EXTRACTION OF KAPPA CARRAGEENAN FROM LOCAL SEAWEED

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ABSTRACT

There are three main types of seaweed, which is red, brown and green seaweed. The most famous is red seaweed, which contains carrageenan. Carrageenan is a generic name of viscosifying and gel forming polysaccharides family. Kappa carrageenan is predominantly produced from Eucheuma cottonii (red seaweed) (Van De Velde et al, 2002) that normally found in the ocean of Philippines, Malaysia and Indonesia. It is widely use since it has excellent physical functional properties such as gelling, thickening and stabilizing abilities (Hoffman et al, 1995). Today, most of Muslim take medicine with gelatin capsules. Gelatin is a mixture of proteins and peptides produced by partial hydolysis of collagen which is extracted from the skin, boiled crushed horn, hoof and bones, connective tissues, organs and some intestines of animals such as domesticated cattle and pigs. An alternative for Muslims to obtain medicine from halal capsules source, the kappa carrageenan from local seaweed is extracted to replace the uses of gelatin in production of medicine capsules (Campo et al, 2009). Kappa carrageenan has a good gel strength which is similar to animal gelatin characteristic. The alkaline treatment is used to modify and promote gel formation in extraction of carrageenan. In this study, carrageenan is extracted through alkaline treatment and follow by KCl precipitation, and the functional group of kappa carrageenan is analysed with using Fourier Transform Infrared, FTIR (Marcela Cerna et al, 2003). The result shows that yield of percentage of carrageenan that treated with the KOH solution is the lowest (17.6%).Meanwhile, the carrageenan that was not treated with any alkaline solution has the highest yield percentage (25.1%). The carrageenan which is treated with KOH solution has a good gel strength since it has the lowest difference weight before and after collapse (0.15 mg). While, carrageenan treated with $Ca(OH)_2$ has the highest difference weight before and after collapse (2.2 mg) indicates it has the weak gel strength compared to others.

PENGEKSTRAKAN KAPPA CARRAGEENAN YANG HALAL DARIPADA RUMPAI LAUT TEMPATAN

ABSTRAK

Terdapat tiga jenis utama rumpai laut yang berbeza dari segi warna iaitu merah , coklat dan hijau. Yang paling terkenal adalah rumpai laut merah, yang mengandungi carrageenan. Kebanyakkannya Kappa carrageenan diekstrak daripada Eucheuma cottonii (rumpai laut merah) (Van De Velde et al, 2002) yang biasanya ditemui di lautan Filipina, Malaysia dan Indonesia. Ia secara meluas digunakan kerana ia mempunyai ciri-ciri fizikal yang sangat baik, iaitu ianya mempunyai struktur gel yang kuat dan digunakan untuk menstabilkan kondisi makanan (Hoffman et al , 1995). Hari ini, kebanyakan orang Muslim mengambil ubat dari sumber kapsul gelatin. Gelatin adalah campuran protein dan peptida yang dihasilkan melalui hidrolisis separa kolagen yang diekstrak daripada kulit , tanduk yang dihancur rebus, kuku dan tulang, tisu penghubung, organ-organ dan beberapa usus haiwan seperti lembu dan babi. Satu alternatif bagi orang Islam untuk mendapatkan ubat dari sumber kapsul yang halal adalah dengan mengekstrak Kappa carrageenan dari rumpai laut tempatan untuk menggantikan penggunaan kapsul gelatin (Campo et al, 2009). Kappa carrageenan mempunyai kekuatan gel yang baik yang mirip dengan ciri gelatin haiwan. Rawatan alkali digunakan untuk mengubah suai dan menggalakkan pembentukan gel dalam pengekstrakan carrageenan. Dalam kajian ini, carrageenan diekstrak melalui rawatan alkali dan diikuti dengan garam KCl, dan seterusnya Fourier Transform Infrared, FTIR digunakan untuk menganalisis kumpulan fungsi Kappa carrageenan (Marcela Cerna et al, 2003). Hasilnya daripada kajian, peratusan carrageenan yang dirawat dengan larutan KOH adalah paling rendah (17.6 %). Sementara itu, carrageenan yang tidak dirawat dengan mana-mana alkali mempunyai hasil peratusan tertinggi (25.1 %). Carrageenan yang dirawat dengan larutan KOH mempunyai kekuatan gel yang baik kerana ia mempunyai perbezaan sebelum dan selepas keretakan adalah yang paling rendah (0.15 mg). Sementara itu, carrageenan yang dirawat dengan $Ca(OH)_2$ mempunyai berat sebelum dan selepas keretakan adalah yang paling tinggi (2.2 mg) dan ini menunjukkan bahawa ianya mempunyai kekuatan gel yg lemah jika dibandingkan dengan carrageenan yang lain yang dirawat dengan alkali yang berbeza.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

In several centuries, seaweed had been used as traditional food in China, Japan and Korea. One of the main active ingredients in seaweed is carrageenan. The carrageenans are extracted from red seaweeds (Van De Velde et al, 2002). They are widely used in the food industry, for their gelling, thickening and stabilizing properties (Dyrby et al., 2004; Mou et. al, 2004). Their main application is in dairy and meat products, due to their strong interactions with protein. There are three types carrageenan, which differ in their degree of sulfation. Kappa-carrageenan has one sulfate per disaccharide. Iota carrageenan has two sulfates per disaccharide. Lambda carrageenan has three sulfates per disaccharide.

Carrageenans are introduced in the industry at early 1930s. They were first used in China around 600 before century. Philippines is the current largest producer of carrageenan where the supply is about 80% cultivated seaweed to the world industry (Freile-Pelrgrin & Robledo, 2010; Bindu and Levine, 2010). In the original method, in the late 1970s to early 1980s the carrageenan is extracted from the seaweed into an aqueous solution. The seaweed residue is removed by filtration and then the carrageenan is recovered from the solution, eventually a dry solid containing little else than carrageenan (Wiratni et al, 2011). In the second method, the carrageenan is never actually extracted from the seaweed. Rather the principle is to wash everything out of the seaweed that will dissolve in alkali and water, leaving the carrageenan and other insoluble matter behind. This insoluble residue, consisting largely of carrageenan and cellulose, is then dried and sold as semi-refined carrageenan.

1.2 PROBLEM STATEMENT

About 1.6 billion of total number of Muslims (DA, 2011) are possibly taking medicine with gelatine capsules. Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, boiled crushed horn, hoof and bones, connective tissues, organs and some intestines of animals such as domesticated cattle, chicken, pigs. To make sure all Muslim obtain medicine from halal capsules source and not from non-halal or pig's gelatin, the kappa carrageenan from local seaweed is extracted to replace the uses of gelatin in production of medicine capsules. Where carrageenan has similar characteristic with the gelatin and suitable to replace gelatin capsules which now is widely used in medical. The kappa-carrageenan characteristics are strong, rigid gel formed with potassium salts, slightly opaque gel and it became clear with sugar addition (Piere Etiene Bost et al, 2002).

1.3 RESEARCH OBJECTIVES

The aims of this research:

1. To extract kappa carrageenan from the local seaweed, Eucheuma Cottonii through the KCl Precipitation method.

2. To determine the effect of different type of alkaline treatment on the carrageenan yield, gel strength and analyze its functional group using FTIR.

1.4 SCOPE OF STUDY

The scope of this study is to extract kappa carrageenan from Eucheuma cottonii seaweed from local sea through KCl Precipitation method. This method is used in determining the effect of different types of alkaline treatment on yield percentage and gel strength of carrageenan. Through this method, the functional group of carrageenan is identified by Fourier Transform Infrared (FTIR). Different functional group, has different absorbance band at wavelength of FTIR result. Types of carrageenan such as kappa, iota and lambda are differentiated from their functional group which is indicated in the absorbance band at FTIR result. Kappa carrageenan has one sulfate per disaccharide. Iota carrageenan has two sulfates per disaccharide. Lambda carrageenan has three sulfates per disaccharide (JECFA, 2001).

1.5 RATIONAL AND SIGNIFICANCE OF STUDY

The extraction of kappa-carrageenan from local seaweed is a continue research work to improve the quality production of plant based capsule. Indirectly, it helps provide a lower cost production (Piere Etiene Bost et al, 2002) to plant based capsules industry and this is reasonable for vegetarians and Muslims people to get their capsules in affordable price. This research is to develop the extraction of plant based capsules technique and allow Muslim people to get their halal capsules. In addition, this study is to identify the functional group of carrageenan and determine the extraction process of carrrageenan which has high quality gel strength and high production yield.

CHAPTER 2

LITERATURE REVIEW

2.1 SEAWEED

There have been many centuries that seaweed is used as daily food among people in China, Republic Korean and Japan. Then, the people from these countries have been migrated to the others countries and this custom have moved with them. Therefore, the consumption of seaweed at this present time is not unusual in many countries. Meanwhile, in tropical climates such as Philippines, Malaysia and Indonesia, the people from these countries use fresh seaweed as salad components (McHugh et al, 2003, FAO). The present uses and application of seaweed are for cosmetics component, human foods, fertilizer, and for the extraction of chemicals and industrial gums. Seaweed has important uses in medical and industrial fields (Edwards et al, 2012).

In recent years, there has been large wave in the France to introduce the seaweed into the European menu or cuisine. But, it is still recognized and regarded as the exotic component in the menu or cuisine. It gains acceptance in California and Hawaii region since there has many Japan people there. In the Canada and U.S. around New Brunswick, Nova Scotia and Maine, some company have started to cultivate seaweed on shore, in tank for their market, export to Japan and human consumption. Now, seaweed is widely marketed in many countries, since it is considered as "sea vegetable" in the cooking books around the world. With the current trend for consumers to embrace

organically grown foods and “natural” foods from the clean environments since seaweed growth in sea water which is free from bacterial contamination.

There a wide variety of seaweed products that estimated has value about 5.5-6 billion U.S. Dollar annually (McHugh et al, 2003, FAO). 800 tonnes of seaweed is supplied to China every month by Fiji (Serafina Silaitoga, 2013). SRI consulting has estimated the world growth rate of hydrocolloid consumption from year 2003-2008 exceed the percentage range (1.5%-2.5%) per year (Feliza Mirasol, 2006). This showed the positive demand of seaweed in the food marketing.

2.1.1 TYPES OF SEAWEED

Seaweed was also known as marine algae. It is not categorized as plant even it has cell wall and can carry out photosynthesis looks like them. The green, brown and red seaweed are classified into three different kingdoms: chromist, plantae and protest. There are various types of seaweed and them different in shapes, colour and sizes.

2.1.1.1 BROWN SEAWEED

Brown seaweed is the complex and largest type of seaweed. This type of seaweed is olive (yellowish brown), brown in colour. Seaweed contains pigment (fucoxanthin) which gives its colour and chlorophyll c and a. Fucoxanthin is not found in other plants or seaweeds. Brown seaweed is different from red and green seaweed. It is in the kingdom chromista.

Brown seaweed are rooted to a stationary structure such as rock, dock or shell by structure called a holdfast, although categorized as free floating species. Many of species of brown seaweed have air bladder which helps brown seaweed blader to float on the surface of ocean and this allow for maximum sunlight absorption. Brown seaweed can be found from tropical to polar zones, in

interdal zones, near coral reefs and deep water region. It commonly use as food stabilizers, thickeners and fillers.

2.1.1.2 GREEN SEAWEED

Green seaweed ranges from simple (one cell) organisms to complex (multicellular) organisms. It lives in colonies and able to carry out photosynthesis. It is classified in the plant (plantae) kingdom. Green seaweed has same amount of chlorophyll a and b as plants, which give dark to light green coloration to it. It usually found in areas where light is abundant such as tide pools and shallow water. It less found in the ocean than the brown and red seaweed. Its pigments such as beta-carotene are used as food colouring and it also used in reducing global warming.

2.1.1.3 RED SEAWEED

Red seaweed has reddish or purplish colour. It ranges from simple (one cell) organisms to complex (multicellular) organisms. It is protists in the phylum Rhodophyta and it gains energy from photosynthesis. It cells lack in flagella which make it different from other seaweed. Red seaweed contain a variety of pigments, but the most important pigment is phycoerythrin which provides the seaweed's red pigmentation by reflecting red light and absorbing blue light waves, it can be found in the deep ocean. It will appear as green or blue when it lack in phycoerythrin. Red seaweeds are found from polar to tropical water, and commonly found in coral reefs and tide pools. Red seaweed is used to produce agars which use in made pudding, as a food additive, as culture medium in the science lab and etc.

2.2 CARRAGEENAN

Carrageenan is a generic name of viscosifying and gel forming polysaccharides family, which obtained by extract it from the red seaweeds (Van De Velde et al, 2002) like Chondrus crispus, Gigartina stellate, Gymnogongrus furcellatus, Cystoclonium purpureum, Kellymenia reniformis, Kappaphycus alvarezii, Eucheuma cottonii, Eucheuma, Eucheuma gelatinae, Furcellaria fastigiata, Hypnea spicifera and etc. The word carrageenan came from the colloquial Irish name, in which the means of carrageen is little rock. Seaweeds which produce carrageenan as their main cell wall component is belong to Rhodophyta.

In industrial application, it is widely use since it has excellent physical functional properties such as gelling, thickening and stabilizing abilities. It has been used to improve the texture of cottage cheese, to control the texture and viscosity of dairy desserts and pudding. It also acts as binder and stabilizer in meat processing industry. Carrageenan is also used in various non-food products such as in cosmetics, pharmaceutical, printing and textile formulations (Imelson, 2000). It is used to absorb body fluids when formulated in wound dressings and to stabilize toothpaste preparation. And in lotions and shampoos, it interact with human carotene in order to give soft skin and silky hair. It has proved, that it is suitable as tableting excipients since high robustness, good compatibility and persistent viscoelasticity of the tablet during compression. These properties showing that carrageenan is suitable excipient for sustained release formulation (Bhardwaj et al, 2000).

The market for carrageenan is consistently growth at about 5% per year, from 5500 tonnes in 1970 to 20000 tonnes in 1995 (Bixler, 1996). Meanwhile, in 2003 it was 35000 MT/year with value at around $300 million (McHugh, 2003). Nowadays, the demand of carrageenan in the international market is increase with annual market at 450 million US Dollar (Robled et al, 2010). The carrageenan industry has dominated by very large and multi-product companies with factories in Philippines, US, Europe, Canada, etc. The sales of carrageenan in the Europe and US is holding up reasonably well despite the ongoing global recession.

2.2.1 CHEMICAL STRUCTURES OF CARRAGEENAN

Carrageenan is a sulfated polygalactan which contain ester sulfate at about 15-40%. It is formed by alternating the units of 3,6-anhydro-galactose and D-galactose which joined by β-1,4 and α-1,3 glycosidic linkage. There are many types of carrageenan such as λ, μ, ι, κ, ε, which are containing 22 to 35% sulphate groups. The classification of carrageenan is based on its solubility in the potassium chloride. The properties of carrageenan is influenced by its position and number of ester sulfate groups as well as the content of 3,6-anhydro-galactose. The names are not exactly show its chemical structures but only for general difference in the composition and degree of sulfation at specific location in the polymer. The higher level of ester sulphate in the carrageenan, the lower its solubility temperature and gel strength. Kappa carrageenan has 25-30% ester sulfate and 28-35% 3,6-anhydro-galactose. Lambda carrageenan has 32-39% ester sulfate and has no 3,6-anhydro-galactose. Meanwhile, iota carrageenan has 28-38% ester sulfate and 25-30% 3,6-anhydro-galactose (Barbeyron et al, 2000)

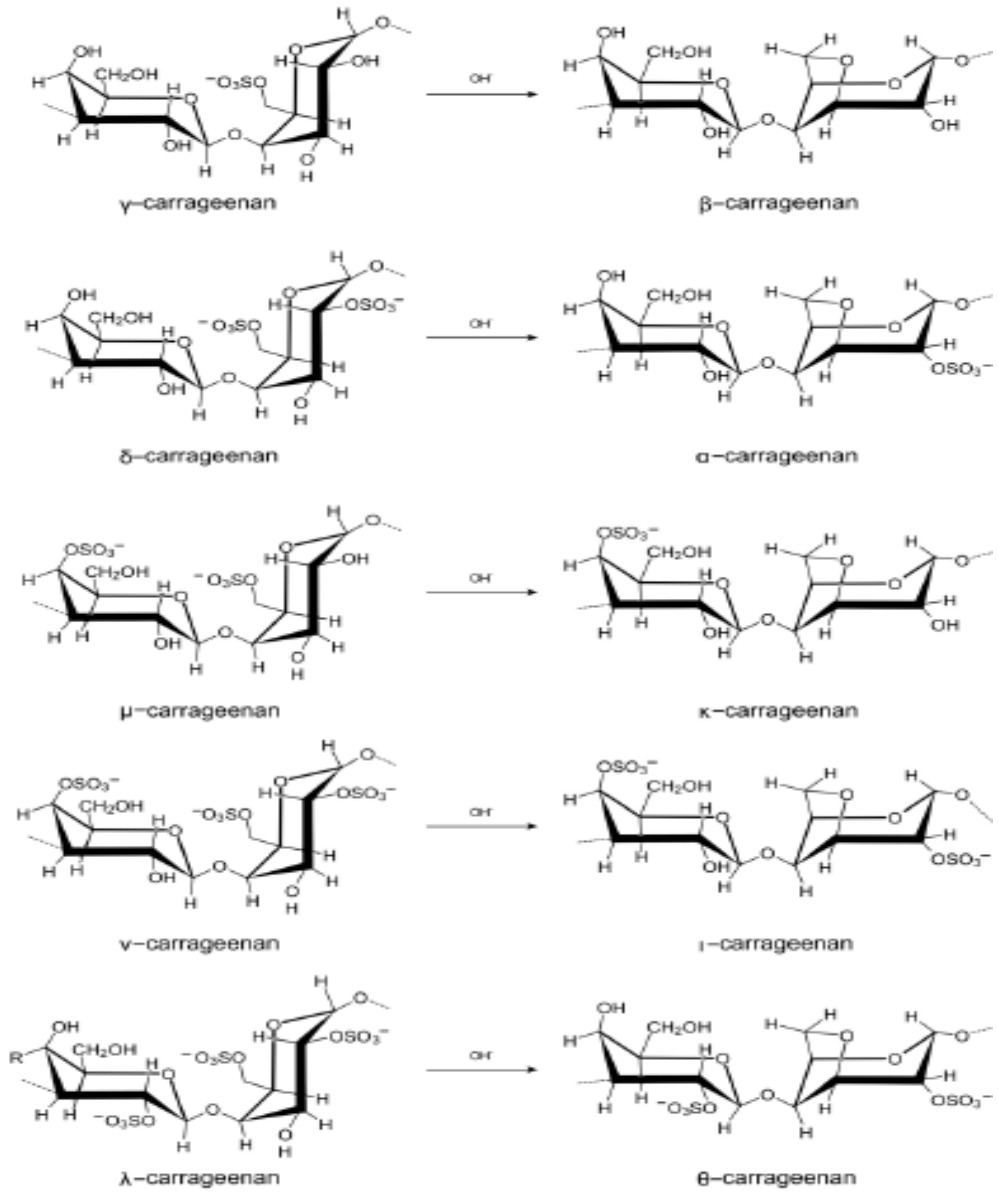


Figure 2.1: Chemical structure of different types of carrageenans (Lenka Bartosikova et al, 2013)

2.2.2 CHARACTERISTICS OF CARRAGEENAN

Table 2.1: Characteristics of carrageenan (Sources from: Tobacman 2001)

Chemical composition	Hydrocolloid of β-D-1,4 and α-D-1,3 galactose residues that sulphated up to 40% of the total weight; strong negative charge over normal pH range; associated with ammonium, sodium, magnesium, potassium, or calcium salts
Gel formation	1. λ carrageenan does not form gel 2. λ and ι carrageenans form right handed helices 3. Potassium chloride promotes gel formation of k carrageenan. 4. Calcium ion promotes gel formation of ι carrageenan
Solubility	1. λ carrageenan is soluble in hot or cold aqueous solution. 2. κ carrageenan is soluble in hot aqueous solution. 3. Treatment of hot aqueous potassium ion will precipitates κ carrageenan
Viscosity	Near logarithmic increase in viscosity with increasing concentration; viscosity of grade carrageenan defined as not less than 5 cps at 75°C for 1.5% solution; viscosity range from 5 to 800 cps for 1.5% solution at 75°C.
Source	Red seaweed; predominantly aqueous extraction from Chondrus, Eucheuma and various Gigartina species
Metabolism	1. Desulfation by sulfatases. 2. Hydrolysis of glycosidic linkage at lower pH (especially pH ≤ 3)
Properties	κ and λ carrageenan combine easily with milk protein to improve solubility and texture; also acts as emulsifier, stabilizer and thickening agent in food
Molecular weight	Discrepancies in definitions; native carrageenan reported to have average molecular weight of 1.5 × 106 to 2 × 107; food-grade carrageenan reported as 100 000–800 000 or 200 000–400 000; degraded carrageenan (poligeenan) has average molecular weight of 20 000–30 000; furcellaran has average molecular weight 20 000–80 000
Synergistic effects	with locust bean gum, increase in gel strength; other hydrocolloids may also affect cohesiveness and gel strength
Concentration in food products	0.005–2.0% by weight
Major uses	Use in processed meats, milk products, dietetic formulations, infants formula, cosmetics, toothpaste, skin preparations, laxatives and pesticides

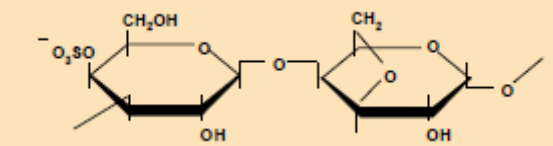
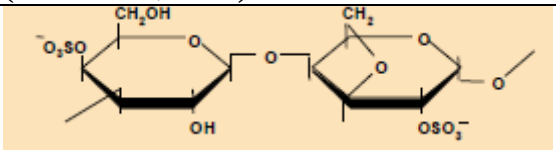
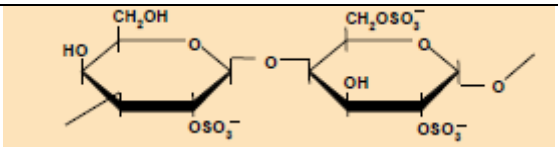
The carrageenan chemical reactivity is primarily due to its half ester sulphate groups which are strongly anionic, being comparable to inorganic sulphate in this respect. The free acid is unstable, and commercial carrageenans are available as stable calcium salts and sodium potassium. The physical properties of the carrageenans can be determined through the associated of cations together with the conformation of the sugar in the polymer chain.

1. Kappa carrageenan form gels in the presence of potassium ions, iota carrageenan form gels in the presence of calcium ions. Meanwhile, lambda carrageenan does not form gels in the presence of potassium or calcium ions (Micheal et al, 1997).
2. Carrageenan is a linear, water soluble, polymer, highly viscous aqueous solution.
3. Viscosity is depend on temperature, concentration, type of carrageenan, the presence of other impurities and solutes, and its molecular weight (Lai et al, 2000).
4. Viscosity of carrageenan decreases with temperature and increases nearly exponentially with concentration.
5. Carrageenan are depolymerized through acid catalyzed hydrolysis.
6. Carrageenan may completely loss functionality at high temperature and low pH (Stanley, 2011).
7. All carrageenan fractions are soluble in water. Insoluble in organic solvents, fats or oil. But carrageenan solubility in water is depend on its level sulphate groups and on its association to cation (Pardonche, 1985)
8. The main ionizable cations found in carrageenan is calcium, potassium, sodium and magnesium. Other ions also can also ionizable but at lower frequency (Pardonche, 1985)
9. The proportion of sulphate fraction and the equilibrium of cation in the water solution determined the gel strength and viscosity of solution that formed by carragenans.

2.2.3 MAIN TYPES OF CARRAGEENAN

There are three main types of carrageenan namely kappa, iota and lambda which are totally different in their number and position of their sulphate group on each sugar and the absence or presence of 3,6 anhydro group on the B monomer. The 3,6 anhydro group helps in promoting α-helix formation which is very important in introduce gelling characteristics in the carrageenan (Lamond, 2004; Whistler et al, 1997). This is a result of increased flexibility that promotes a random coil structure. Then, conformation of the glycosidic bond changes to equatorial (Therkelsen, 1993).

Table 2.2: Molecular structures of kappa, iota, and lambda carrageenan.

Types of carrageenan	structure	Brief description
Kappa carrageenan	 (Gail Fisher, 2009)	Kappa carrageenan has 25-30% ester sulfate and 28-35% 3,6-anhydro-galactose (Barbeyron et al, 2000).
Iota carrageenan	 (Gail Fisher, 2009)	Iota carrageenan has 28-38% ester sulfate and 25-30% 3,6-anhydro-galactose (Barbeyron et al, 2000).
Lambda carrageenan	 (Gail Fisher, 2009)	Lambda carrageenan has 32-39% ester sulfate and has no 3,6-anhydro-galactose (Barbeyron et al, 2000).

2.2.3.1 KAPPA CARRAGEENAN

Kappa carrageenan has one sulfate group for a repeat dimer which is located on the O-3 galactose ring such as seen in the diagram inside the Table 2.2 (Lamond, 2004; Whitler et al, 1997). Based on the X-Ray fiber diffraction, the structure of kappa carrageenan is right-handed double helix of parallel chains (Therkelsen, 1993). This structure allow kappa carrageenan to form its durable thermoreversible gels by itself. In the presence of salts, usually potassium, kappa carrageenan will form strong and rigid gels, although these gels are very susceptible to syneresis. Kappa carrageenan also react with milk proteins through charge complexes (Whitler et al, 1997)

2.2.3.2 IOTA CARRAGEENAN

Iota carrageenan has right handed double helix of parallel chains. It has two sulphate groups per repeat dimer, which each of sulphate group is located on each of the sugar units as seen in the Table 2.2. Iota carrageenan can forms elastic, strong, thermoreversible gels with limited syneresis. Iota carrageenan form gels in the presence of calcium ions, where calcium ions forms ionic bridges between iota carrageenan chains (Gail Fisher, 2009).

2.2.3.3 LAMBDA CARRAGEENAN

Non-gelling lambda carrageenan has three sulphate groups per repeat dimer units of D galatose-2-sulphate-D-galactose-2, 6-disulphate.It does not contain the 3, 6 anhydro group which is necessary in formation of the double helix. Lambda carrageenan does not form gels but is widely used as a viscosifier in many food applications (Whitler et al, 1997).

2.2.4 USES AND APPLICATION OF CARRAGEENAN

2.2.4.1 INDUSTRIAL USES OF CARRAGEENAN

Carrageenan acts as a support material for immobilisation of both enzymes and whole cell systems which is importance in the increasing of the stability and activity of the biocatalysts. This is proven by several applications in different industrial fields. The carrageenan also has been promoted as a food grade additive in the food industries. The mild immobilisation and reaction conditions of carrageenan in immobilization of whole cells as factor it apply and use in highly selective production processes for pharmaceutical compounds (Van de Velde et al. 2002).

2.2.4.2 INDUSTRIAL FOOD APPLICATIONS

Recently, continuous production of vinegar was using a bubblemixed tabletop bioreactor with κ-carrageenan immobilized Acetobacter suboxydans cells (Tosa and Shibatani, 1995). Fermented milk products can be obtained by simultaneous acidification and inoculation of skimmed milk by immobilised mixed cultures in κ-carrageenan/locust bean gum and used in a 2-L stirred reactor (Sodini et al, 1997). In the beer production, beer is produced continuously by using κ-carrageenan beads in the static mixer (Mensour et al. 1996 & 1997). In the ethanol production from glucose, kappa carrageenan is use to immobilized cells of Zymomonas mobilis in a fluidised bed fermenter (Krishnan, 1999). In the ethanol production from pineapple cannery waste, yeast cells is used and immobilised in κ-carrageenan (Nigam 2000).

2.2.4.3 PHARMACEUTICAL APPLICATIONS

Tetracycline is one of the most important antibiotic group which produced through fermentation reaction in the industry. Then, the kappa carrageenan is used to immobilized the Streptomyces aureofaciens in order to improve the production of tetracycline and chlorotetracycline (Asanza-Teruel et al, 1997)

Kappa carrageenan also acts as a support material for production of 6-aminopenicillanic production which was tested with E.coli cell with penicillin amidase activity (Nagalakshmi and Pai, 1997). Kappa carrageenan also was used to immobilize dihydropyrimidinase and carbamoylase in the Recombinant E.coli (Chao et al, 1999)

2.2.4.4 OTHER USES OF CARRAGEENAN

In the food chemists' field, carrageenan is known well as stabilizer, emulsifier, gum or colloid. Many of products that people now take for granted such as dairy products, milks, soy milks, infant formulas and nutritional supplement are made, stored and packaged for long period of time with this carrageenan. Carrageenan is used to gel, suspend or thicken foods. Besides that, it used in emulsion, stabilization, for syneresis control, and for bodying, binding and dispersion of food, particularly dairy food applications (Lenka Bartosikova et al, 2013).

Today, the special properties of excellent gel texture and flavor release make kappa carrageenan a preferred product for use in milk pudding powder (Lenka Bartosikova et al, 2013). Iota carrageenan has similar textures to gelatin gels that use in dessert gel formulations. It has an advantage over gelatin gels in that its melting point is higher, making it more suitable in tropical climates or where refrigeration is not available. In toothpastes carrageenan acts as binder to impart the desired rheological properties to the paste.

2.3 EXTRACTION OF KAPPA CARRAGEENAN

As mentioned previously, kappa carrageenan has good gelling properties and protein reactivity which let it use widely in pharmaceutical and as food additives. It predominantly produce and extracted from seaweed that really well known which is called as species Eucheuma cottonii. Kappa carrageenan also can extract from other species of seaweed namely Chondrus crispus, Gigartina stellate, Furcellaria fastigata and Hypnea. But in this study, Eucheuma cottonii is used since it is predominantly produce kappa carrageenan if compare to the other seaweed species (Wiratni et al, 2011). There are many types of extraction method for kappa carrageenan including ethanol precipitation, KCl precipitation and etc. But in this study, KCl precipitation method is used to extract kappa carrageenan since KCl salt is better than ethanol alcohol in term of precipitate the carrageenan solution into gel form (Rideout et al, 1998).

2.3.1 PRECIPITATION OF KAPPA CARRAGEENAN USING KCl SALT

Eucheuma cottonii seaweed is first washed using distilled water or deionized water as option to remove impurities in the seaweed. Seaweed is washing for 10-30 minute until satisfy clean. Then, slice and chopping it into about 1,2 or 3 centimeter in length (Rideout et al, 1998). Prepare Base solution and use distilled water during dilution to make sure the purities of carrageenan is not affected by the impurities that perhapsly exists in the water. Then, the chopping seaweed is boiled in base solution at recommended temperature. Generally, the base used in this study is the alkali or alkaline earth metal such as sodium hydroxide, calcium hydroxide and potassium hydroxide. This aqueous base lead to the formation of 3,6-anhydro linkages in the galactose units of carrageenan (Christopher et al, 2012). After modification step, the hot extract is filtered using filter cloth and filter paper to remove the insoluble material such as hemicelluloses, cellulose and other particulates. Then add acid to control pH to 7.5 to 10.5 (Rideout et al, 1998). Next, cold KCl salts solution is used to coagulate and precipitate the carrageenan and drying it for whole day at room (25-35℃) temperature in the oven or for 16 - 18 hours at oven with optimal temperature.

2.4 FOURIER TRANSFORM INFRARED (FTIR)

FTIR is a valuable tool in determine the functional group by virtue of their characteristics vibrational frequencies. It was a technique used to identify the structural of material before the invention of nuclear magnetic resonance (NMR) spectroscopy is introduced. The FTIR gives an absorbance that can detect much higher absorbance than the UV-visible spectrometer. Infrared spectroscopy is one of the important standard techniques use for characterisation of the carrageenans especially kappa carrageenan in this study. It also used for analysis of the spectra as what had been reported by many author (Prado, 2001), in which the different structural elements of carrageenan are assigned to different absorption bands. Based on Prado, 2002. The carrageenans has the total sulphate content at the absorbance band at 1250 cm^{-1}, which was decreased from lambda carrageenan to iota carrageenan to kappa carrageenan (Chopin et al, 1993). Kappa carrageenan spectrum displayed a band at 845 cm^{-1} due to the galactose-4-sulphate (Seekal and Legrand, 1993). Meanwhile, iota carrageenan spectrum present a band at 845 cm^{-1} arising from the galactose-4-sulpahate (Seekal and Legrand, 1993) and one another band at 805 cm^{-1} due to the 3,6-anhydrogalactose-2-sulphate (Seekal et al, 1993). In lambda carrageenan, two bands of spectrum appear at 830 cm^{-1} and 820 cm^{-1} which corresponding to galactose-2-sulphate and to galactose-6-sulphate, respectively (Chopin et al, 1993).

2.4.1 PRINCIPLES OF FTIR

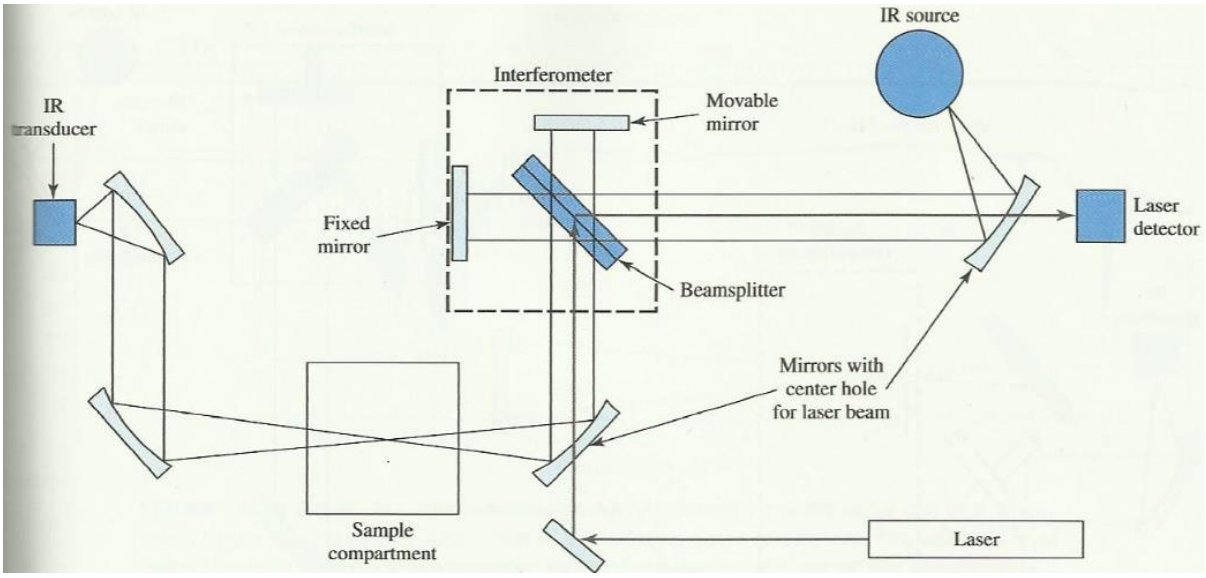


Figure 2.2: Single beam FTIR spectrometer (Sources: F. James Holler et al, 2006)

Based on Figure 2.2, in one arm of the interferometer, the IR source radiation travels via the beamsplitter to the fixed mirror and back to the beamsplitter, and through the sample to the IR transducer. Meanwhile, in the other arm, the IR radiation travels to the beamsplitter is reflected to the movable mirror and travels back through the beamsplitter to the sample, then to the ransducer. When the two beams meet again at the beamsplitter, they will interfere with each other if the phase or path difference is appropriate. A plot of the signal against mirror displacement is the interferogram that contains information of all the frequencies present. The spectrum which is intensity versus wavenumber is the fourier transform of the interferogram. It can calculated in a computer from the signal versus mirror displacement. The reference spectrum is calculated when sample compartment is empty. Then, the sample is placed in the sample compartment and the sample spectrum is collected or obtained. The absorbance is then calculated at each wavenumber from the ratio of the sample intensity to the reference intensity (F. James Holler et al, 2006).