religious reasons. A vegetarian gelatine that available is costlier than the animal-sourced gelatine. It can be seen that, kappa carrageenan has the same characteristic with gelatin in terms of thermoreversible, strength and elasticity. Thus it is the most suitable substance to use as alternative gelatine.

D. Non-food Applications

Gel formation, thickening effect, film forming ability, and diffusion rate in carrageenan gels are some of the propeties which make carrageenan suited in many non-food applications. The ability of binding water effectively and forming weak water gels which are very stable against enzymatic degradation makes carrageenan unique as a thickener in toothpaste, the gel imparting excellent stand up of the paste and excellent flavour release and rinsability. The film forming ability of carrageenan makes carrageenan an excellent conditioner in shampoo, as well as a suitable tablet coating agent. The ability to form strong water gels in which solutes diffuse rapidly makes carrageenan a possible gelling agent for immobilization of enzymes and living cells. Special carrageenans, which are chemically gelled without applying heat may find use as gelling agents for solid bacteriological media (Cp Kelco ApS, 2001).

3 MATERIALS AND METHODS

3.1 Overview

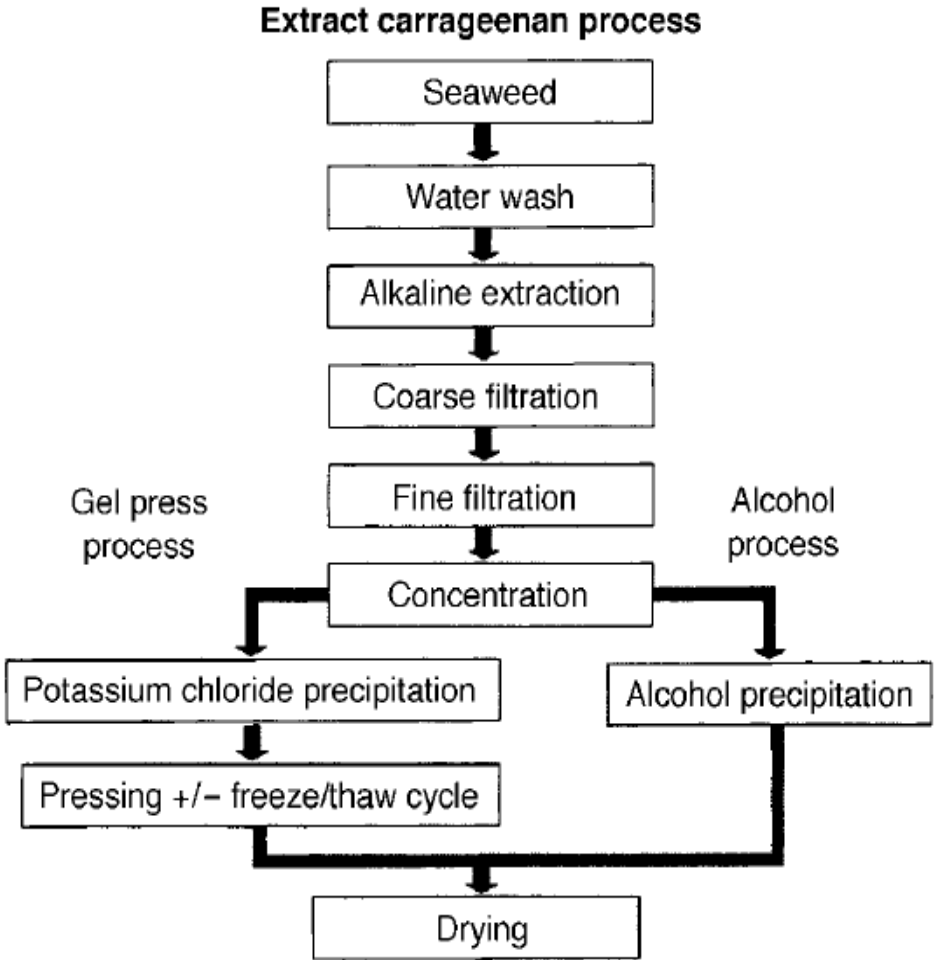

Figure 3. 1: Extract carrageenan process

Source: Philips *et al.*, (2000)

The overall process to extract seaweed is illustrated in Figure 3.1. Carrageenan can be separated not only by using KCl precipitation but also with alcohol. Using alcohol precipitation and freeze-thaw process are high cost, thus for this study, the gel press method is chosen compared to the alcohol process because the use of KCl as a precipitating agent is relatively cheaper compared to other methods. The K+ ion could increase gel strength, but if excessive, K+ ion could decrease gel strength, the optimum KOH concentration as extracting agent has to be investigated as well the temperature and time of extraction.

3.2 Chemicals

Potassium Hydroxide (KOH), Potassium Chloride (KCl), Hydrochloric Acid (HCl), Barium Chloride (BaCl) , Nutrient Agar, Potato Dextrose Agar (PDA) were purchased from Sigma-Aldrich.

3.3 Seaweed material

Red seaweed (*Eucheuma Cottoni*) (Figure 3.2) was purchased from Sabah. Seaweed were washed to remove sand and reduce the salt content using tap water, and then cut into $\pm$ **1** $\boldsymbol{cm}$ length, and later dried in the oven at 60℃ for two days untill constant weight (Distantina *et al*., 2011). The process seaweed samples were kept in a dried state inside the desiccators until analysis.

Figure 3. 2: *Eucheuma cottoni*

3.4 Alkaline Extraction

Carrageenan extraction was performed as described by Mustapha *et al*., (2011) with modifications. Pretreated seaweeds were extracted using three different concentrations of KOH (0.03, 0.05, 1.0 M) at different temperatures of 80, 90 and 100°C and various extraction time (1h, 2h, 3h and 4h) . During extraction process the samples were stirred using magnetic bar and hot plate stirrer (Figure 3.3) for 10 minutes for every half an hour. Throughout the experiments the ratio of seaweed to solvent volume (1:20 g/ml) were remained constant. Extraction was performed in the oil bath as a heating medium at a specific temperature, as stated.

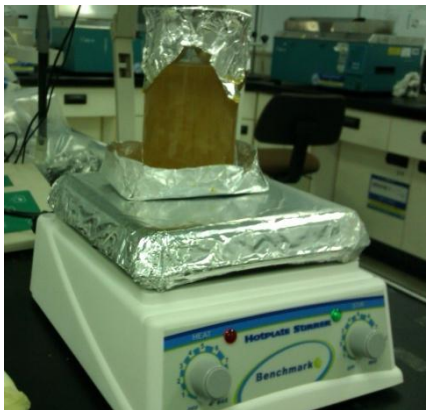

Figure 3. 3:Alkaline extraction

3.5 Isolation

After extraction, filtrate was separated from its residue by using filter cloth. It then immediately poured into a 2% (w/v) concentrated solution of potassium chloride (KCl) which lead to precipitation process(Basmal *et al*., 2009) (Figure 3.4). The precipitated mass then undergo filtration (Figure 3.5) and dewatered proses under pressure to make 'gel press' carrageenan. To isolate kappa carrageenan from other component, such as iota. In the gel pressing method, the gel was cut and put between two cloths. Then will be squeezed between absorbent papers and cardboards for 2 days (the papers were changed regularly until the water was completely removed, the gel was then leaved to dry at room temperature (Istina *et al*.,1994).


Figure 3. 4:Mixture of seaweed extract and KCl to form precipitate

Figure 3. 5: Precipitation of Kappa carrageenan was filter from KCl solution

3.6 Analysis of yield

Yield is a ratio of dried carrageenan weight to dried seaweed weight. Percent sulphate content was determined using the method of sulphate hydrolysis followed by precipitation of sulphate as barium sulphate . A known amount of dried carrageenan (W1, g) was hydrolyzed with 10 ml 1N of HCl for 6 minutes at boiling temperature. 2 ml BaCl2 0.25M was added dropwise within boiling temperature for 3 minutes. The BaSO4 precipitates (Figure 3.7) were filtered using using ashless paper filter (Whatman no. 41) and cooled at room temperature for 5 hours, then burnt in a furnace for 1 hour at 700°C (Distantina *et al*., 2011.) (Figure 3.8). The white ash is weight (W2). The percent of sulphate content was calculated as in Equation (1).

$$\% \text{ Sulphate} = (W2/W1) \times 100 \times 0.4116 \quad (1)$$

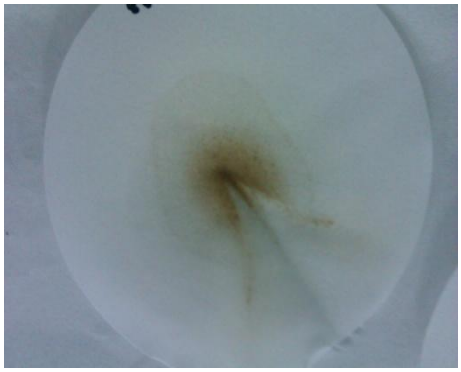

Figure 3. 6: BaSO4 precipitate

Figure 3. 7: White ash

3.7 Analysis of gel strength

Gel strength is defined as a ratio difference weight of before and after gel collapse to the surface area of the rod. The gel strength was calculated on an average of two determinations on the same sample. The dried extracted carrageenan was diluted in distilled water with slow heating to obtain in a ratio of 1.5% (w/v) extracted carrageenan solution. For determining the gel strength (GS), ten-ml solution was poured into a container (diam. 3.2 cm) and height was around 0.2-0.4 cm. After cooling overnight at room temperature, the container was placed on the balance. A stainless rod (surface area 1.2 cm^2) was pressed by hand into the gel surface until it collapse, the maximum balance was noted (Distantina *et al*., 2011). The gel strength was calculated as in Equation (2).

$$\text{Gel strength (g/ } cm^2) = \frac{Weight\ before\ \ gel\ collapse - Weight\ after\ \ gel\ collapse}{\text{surface of the rod}} \qquad (2)$$

3.8 Microbiology Test

Microbiological test was carried out using the total plate count (TPC). Microbial test was measured by using nutrient agar and potato dextrose agar (PDA) as solid medium. The extracted carrageenan (1g) sampel was mixed with 10ml sterilized distilled water in a ratio 1:10 (w/v)(Figure 3.9) , mixed for 5 to 10 minutes. Then, 1ml of the sampel was poured into each agar plate TPC and PDA separately (Figure 3.8). The sampel was spread over the agar using hockey stick , and incubated at 37 ° C for 24 hours.

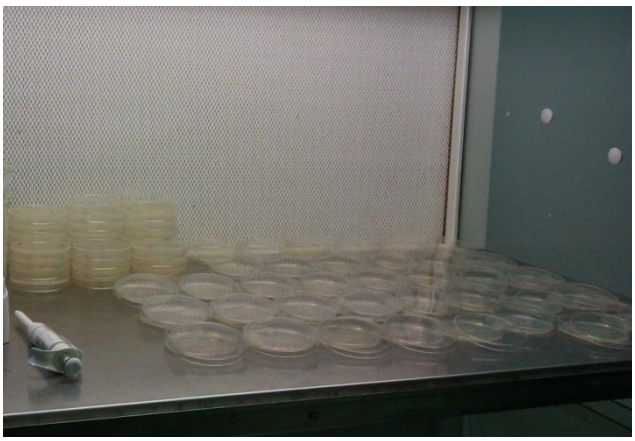

Figure 3. 8: Nutrient agar and PDA plates

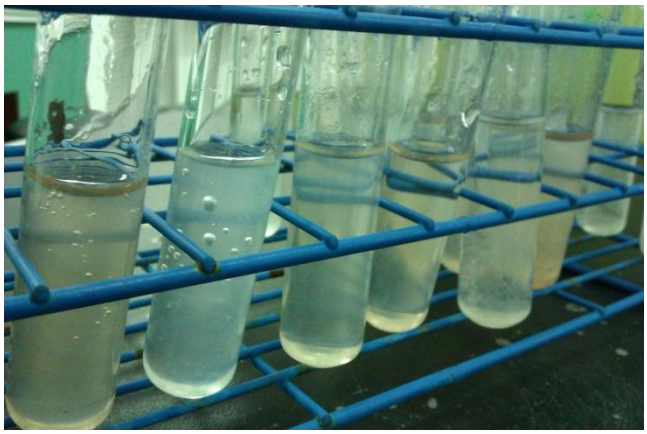

Figure 3. 9: Mixture of samples with sterilised water

4 RESULT AND DISCUSSION

4.1 Overview

There were total of 36 samples that were used in this research, each with different parameters. Table 4.1 shows summary of all parameters that were investigated in this experiments.

Table 4. 1: Samples name

Concentration (M)	Temperature (℃)	Time (h)	Sample
0.03	80	1	A1
		2	B1
		3	C1
		4	D1
	90	1	E1
		2	F1
		3	G1
		4	H1
	100	1	I1
		2	J1
		3	K1
		4	L1
0.05	80	1	A2
		2	B2
		3	C2
		4	D2
	90	1	E2
		2	F2
		3	G2
		4	H2
	100	1	I2
		2	J2
		3	K2
		4	L2
0.1	80	1	A3
		2	B3
		3	C3
		4	D3
	90	1	E3
		2	F3
		3	G3
		4	H3
	100	1	I3
		2	J3
		3	K3
		4	L3

4.2 Effect of Alkaline (KOH) Concentration and Yield Analysis

The effect of KOH concentrations on carrageenan were investigated. A known amount of seaweed was soaked at different concentrations, times and temperatures, separately. Figure 4.1 until Figure 4.4 show the yield results for every hour. Results (Figure 4.3) show that the highest yield of carrageenan was 91.20% at 0.05M, 80 ℃ for 3h. Mustapha *et al* (2010) mentioned that at low concentration of alkaline solution($\leq$ 0.01 *M*) was unable to form a gel, consequently low viscosity. This proved that kappa carrageenan requires high concentration of alkaline solvents. Some samples from 0.1M KOH shown the least yield. This situation occurred due to the polymer destruction by high solvent concentration. Polymer destruction produced the low molecular weight which cannot be precipitated by KCl (Distantina *et al*.,2011). This suggested that extraction with high alkali concentration can cause more degradation of polymer.

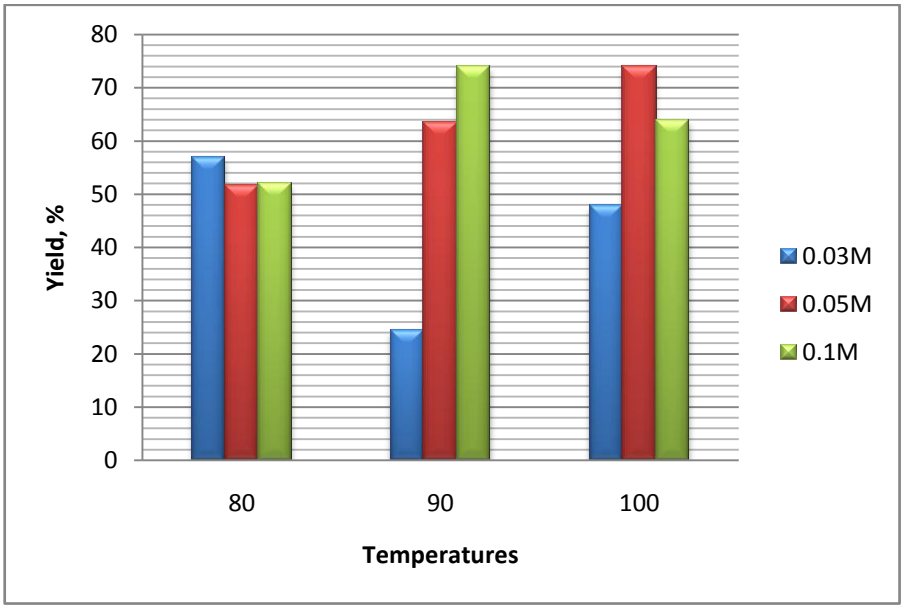

Figure 4. 1: Yield for 1 hour extraction

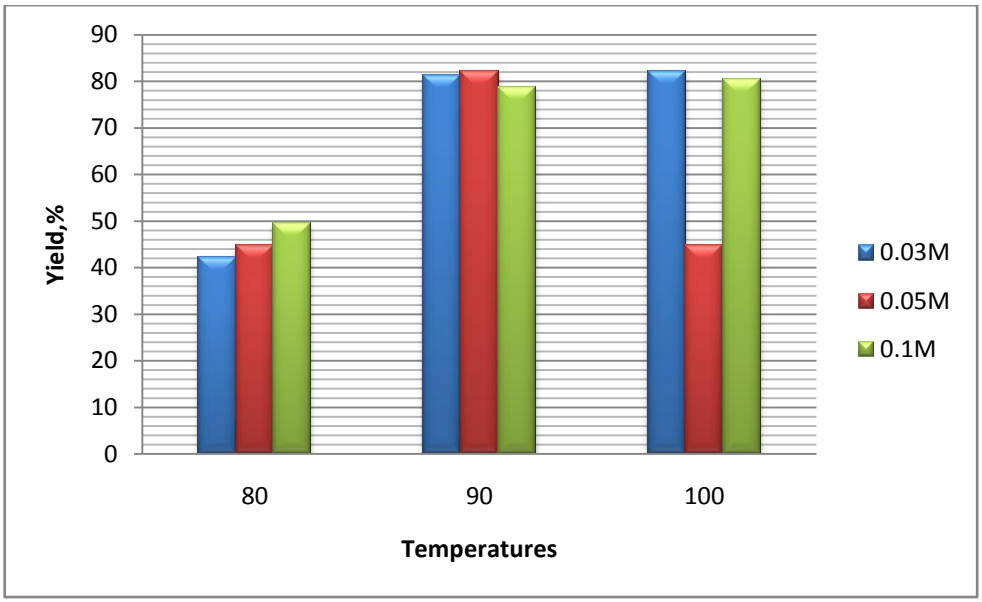

Figure 4. 2: Yield for 2 hours extraction

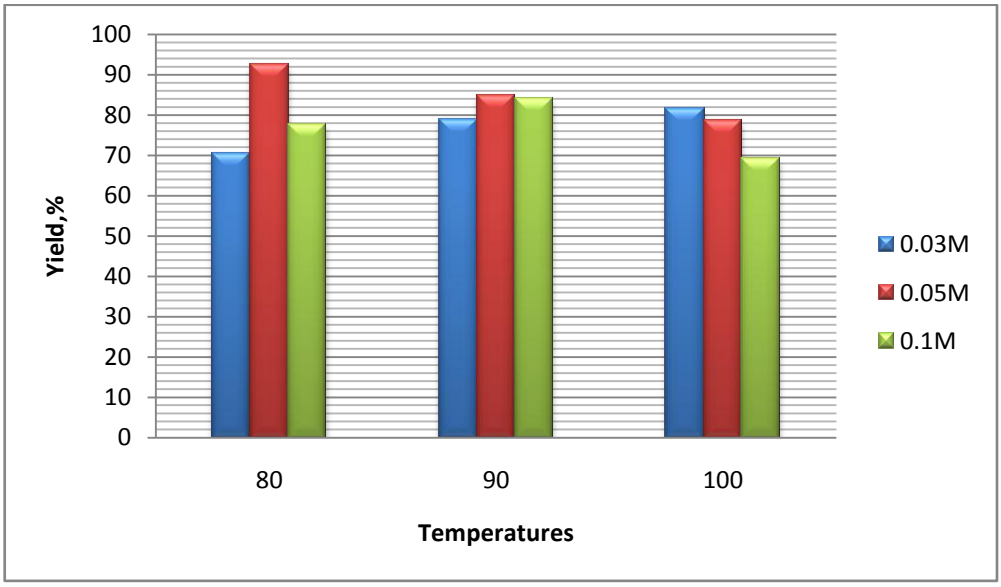

Figure 4. 3: Yield for 3 hours extraction

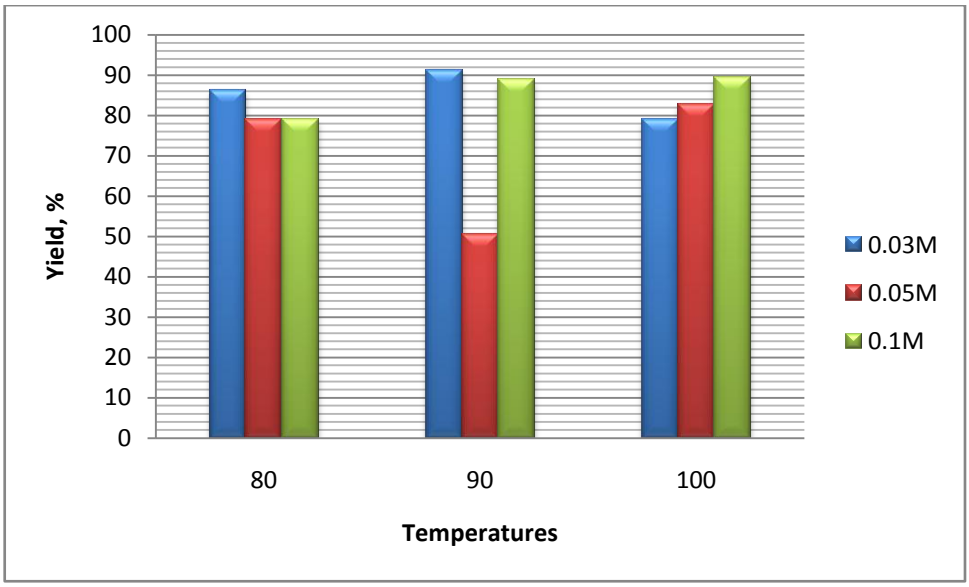

Figure 4. 4: Yield for 4 hours extraction

During extraction process, KOH solution change its' color, from colorless to yellowish within the first 30 minutes of extraction for all concentration at high temperature of 90-100 ℃. As the temperature reduced to 80℃, color change was observed only after 30 minutes of extraction process. Seaweed extraction at higher concentration (0.1M of KOH) dark yellow and hard seaweed was formed. Whereas, when seaweed extract at lower concentration (0.03M) seaweed formed was soft and whitish (Figure 4.5). Based on report by Distantina *et al* (2011) this situation showed that the seaweed formed has been disintegrated and resulted in the loss of carrageenan.

(a) At 0.03M (b) at 0.05M (c) at 0.1M

Figure 4. 5: (a), (b), (c) Color of solution based on their concentration

4.3 Effect of temperatures

Gel formation occurred at high temperatures of 80-100℃, the color of the extraction solvent had changed from colorless to dark brown. The dissolved seaweed obtained was lighter brown and hard. After additional of potassium chloride (KCl) of (2% w/v) the solution became cloudy viscous suspension. When KCl was added, the solution became gel, yellowish and slight opaque gel because of cellulose in seaweed (Figure 4.6). The addition of K^+ may induced the structural change from coil (disordered) structured to helix (ordered) conformational transition, followed by aggregation and network formation between ordered helices in carrageenan solution (Hermansson *et al*., 1991) . The process of shielding charge of sulphate groups, followed by ion-dipole binding with polysaccharides into aggregates were improved gel formation and strength.

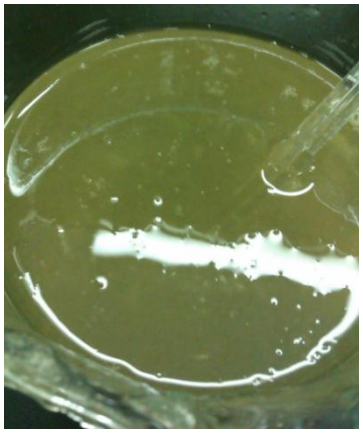

Figure 4. 6: Sample after mix with KCl

4.4 Gel strength

On the gel strength investigation, gel from sample L3 (0.1M, 100℃, 4h) recorded the highest value (Figure 4.9). Figure 4.7 until Figure 4.10 show the results of gel strength for every hours of extraction. Gel formation involves formation of a 3-dimensional matrix and allows immobilization of water within the gel structure. Hermansson *et al* (1991) stated that the gel strength influenced by the type and quantity of counter-ions in gel solution.

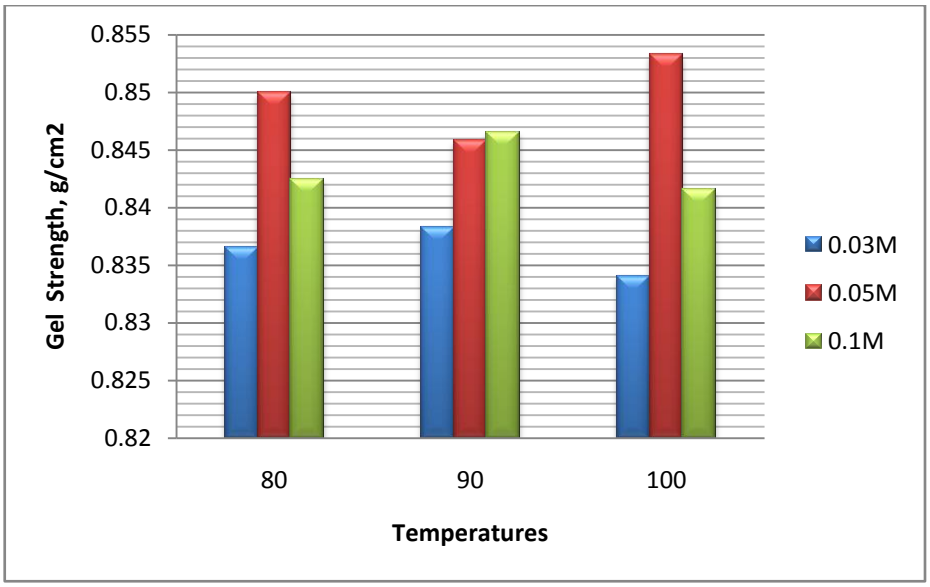

Figure 4. 7:Gel strength for 1 hour extraction

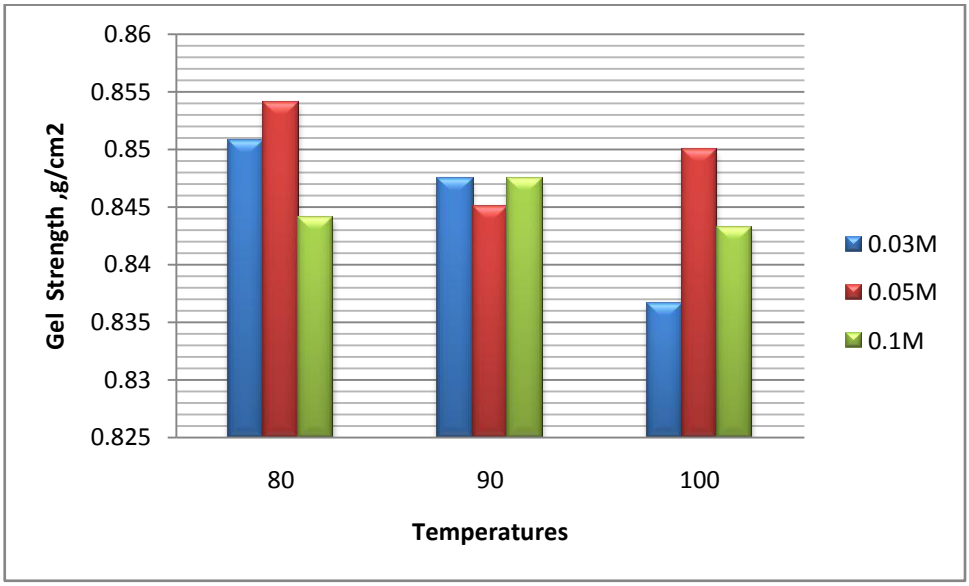

Figure 4. 8: Gel strength for 2 hours extraction

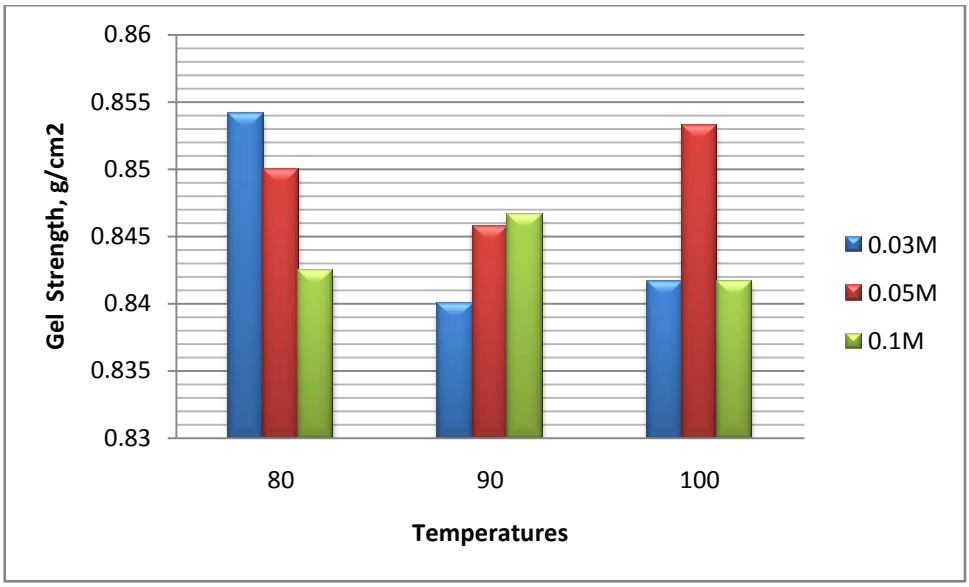

Figure 4. 9: Gel strength for 3 hours extraction

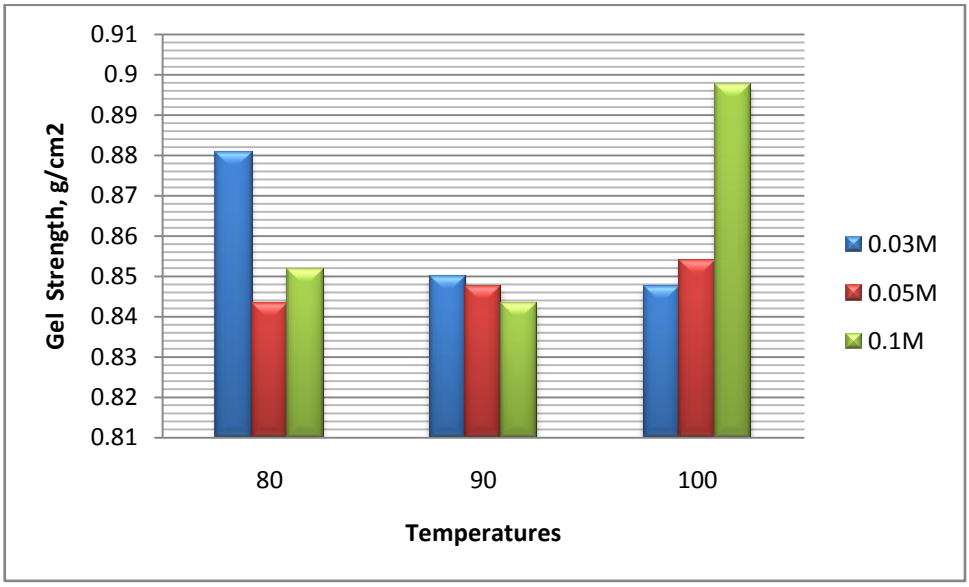

Figure 4. 10: Gel strength for 4 hours extraction

However, since fixed quantity of KCl was used in this research, thus this cannot be used to explain the differences in gel strength of different samples. The composition of polysaccharides in samples affect their gel strength. Due to the higher KOH concentrations which referring to the greater amount of K^+ and OH^- ions. OH^- ions can replace sulphate groups to form more 3,6 anhydrogalactose. It was reported by Mustapha *et al* (2010) that high amount of 3,6 anhydrogalactose may increases the gel strength. Higher temperature process increased reaction rate to form 3,6 anhydrogalactose. Gels formed at extraction temperatures above 80℃ were firm and

brittle, this situation occurred could be due to the aggregation process which occurred simultaneously with conformational transition of carrageenan structure (Kang *et al*., 1991).

4.5 Analysis of sulphate content

In determining the kappa carrageenan, sulphate content on seaweed extractions were investigated. Figure 4.11 until Figure 4.14 also show the effect of potassium hydroxide on sulphate content. Sulphate content for sampel 0.1M KOH (sample L3) show lowest sulphate content. Based on the result, the sulphate content also related to the gel strength value. It can be seen that the trend where the increasing potassium hydroxide concentration caused the value of sulphate content decreased and the gel strength increase. Based on worked by Distantina *et al* (2011) the reduction of sulphate content by potassium hydroxide indicates that there was a carrageenan reaction which converted precursor carrageenan (mu carrageenan) into kappa carrageenan. The cyclization process involves release of sulphate groups, thus after alkali treatment the sulphate content should be lower.

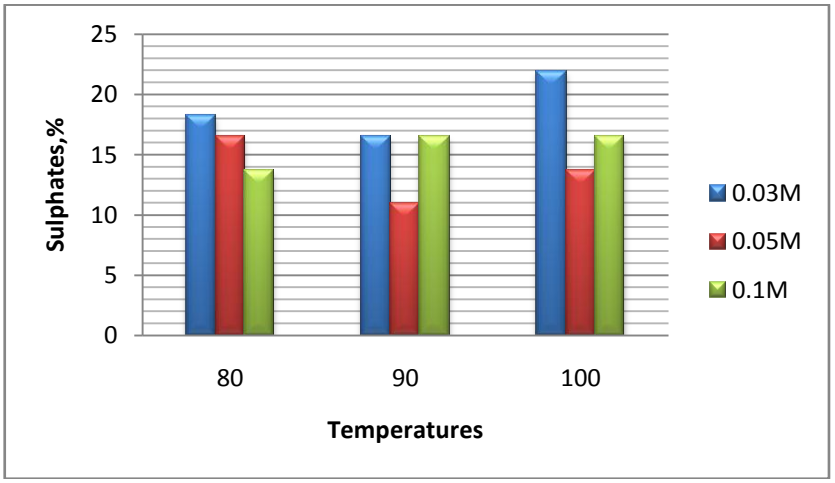

Figure 4. 11: Sulphates content for 1 hour extraction

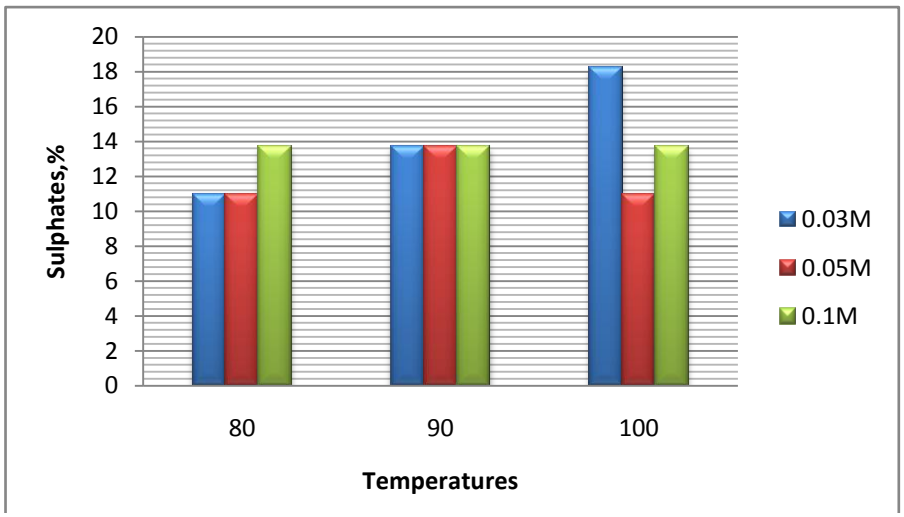

Figure 4. 12: Sulphates content for 2 hours extraction

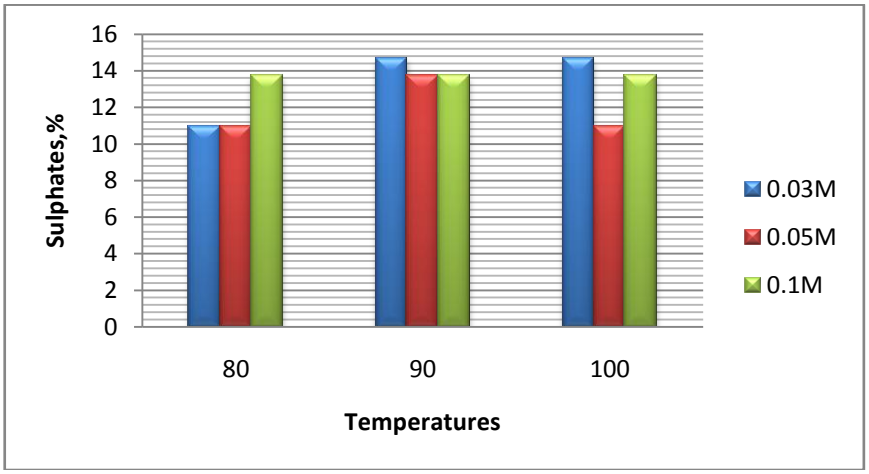

Figure 4. 13: Sulphates content for 3 hours

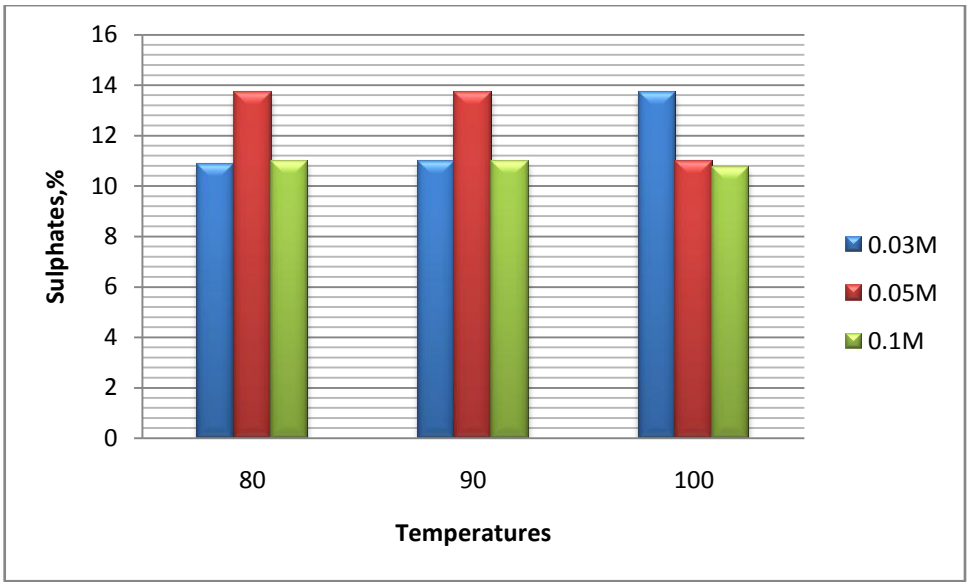

Figure 4. 14: Sulphates content for 4 hours extraction

4.6 Microbiological test

For this test, nutrient agar and Potato Dextrose Agar (PDA) were used as the solid medium. General purpose of Potato dextrose agar was as medium for yeast and molds that can be supplemented with acid or antibiotics to inhibit bacteria growth. It was used in plate count methods when testing food. Data in Table 4.3 showed that most of the samples were free from any bacterial growth, but there were some samples where bacteria and fungi growth, thus kappa carrageenan extracted were considered unsafe to be used in food or pharmaceutical products.

Table 4. 2: Microbiological Test

Concentration (M)	Temperature (℃)	Time (h)	Sample	Medium	
				Nutrient agar Bacterial growth (Yes/No)	PDA Fungi growth (Yes/No)
0.03	80	1	A1	No	No
		2	B1	No	No
		3	C1	No	No
		4	D1	No	No
	90	1	E1	No	No
		2	F1	No	No
		3	G1	No	No
		4	H1	No	No
	100	1	I1	No	No
		2	J1	No	No
		3	K1	No	No
		4	L1	No	Yes
0.05	80	1	A2	No	No
		2	B2	No	No
		3	C2	No	No
		4	D2	No	No
	90	1	E2	No	Yes
		2	F2	No	No
		3	G2	No	No
		4	H2	No	No
	100	1	I2	No	No
		2	J2	No	No
		3	K2	No	No
		4	L2	No	No
0.1	80	1	A3	No	No
		2	B3	Yes	No
		3	C3	No	No
		4	D3	No	No
	90	1	E3	Yes	Yes
		2	F3	No	No
		3	G3	No	No
		4	H3	No	No
	100	1	I3	No	No
		2	J3	No	No
		3	K3	No	No
		4	L3	No	No

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion it can be seen that the best parameter to extract kappa from seaweed was by using high temperature, 100℃ , 0.1M KOH and for 4 hours (sampel L3). Even though kappa can be extracted at low temperature (80 ℃) but the gel produce was soft compared to gel at 100℃, which more hard and rigid gel. The highest gel strength was sample L3 with gel strength value of 0.8975 g/cm^2 . Other than high temperature, high concentration and long extraction time to extract also maximize the extraction of kappa carrageenan. This showed that most of sampel were extract at 0.1M of KOH can produce yield which higher than 40%. The highest yield was at 0.05M KOH, 80 ℃ for 3 hours (sample C2) which was 92.6% . Based on research by Distantina *et al* (2011) the value of yield for extraction of 0.1M KOH for 45 minutes (stirred at 275 rpm) at 85℃ using ratio of 1:50 (seaweed weight to extraction solvent) were 44.63%. This research showed that extraction at higher temperature and longer time yield more kappa carrageenan.

For the microbiology test, there were some samples where bacteria and fungi growth, thus kappa carrageenan extracted were considered unsafe to be used in food or pharmaceutical products. The objective was achieved.

5.2 RECOMMENDATION

For future study, it is recommended to use equipment that equip with stirrer so that we would not disturb the extraction process as in this research the sample need to take out from oil bath and stirred it at room temperature for 10 minutes.

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APPENDICES A

Table A1 Extraction of seaweed at 0.03 M

Temperature (℃)	Time (hour)	Dried carrageenan (g)	Sampel name
80	1	5.719	A1
	2	4.041	B1
	3	4.253	C1
	4	5.860	D1
90	1	1.628	E1
	2	5.152	F1
	3	2.757	G1
	4	4.371	H1
100	1	1.728	I1
	2	3.917	J1
	3	2.609	K1
	4	4.344	L1

Table A2 Extraction of seaweed at 0.05 M of KOH

Temperature (℃)	Time (hour)	Dried carrageenan (g)	Sampel name
80	1	3.857	A2
	2	1.468	B2
	3	4.633	C2
	4	5.020	D2
90	1	4.966	E2
	2	1.341	F2
	3	2.005	G2
	4	1.717	H2
100	1	3.678	I2
	2	2.722	J2
	3	3.615	K2
	4	4.863	L2

Table A3 Extraction of seaweed at 0.1 M of KOH

Temperature (℃)	Time (hour)	Dried carrageenan (g)	Sampel name
80	1	3.864	A3
	2	4.984	B3
	3	3.970	C3
	4	5.428	D3
90	1	5.550	E3
	2	4.077	F3
	3	4.904	G3
	4	4.277	H3
100	1	2.215	I3
	2	3.810	J3
	3	3.421	K3
	4	4.542	L3

Table A4 Analysis of sulphate content

Sampel	Dried carrageenan (W1)	White ash(W2)	% Sullfate
A1	0.15	0.03	18.232
B1	0.15	0.04	10.976
C1	0.15	0.04	10.976
D1	0.15	0.03	10.832
E1	0.15	0.05	16.464
F1	0.15	0.06	13.720
G1	0.15	0.09	14.694
H1	0.15	0.04	10.976
I1	0.15	0.08	21.952
J1	0.15	0.03	18.232
K1	0.15	0.05	14.694
L1	0.15	0.04	13.720
A2	0.15	0.05	16.464
B2	0.15	0.04	10.976
C2	0.15	0.04	10.976
D2	0.15	0.06	13.720
E2	0.15	0.04	10.976
F2	0.15	0.05	13.720
G2	0.15	0.03	13.720
H2	0.15	0.05	13.720
I2	0.15	0.04	13.720
J2	0.15	0.05	10.976
K2	0.15	0.04	10.976
L2	0.15	0.04	10.976
A3	0.15	0.05	13.720
B3	0.15	0.05	13.720
C3	0.15	0.04	13.720
D3	0.15	0.05	10.976
E3	0.15	0.05	16.464
F3	0.15	0.06	13.720
G3	0.15	0.05	13.720
H3	0.15	0.05	13.720
I3	0.15	0.05	16.464
J3	0.15	0.05	13.720
K3	0.15	0.06	13.720
L3	0.15	0.05	10.720