EXTRACTION OF KAPPA CARRAGEENAN FROM LOCAL SEAWEED

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SUPERVISOR DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering

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STUDENT DECLARATION

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

Signature

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Date

In The Name of Allah, Most Gracious, Most Merciful

Love special dedicated to…

Special inspiring and special encouraging of my lovely parent: Helima Binti Anwar and Husin Bin Gemak, my siblings and also my truly best friends

Those who has influenced my life on the right course

Thank you so much

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EXTRACTION OF HALAL KAPPA CARRAGEENAN FROM LOCAL SEAWEED

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)

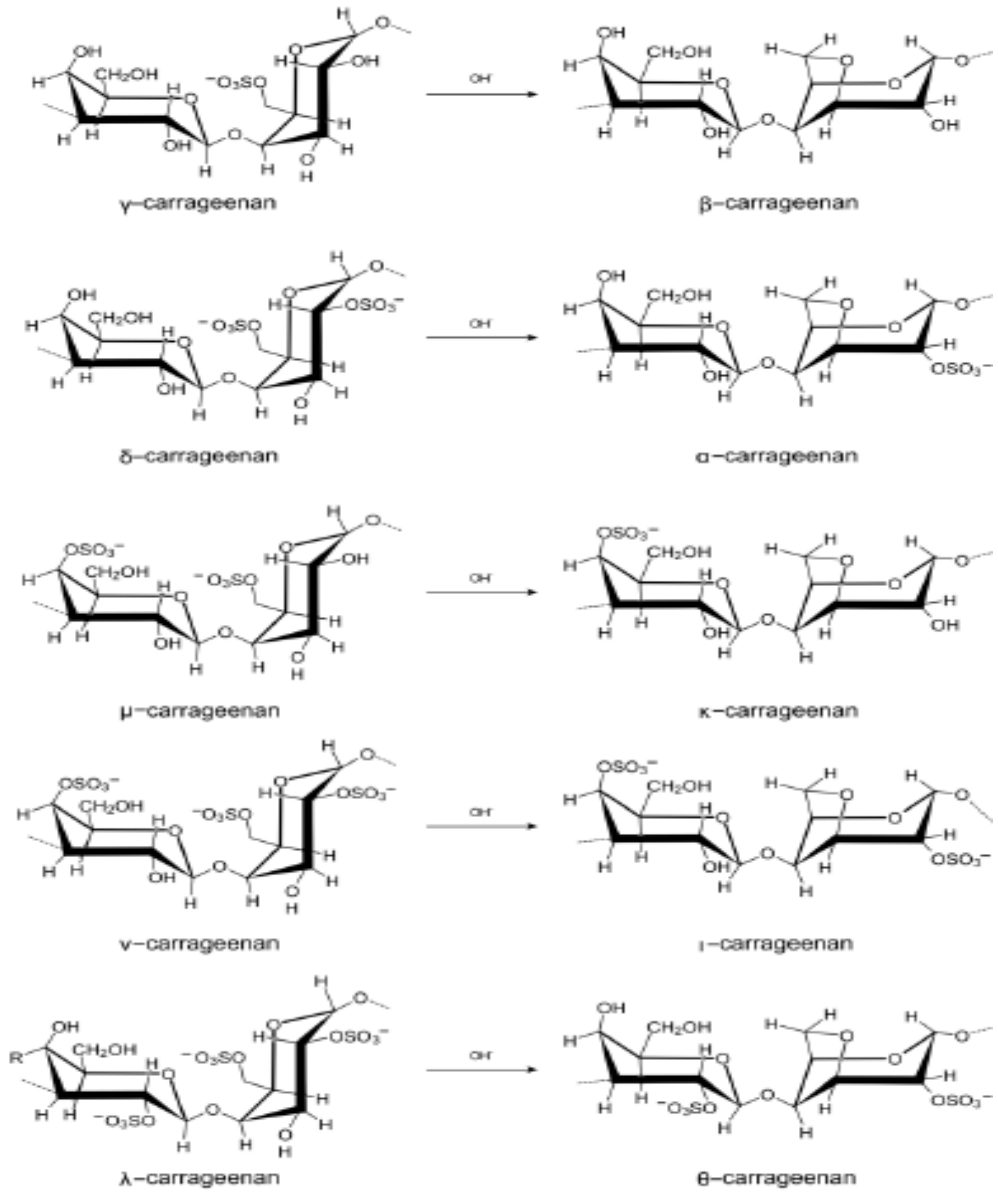


Figure 2.1: Chemical structure of different types of carrageenans (Lenka Bartosikova et al, 2013)

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

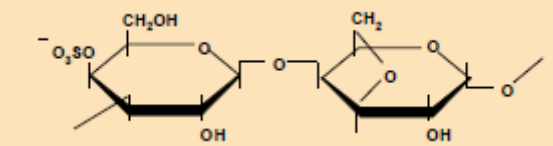
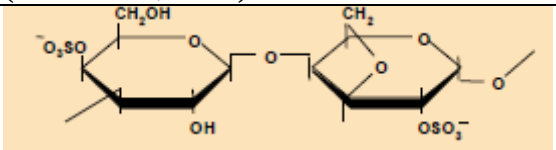
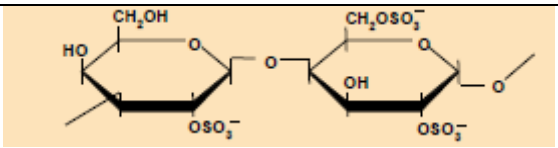
The carrageenan chemical reactivity is primarily due to its half ester sulphate groups which are strongly anionic, being comparable to inorganic sulphate in this respect. The free acid is unstable, and commercial carrageenans are available as stable calcium salts and sodium potassium. The physical properties 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

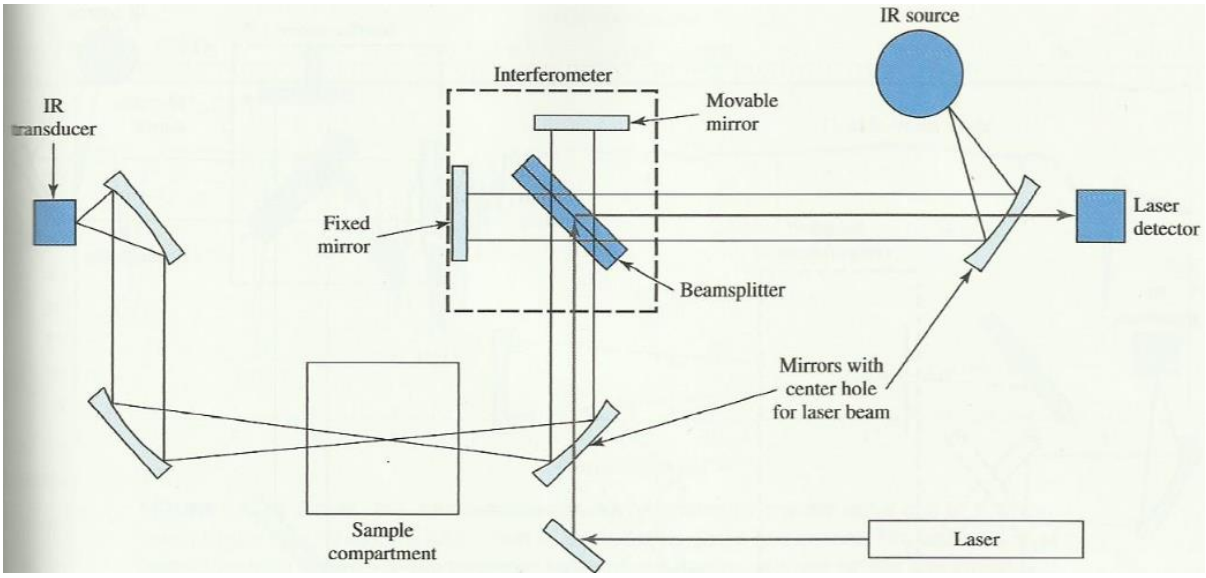


Figure 2.2: Single beam FTIR spectrometer (Sources: F. James Holler et al, 2006)

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).

CHAPTER 3

METHODOLOGY

3.1 APPARATUS AND EQUIPMENT

Beaker, conical flask, filter funnel, dropper, syringe, spatula, glass rod, thermometer, glass bottle, hot plate stirrer, stop watch, aluminum foil, filter cloth, filter paper, litmus paper, magnetic stirrer, micro slide, powder jar, measuring cylinder, volumetric flask, water bath, FTIR, fumes, oven, refrigerator, desiccator, test tube, knife and scissors, weighing boat and analytical balance.

3.2 MATERIAL AND CHEMICAL

Eucheuma cottonii seaweed, distilled water, deionized water, tap water, potassium hydroxide, sodium hydroxide, calcium hydroxide, potassium chloride salts and dilute hydrochloric acid.

3.3 EXTRACTION METHOD OF CARRAGEENAN (KCl PRECIPITATION METHOD)

CLEAN & WASH SEAWEED

1. 10 g of seaweed (Eucheuma Cottonii)
2. Washing time – (10- 30 minutes)
3. Use distilled or deionized water

ALKALINE TREATMENT

1. 0.05 M (KOH / NaOH / $Ca(OH)_2$
2. Initial temperature = 90℃, Operating temperature = 60℃-70℃
3. Time = 2 hours and 30 minutes

COARSE & FINE FILTRATION

1. Use filter cloth and filter paper to separate precipitate (carrageenan) from seaweed

KCl PRECEPITATION

1. 4 g KCl / 200 mL H_2O
2. Time = 10-30 minutes, Temperature = 10℃-15℃
3. Recommendation: Stir gently using hand

DRYING

1. Drying precipitate carrageenan at oven for 16-17 hours at temperature 60℃

DESCRIPTION OF PROCEDURES OF EXTRACTION

A) Clean & Wash Seaweed

1. First 10 g of seaweed (Eucheuma Cottonii, which come from Sabah region) is washed using distilled water or deionised water for 10-30 minutes until it satisfy clean. Use distilled water or deionised to remove all impurities from seaweed.
2. Then cut seaweed into 1.0 cm -1.5 cm before treat it with alkaline solution.

B) Alkaline Treatment

1. First 0.05 M KOH is prepared (such as 0.5611 g KOH / 200 Ml distilled water), make sure KOH in pellet form is completely dissolve in distilled water. Recommendation: use distilled water for dilution, since it is free from impurities.
2. Then, heat up the alkaline solution (KOH solution) to 90℃ as initial temperature in the water bath. Make sure water bath in a good and maintain condition to avoid the temperature deviated from initial setting temperature.
3. When, temperature of alkaline solution reached 90℃. The seaweed slices are mixed with alkaline solution, and turn on the hot plate stirrer to 60℃ constantly, and use magnetic stirrer to stir this mixture solution. Let the seaweed is boiled in the hot plate stirrer for 2 hours 30 minutes.

C) Coarse & Fine Filtration

1. When, seaweed finish boiled at 2 hours 30 minutes, separate the carrageenan from seaweed solid by using filter funnel and filter cloth.
2. Then, these carrageenan is precipitated using cold KCl solution.

D) KCl Precipitation

1. First, KCl solution is prepared. Dissolve 4 g KCl in 200 mL distilled water. Make sure it dissolved completely. Then, store and keep the KCl solution in the refrigerator at temperature between 10-15℃.
2. When, the KCl solution reached at that desired temperature (10-15℃), it is ready use for precipitation purpose. Then, this cold KCl solution is used to precipitate the carrageenan that filtered from previous step. Then, use filter paper to separate precipitate from excess KCl solution.

E) Drying

1. The precipitated carrageenan is dried on the oven at temperature 60℃ for 16-17 hours, this to make sure it completely dry and free from water.

F) Repeat

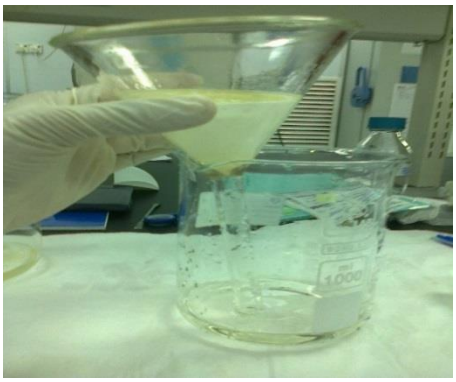
1. Then, run next or other sample, by repeat step A to E and replace the alkali of alkaline solution in the step B. The alkali is NaOH and $Ca(OH)_2$. Then the data is recorded as shown in the chapter result and discussion.

DIAGRAM OF EXTRACTION PROCESS

Heat up the alkaline solution (such as KOH / NaOH / $Ca(OH)_2$ solution) to 90℃ as initial temperature

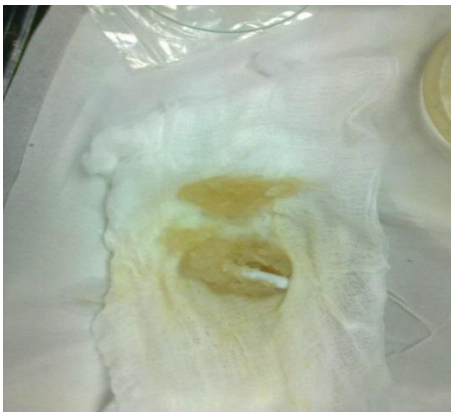
The seaweed slices are mixed with alkaline solution, and turn on the hot plate stirrer to 60℃ and boiled in for 2 hours 30 minutes.

Cold KCl solution is used to precipitate the carrageenan

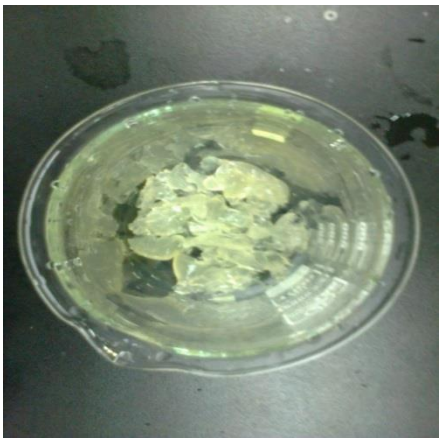


Filter paper is used to separate precipitate of carrageenan from excess KCl solution.

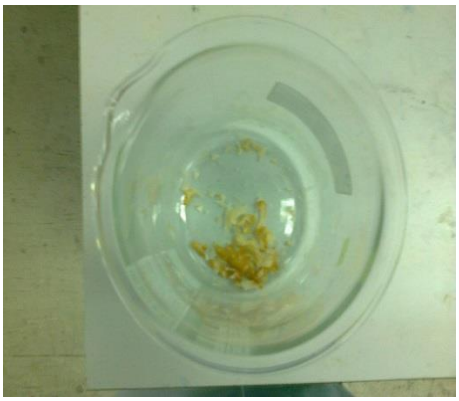
Precipitate of carrageenan



Seaweed solid waste (Residue)



Wet carrageenan before drying it on oven at 60℃ for 16-17 hours.



Dried carrageenan

Cold KCl solution (10-15℃) is used to precipitate carrageenan

3.4 ANALYSE METHOD

3.4.1 ANALYSE CARRAGEENAN YIELD

Yield is defined as ratio of dried carrageenan weight to dried seaweed weight (Wiratni et al, 2011)

Percentage Yield (%) = [Dried carrageenan/Dried seaweed] X 100%

3.4.2 ANALYSE CARRAGEENAN GEL STRENGTH

Gel strength is determined by using the method described by Falshaw (Falshaw et al, 1998) with minor modifications. The wet carrageenan precipitate is stick on microslide and dried it on the oven for 5 hours. Measure the weight of microslide, the weight of the dried carrageenan before collapse, and the weight of dried carrageenan after collapse. Use small stainless rod (surface area 0.2 cm^2) to press the surface area of dried carrageenan until it collapse by using hand. Then measure the difference before and after dried carrageenan collapse (Wiratni et al, 2011). The large difference, the weak of carrageenan gel strength (Wiratni et al, 2011).

Difference Weight of dried carrageenan	= [Dried carrageenan before collapse (weight) – Dried carrageenan after collapse (weight)]

3.5 FTIR ANALYSIS

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).

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 display one band at 845 cm^{-1} due to the galactose-4-sulphate (Seekal and Legrand, 1993). Meanwhile, iota carrageenan spectrum present one 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 are appear at 830 cm^{-1} and 820 cm^{-1} which are corresponding to galactose-2-sulphate and to galactose-6-sulphate, respectively (Chopin et al, 1993).

CHAPTER 4

RESULT AND DISCUSSION

4.1 INTRODUCTION

This chapter will explain and discuss more detail about the gel strength and yield of carrageenan at different types of alkaline treatment (Falshaw et al, 1998; Wiratni et al, 2011) and analysis of carrageenan functional group through Fourier Transform Infrared, FTIR (Prado, 2001).

4.1 YIELD OF CARRAGEENAN AT DIFFERENT TYPES OF ALKALINE TREATMENT

In this study, different chemical treatment was applied to the extraction process such NaOH, KOH, $Ca(OH)_2$. And distilled water. The extraction yield is defined as ratio of dried carrageenan weight to dried seaweed weight (Wiratni et al, 2011), Percentage Yield (%) = [Dried carrageenan/Dried seaweed] X 100%. The percentage yield of carrageenan at different types of alkaline treatment is shown as in Graph 4.1 below. Yield of carrageenan that treated with KOH solution is the lowest one, which is about 17.6%. Yield of carrageenan that treated with NaOH solution is about 23.7%. Meanwhile, carrageenan that treated with no alkaline solution (distilled water) has 25.10% which is the highest yield. Yield of carrageenan that treated with $Ca(OH)_2$ solution is 21.7%. Therefore, yield of carrageenan that treated with no solution (distilled water) > NaOH solution > $Ca(OH)_2$ solution > KOH solution. US Patent Application Publication (US 2008/0317926 A1) reported that Eucheuma cottonii seaweed which treated using calcium hydroxide has higher yield of

carrageenan after seaweed treated with no alkaline solution. Based on US 2008/0317926 A1 data, seaweed that treated with no alkaline solution has 100% yield of carrageenan, treated with $Ca(OH)_2$ solution is 90%, treated with NaOH solution is 25% and seaweed that treated with KOH solution is 20%.

The percentage yield of carrageenan which treated with no alkaline solution and calcium hydroxide solution in the experiment of this study is contrary to the finding reported from the US 2008/0317926 A1 data. This contrary is might be effected by several factor during extraction of carrageenan and this factor may come from unrealized human error that conduct experiment. The factor may come from different temperature setting (± 5℃), efficiency of hot plate stirrer on maintaining temperature, error of analytical balance, efficiency of water bath in term of maintaining of temperature, a bit different washing time (± 5 minutes), a bit different alkaline treatment time (± 5-10 minutes), unrealized impurities and etc.

In this study, 10 g of Eucheuma cottonii seaweed sample was used for extraction of carrageenan (US 2008/0317926 A1, US 2002/ 0098553 A1). The washing time of Eucheuma cottonii seaweed can affect the yield of carrageenan (Jens Eskil Trudsoe, 2008). Besides that, according to Jens Eskil Trudsoe different washing temperature can affect the result of carrageenan yield. After washing, seaweed is cut into 1.0 cm -1.5 cm before treat it with alkaline solution, ±0.5 of length cutting may also give little effect on the result, the small cutting is better and provide large surface area contact for extraction mechanism. Distilled water is recommended for washing seaweed, since it is free from contamination of impurities and deionized water as option cleanser (Jens Eskil Trudsoe, 2008).

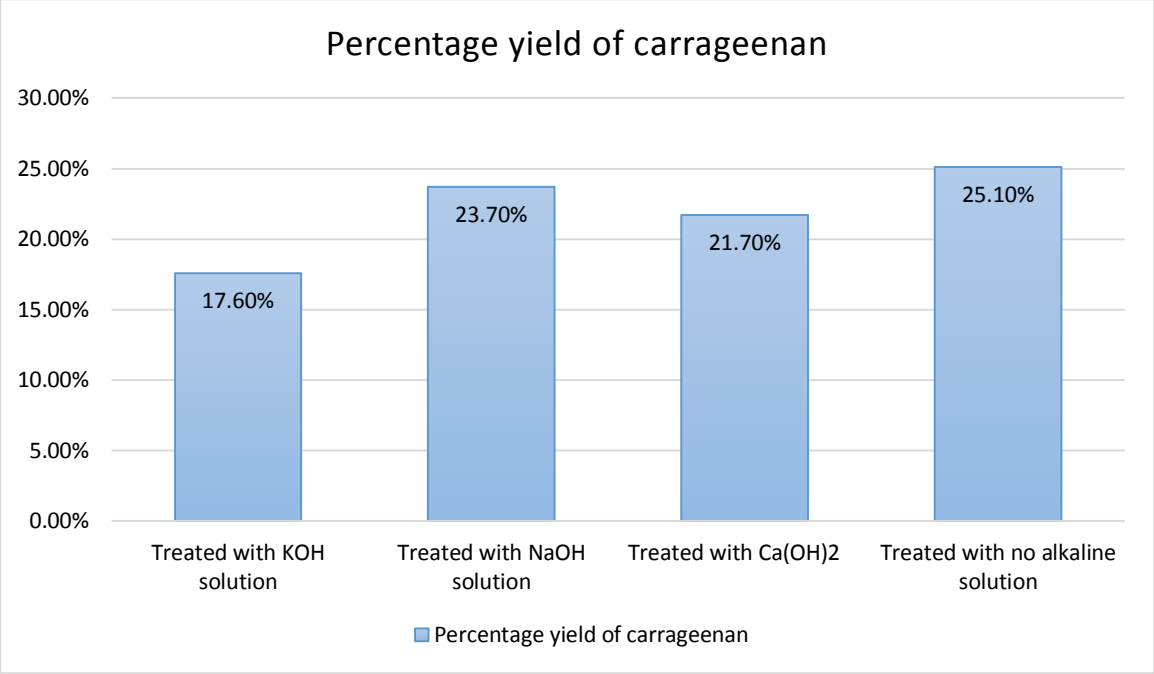
Then, seaweed is treated with different alkaline solution. First, it treated with KOH solution, NaOH solution, $Ca(OH)_2$ solution and followed by no alkaline solution. These alkali metal oxide are used to modify the carrageenan. KOH solution is more effective in converting mu-carrageenan into kappa-carrageenan (Gabriela Azevado et al, 2013). Meanwhile, NaOH solution is effective in converting nu-carrageenan into iota-carrageenan (Gabriela Azevado et al, 2013). Where kappa

carrageenan has a good and strong gels which necessary to replace gelatin function in the medicine capsules (Piere Etiene Bost et al, 2002). Kappa and iota carrageenans have valuable characteristics or properties as food additives and usually used as gelling, thickening and emulsifying agents. Kappa carrageenan which produced from Eucheuma cottonii has strong rigid gels if compared to other species of red seaweed such as Gigartina or Chondrus crispus (Piere Etiene Bost et al, 2002).

Higher concentration and longer pre-treatment durations of KOH will favour the conversion of nu-carrageenan into kappa-carrageenan, and consequently favour the formation of stronger rigid gels (Gabriela Azevado et al, 2013). In this study, the concentration of all types of alkaline solution are keep constant to control the process variable. The concentration of alkaline solution used in this study is 0.05M (Wiratni et al, 2011). The Eucheuma cottonii seaweed is treated in the alkaline solution at controlled initial temperature of 90℃, operating temperature in between 60℃-70℃ (Christopher S. Rideout et al, 1998), and at 2 hours 30 minutes of treatment period. The error may happen in operating temperature, which ± 5℃ may affect the yield percentage of carrageenan from seaweed. Besides that, the efficiency of water bath in term of temperature may cause the initial temperature of alkaline solution that used in treatment is deviated from the actual setting temperature of initial temperature of treatment. In this study, period of treatment is fixed for 2 hours 30 minutes, according to Gabriela Azevado et al, 2013, the longer or shorter of pre-treatment will affect the yield and gel strength of carrageenan.

After seaweed is treated with alkaline solution, the carrageenan is treated with cold KCl salt solution which has temperature in between 10℃ -15℃. The function of KCl salt is to coagulate and precipitate the carrageenan (Christopher S. Rideout et al, 1998). Where the KCl salt provide monovalent cation to prevent diffusion of calcium, potassium and magnesium ions from diffuse into carrageenan, it helps reduce the aqueous solubility of the carrageenan from dissolve into water (Jens Eskil Trudsoe, 2008). During this stage, a little of carrageenan is stick on the filter cloth and filter funnel, and this might affect the percentage yield of carrageenan. Next, the precipitate of carrageenan is filtered and separated from KCl salt solution using filter paper, and this precipitate is drying on the oven. In the oven, the precipitate of carrageenan is dried for 16-17 hours at

temperature 60℃ to avoid the carrageenan from denaturation (Christopher S. Rideout et al, 1998; Wiratni et al, 2011).



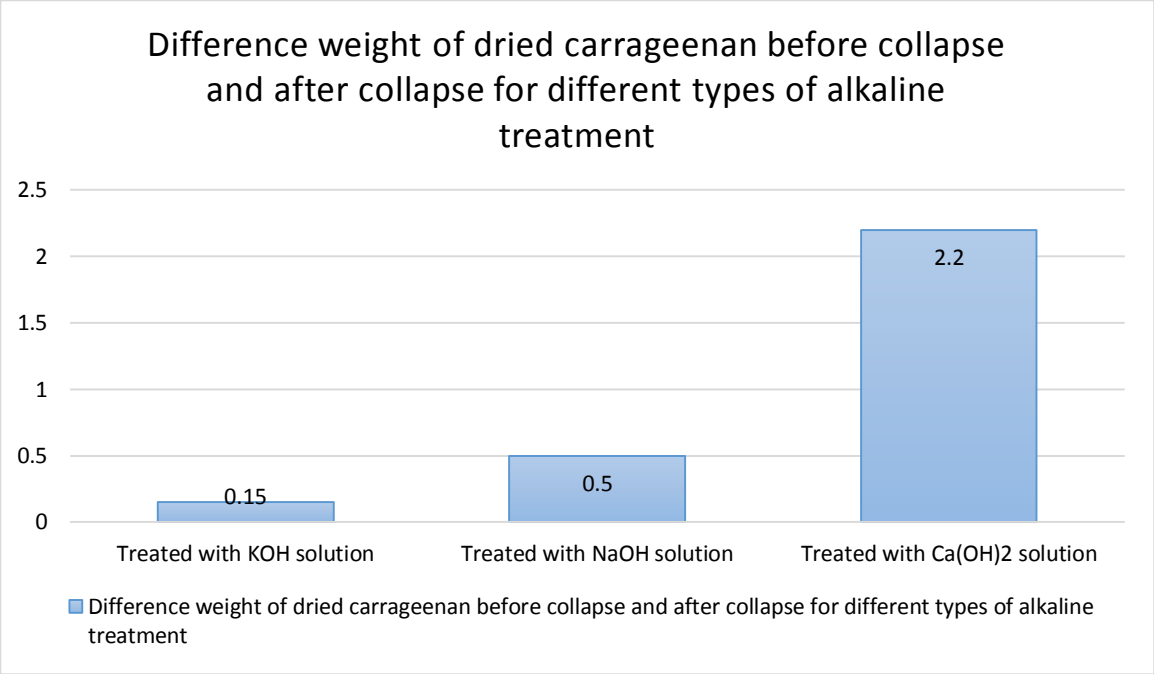
Graph 4.1: Percentage yield of carrageenan at different alkaline treatment

4.2 GEL STRENGTH OF CARRAGEENAN AT DIFFERENT TYPES OF ALKALINE TREATMENT

Based on Graph 4.2, the carrageenan that treated with calcium hydroxide, $Ca(OH)_2$ has the largest difference weight before and after collapse which is about 2.2 mg, and followed by carrageenan that treated with sodium hydroxide (NaOH) has difference weight at 0.5 mg. Meanwhile, carrageenan that treated with potassium hydroxide (KOH) solution has the smallest difference weight, 0.15 mg. This indicate that carrageenan treated with KOH solution has a good and strong of gel strength if compared to other carrageenan that treated with other alkaline solution. The large difference, the weak of carrageenan gel strength (Wiratni et al, 2011; Falshaw et al, 1998).

The presence of KOH concentration and longer KOH treatment favour the conversion of nu-carrageenan into kappa carrageenan in the Eucheuma cottonii seaweed, and consenquently favour the formation of stronger gels (Gabriela Azevado et al, 2013). In addition, NaOH solution is more effective in converting nu-carrageenan in the Eucheuma cottonii seaweed into iota-carrageenan if compared to KOH solution. Kappa carrageenan has a better gel strength if compared to iota carrageenan (Gabriela Azevado et al, 2013; Piere Etiene Bost et al, 2002). Therefore, the small difference weight before and after collapse of carrageenan that treated with KOH solution as shown in Graph 4.2, indicate it has good gel strength than other carrageenan that treated with different alkaline solution. The KOH solution will use and reduce the sulfate content of carrageenan, order to convert mu-carrageenan (precursor carrageenan) into kappa-carrageenan (has strong rigid gels). The carrageenan that extracted by calcium hydroxide has weak gel, it may be caused by the lower cation in the carrageenan (Jens Eskil Trudsoe, 2008).

From this study, carrageenan that treated with 0.05 M of KOH solution has good and strong gel strength due to the of presence of kappa carrageenan. As a result, from the conversion of Mu-carrageenan into kappa carrageenan (Wiratni et al, 2011; Jens Eskil Trudsoe, 2008). Carrageenan that treated with 0.05 M of NaOH solution has a weak gel strength, since Nu-carrageenan in the Eucheuma cottonii seaweed are converted to iota carrageenan that has weak gel properties if compared to kappa carrageenan (Gabriela Azevado et al, 2013; Piere Etiene Bost et al, 2002). Meanwhile, the carrageenan that treated with calcium hydroxide, $Ca(OH)_2$ has the largest difference weight before and after collapse indicated that it has the weakest gel strength among other carrageenans which were treated by different types of alkaline solution. Gel strength is important to replace the gel behavior of gelatin in the manufacturing of medicine capsules and provided halal capsules to Muslim, vegetarian and also non-vegetarian with low cost of production than other plant based capsules (Piere Etiene Bost et al, 2002).



Graph 4.2: Difference weight of dried carrageenan before and after collapse for different types of alkaline treatment

4.3Analysis of functional group of carrageenan by FTIR

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 contains 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 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)

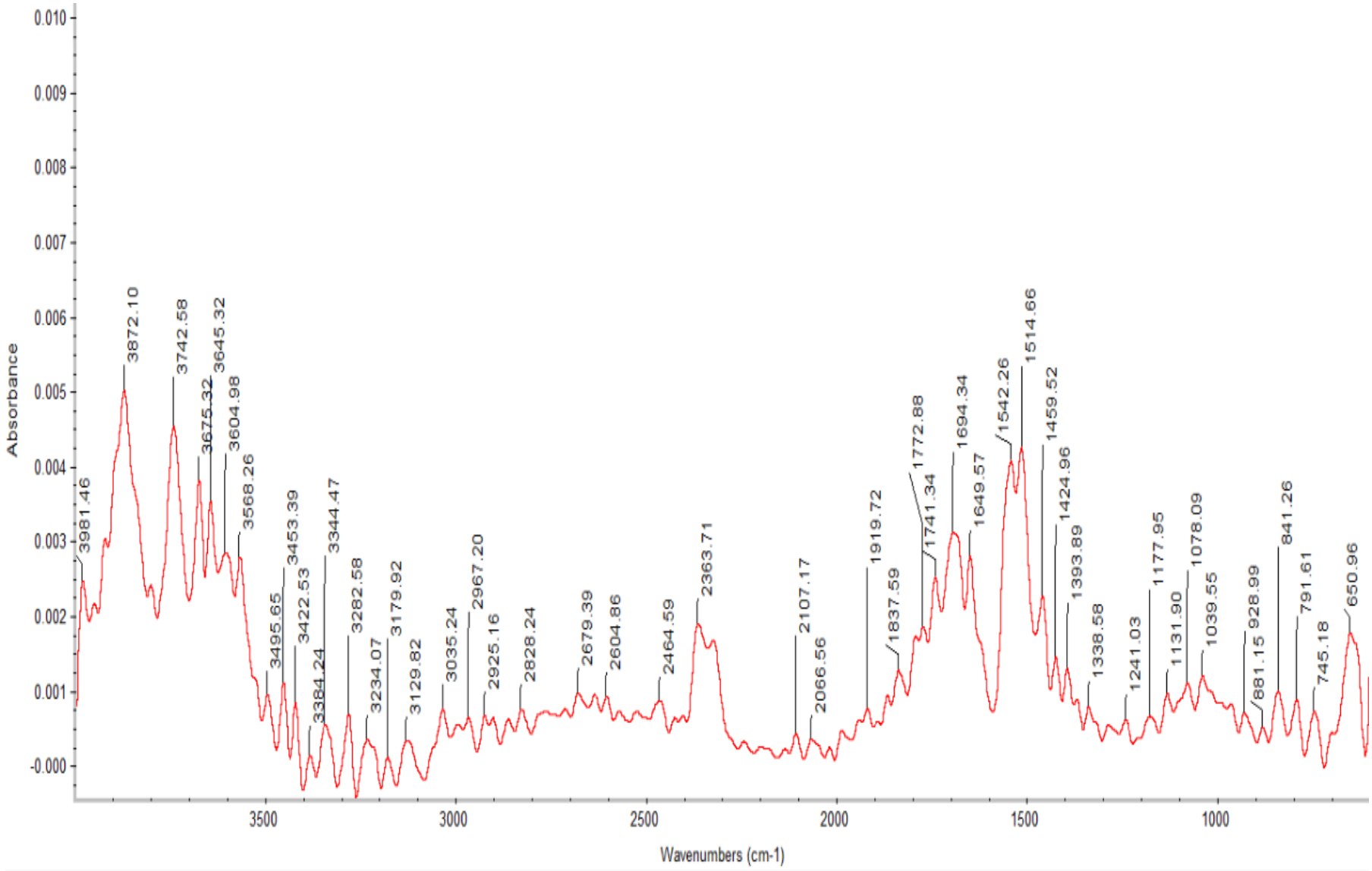
Prado, 2002. The carrageenans has the total sulphate content at the absorbance band at 1250 cm^{-1}, which was decreased from lambda carrageenan to iota carrageenan to kappa carrageenan (Chopin et al, 1993). Kappa carrageenan spectrum display a band at 845 cm^{-1} due to the galactose-4-sulphate (Seekal and Legrand, 1993). Meanwhile, iota carrageenan spectrum demonstrates a band at 845 cm^{-1} arising from the galactose-4-sulpahate (Seekal and Legrand, 1993) and 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 are corresponding to the galactose-2-sulphate and to galactose-6-sulphate, respectively (Chopin et al, 1993).

Table 4.1: Characteristics of Infrared Absorption Frequencies

CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES	
Wave number (cm^{-1})	Compound Type
800-805	3,6-anhydrogalactose-2-sulfate
810-820	Galactose-6-sulfate
825-830	Galactose-2-sulfate
840-850	Galactose-4-sulfate
928-933	3,6-anhydrogalactose
1000-1100	Polysaccharides
1220-1260	Ester sulfate

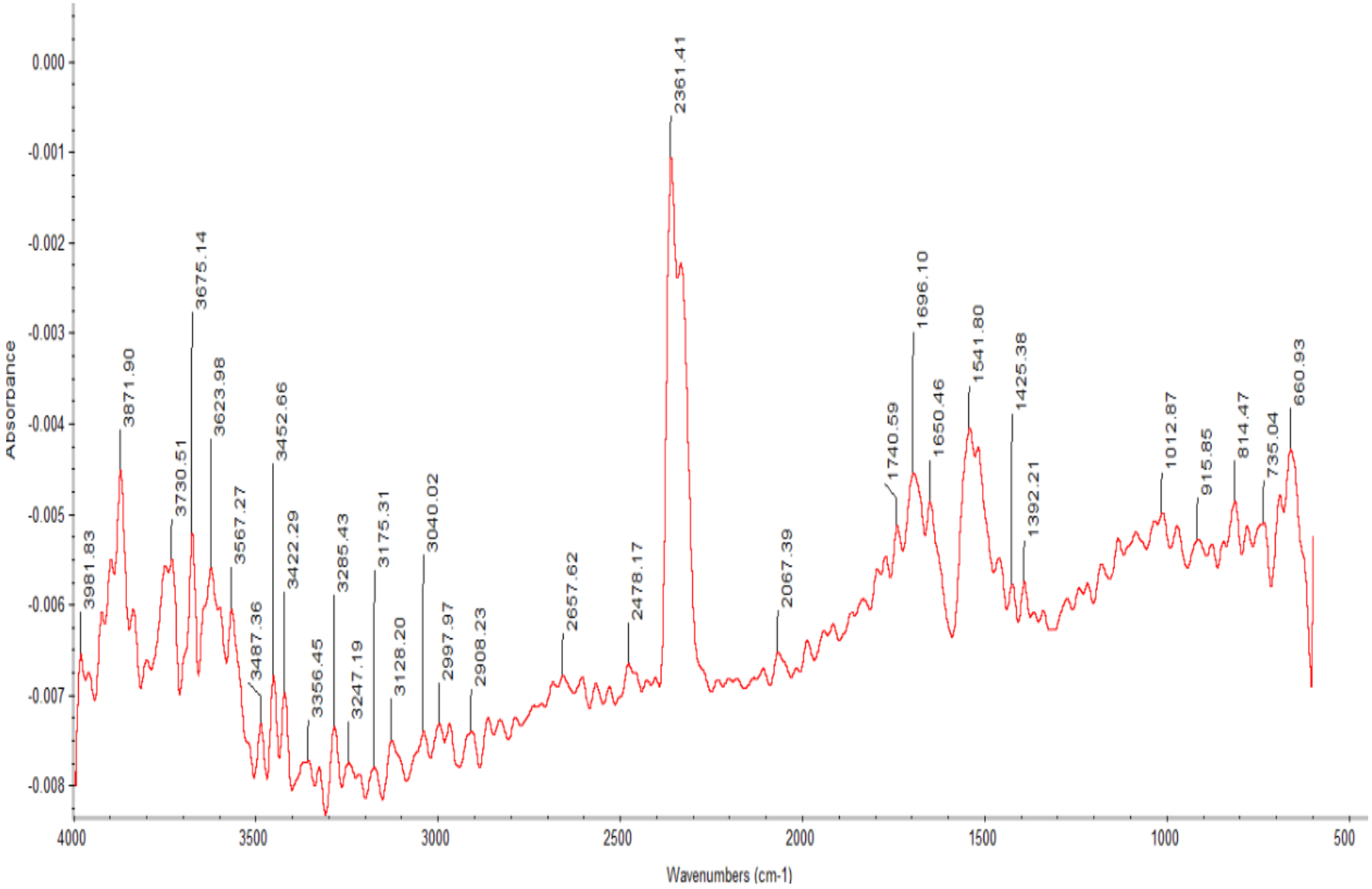
Source: JECFA, 2001

Based on Graph 4.3, the carrageenan which treated with KOH solution has the total sulphate content at the absorbance band at 1241.03 cm^{-1}, 3,6-anhydrogalactose was found the at the absorbance band at 928.99 cm^{-1}, and the galactose-4-sulfate at the absorbance band of 841.26 cm^{-1}. The presence of galactose-4-sulfate on this graph indicated the existence of kappa carrageenan as mentioned by Prado, 2002. In the Seekal and Legrand study, Kappa carrageenan spectrum displayed a band at 845 cm^{-1} due to the galactose-4-sulphate (Seekal and Legrand, 1993). This strong enough to support the carrageenan that treated with KOH solution has a good gel strength, since kappa carrageenan has good gel strength if comparing to iota carrageenan (Gabriela Azevado et al, 2013; Piere Etiene Bost et al, 2002). In addition, the presence of KOH concentration and longer KOH treatment favour the conversion of nu-carrageenan into kappa carrageenan in the Eucheuma cottonii seaweed, and consenquently favour the formation of stronger gels (Gabriela Azevado et al, 2013). The KOH solution will reduce the sulfate content of carrageenan, to convert mu-carrageenan (precursor carrageenan) into kappa-carrageenan (has strong rigid gels). As a result, the carrageenan that treated with KOH solution has small of weight difference before and after collapse, and this indicate, it has good gel strength among other carrageenan.



Graph 4.3: Result of FTIR of carrageenan that treated with KOH solution, A1

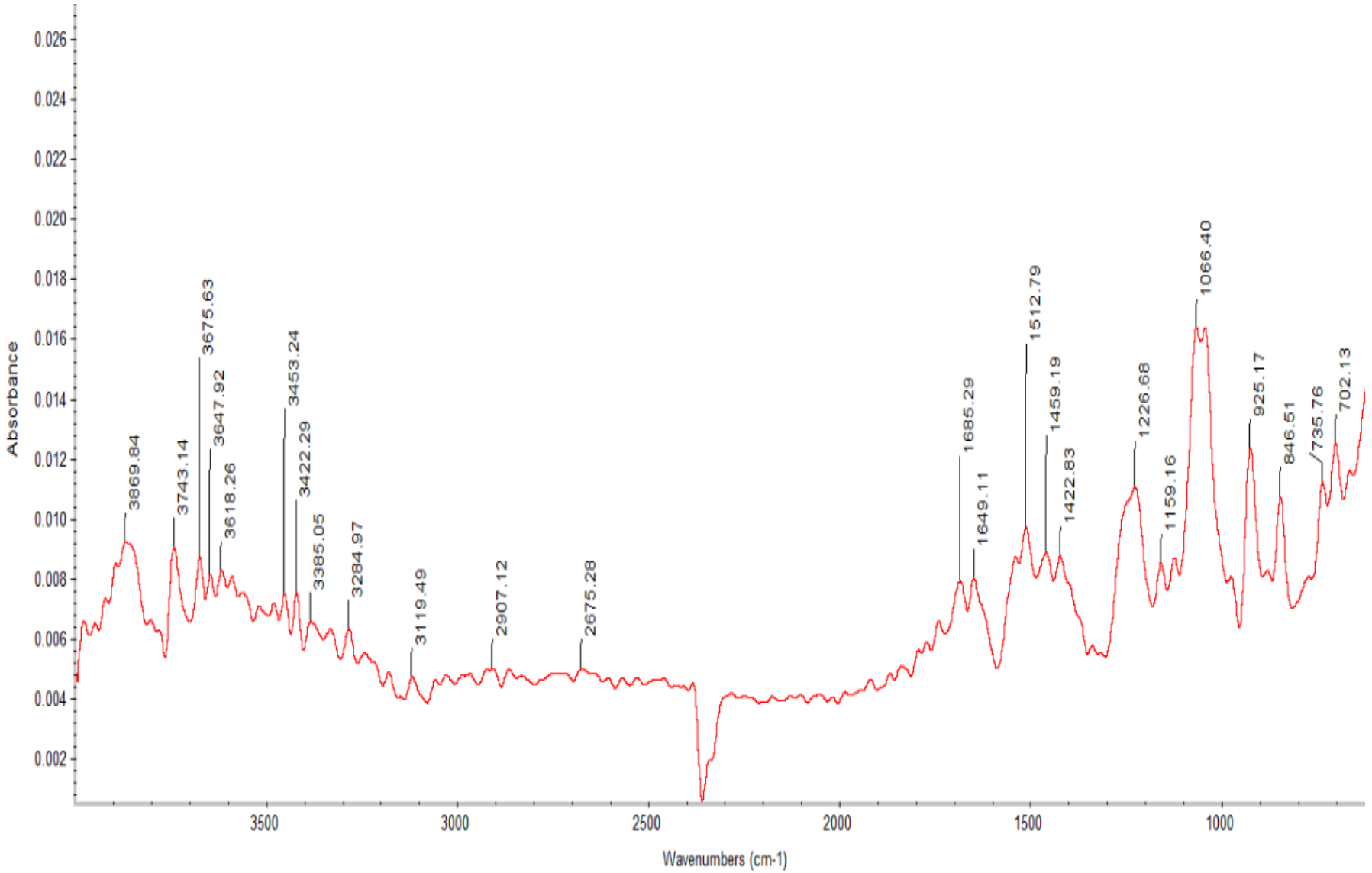
Based on the Graph 4.4 from this study, the carrageenan that treated with NaOH solution has the total polysaccharides content at the absorbance band at 1012.87 cm^{-1} and the galactose-6-sulfate at the absorbance band at 814.47 cm^{-1}. The present of galactose-6-sulphate indicate the existed of carrageenan group, especially lambda carrageenan. In lambda carrageenan, two bands of spectrum are appear at 830 cm^{-1} and 820 cm^{-1} which are corresponding to galactose-2-sulphate and to galactose-6-sulphate, respectively (Chopin et al, 1993). Lambda carrageenan does not form gels but is widely used as a viscosifier in many food applications (Whitler et al, 1997). Suppose FTIR result show the absorbance band in range 800-805 cm^{-1} (3,6-anhydrogalactose-2-sulfate) to indicate the existing of iota carrageenan, but from result of study, FTIR was not show any presence of 3,6-anhydrogalactose-2-sulfate. NaOH solution is effective in converting nu-carrageenan in the Eucheuma cottonii seaweed into iota-carrageenan if compare to KOH solution (Gabriela Azevado et al, 2013). According to Prado, 2002, iota carrageenan has absorbance band at 805 cm^{-1} which represent the exist of 3,6-anhydrogalactose-2-sulfate. The carrageenan sample that treated with NaOH solution do not show the presence of 3,6-anhydrogalactose-2-sulfate in FTIR peak, this may cause by lack of NaOH concentration to convert nu-carrageenan into iota-carrageenan. Therefore, it is recommended to further study on effect of NaOH concentration to yield and gel strength of iota carrageenan. In this study, the concentration of NaOH is 0.05 M, it means is essential to increase the concentration of NaOH toward carrageenan treatment on the further study.



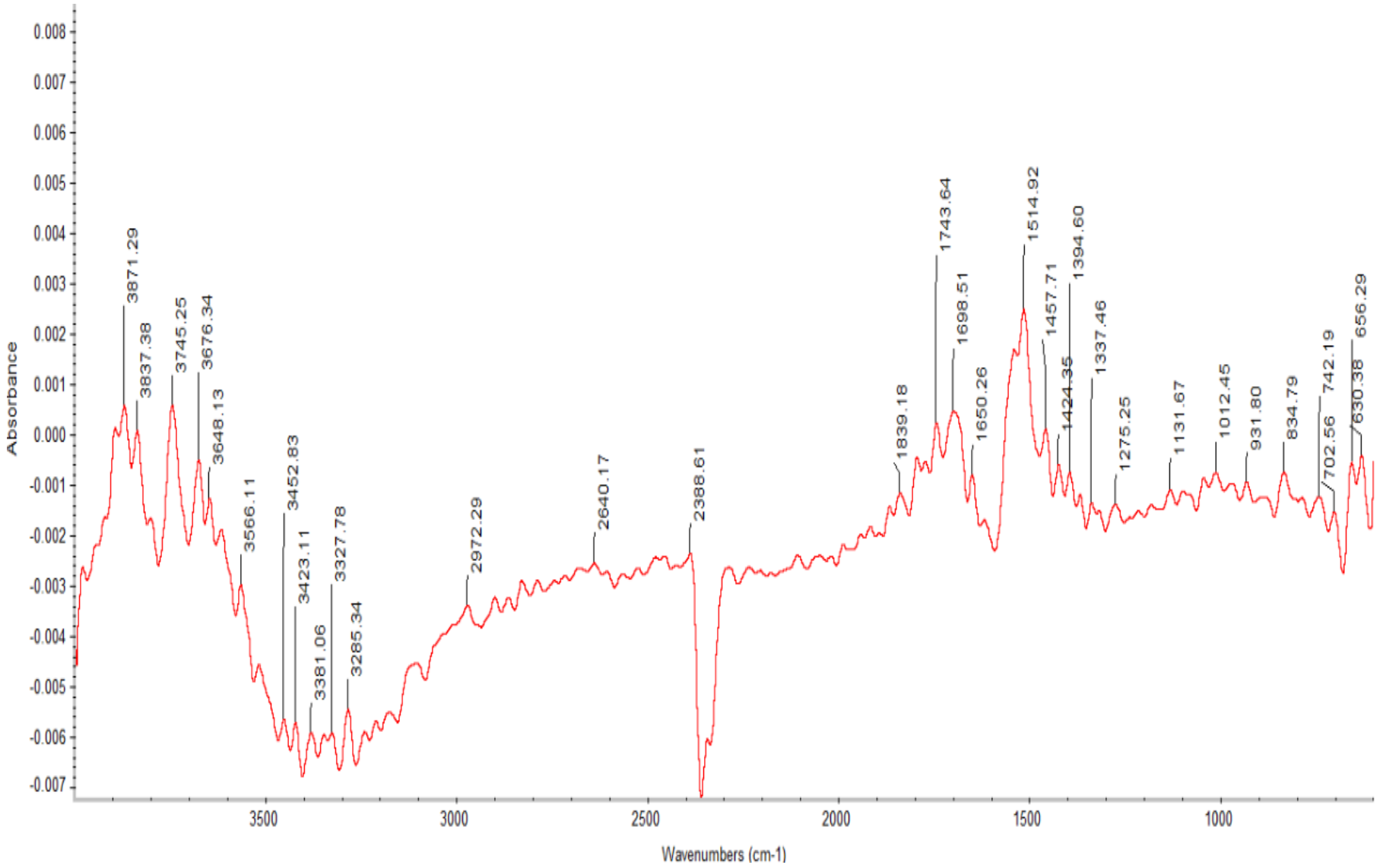
Graph 4.4: Result of FTIR of carrageenan that treated with NaOH solution, B1

Based on Graph 4.5 from this study, the carrageenan that treated with $Ca(OH)_2$ solution has the total sulphate content at the absorbance band at 1226.68 cm^{-1}, the 3,6-anhydrogalactose at the absorbance band at 925.17 cm^{-1}, the total polysaccharides content at the absorbance band at 1066.40 cm^{-1} , and the galactose-4-sulfate at the absorbance band at 846.51 cm^{-1}. The presence of galactose-4-sulfate on this graph indicate the existed of kappa carrageenan as mentioned by Prado, 2002. But, the kappa carrageenan that treated with calcium hydroxide is much brittle (weak gel strength) than the kappa carrageenan that treated with KOH solution. As a result seen in the Graph 4.2 from this study, it has the largest weight difference before and after collapse, and this indicate it has weak gel strength. To get an accurate and precise result, it is recommended to use the most common and convenient texture analyser, which is Stevens LFRA Texture analyser or Texture analyser TA-XT2 (Source: Rousselot a Vion Company, July 2007), they commonly used to measure the gel strength of hydrocolloid especially gelatin sample.

Based on Graph 4.6 , the carrageenan that treated with no alkaline solution (distilled water) has the total sulphate content at the absorbance band at 1275.25 cm^{-1}, the 3,6-anhydrogalactose at the absorbance band at 931.80 cm^{-1}, the total polysaccharides content at the absorbance band at 1012.45 cm^{-1}. From the FTIR result of this study, the carrageenan that treated with distilled water is still categorized as carrageenan group since the present of ester sulphate and the 3,6-anhydrogalactose (Gail Fisher, 2009).



Graph 4.5: Result of FTIR of carrageenan that treated with $Ca(OH)_2$ solution, C1



Graph 4.6: Result of FTIR of carrageenan that treated with no alkaline solution, D1

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, the objective of this study to extract kappa carrageenan from the local seaweed, Eucheuma Cottonii through the KCl Precipitation method, and to determine the effect of different type of alkaline treatment on the carrageenan yield, gel strength and analyze its functional group using FTIR is successfully done and achieved. Based on the Graph 4.1, the percentage yield of carrageenan that treated with KOH solution is lowest, which is about 17.6%. From this study, the percentage yield of carrageenan that treated with no solution (distilled water) > NaOH solution > $Ca(OH)_2$ solution > KOH solution. The yield percentage of carrageenan that treated with no alkaline solution and calcium hydroxide solution in the experiment are totally deviated from the US 2008/0317926 A1 data. This deviation may effected by several factor during extraction of carrageenan and this factor may come from unrealized human error that conduct experiment.

From Graph 4.2 of this study, the carrageenan that treated with KOH solution has the smallest difference weight before and after collapse if compare to others carrageenan that treated with different alkaline solution, this indicate that carrageenan that treated with KOH solution has good gel strength (Gabriela Azevado et al, 2013; Piere Etiene Bost et al, 2002). From the FTIR result (as shown in Graph 4.3), the carrageenan that treated with KOH solution has the galactose-4-sulfate at the absorbance band at 841.26 cm^{-1}. The present of galactose-4-sulfate on this graph

indicate the existed of kappa carrageenan as mention by Prado, 2002. In the Seekal and Legrand study, Kappa carrageenan spectrum display one band at 845 cm^{-1} due to the galactose-4-sulphate (Seekal and Legrand, 1993). Therefore, the carrageenan that treated with KOH solution has a good gel strength, since kappa carrageenan has good gel strength if comparing to iota carrageenan (Gabriela Azevado et al, 2013; Piere Etiene Bost et al, 2002). Finally, extraction of kappa carrageenan from Eucheuma cottonii seaweed, based on functional group is achieved.

5.2 RECOMMENDATIONS

According to Prado, 2002, iota carrageenan has absorbance band at 805 cm^{-1} which represent the exist of 3,6-anhydrogalactose-2-sulfate. Based on Graph 4.4, the carrageenan sample that treated with NaOH solution do not show the presence of 3,6-anhydrogalactose-2-sulfate in FTIR peak, this may cause by lack of NaOH concentration to convert nu-carrageenan into iota-carrageenan. Therefore, it is recommended to further study on the effect of NaOH concentration to yield and gel strength of iota carrageenan. In this study, the concentration of NaOH is 0.05 M, it means is essential to increase the concentration of NaOH toward carrageenan treatment on the further study. This is important in determining the presence of iota carrageenan in the carrageenan precipitation and how it effects the gel strength of carrageenan.

In this study, gel strength of carrageenan is determined by using the method described by Falshaw (Falshaw et al, 1998) with minor modifications. For a better and accurate result, it is recommended to use the most common and convenient texture analyser, which is Stevens LFRA Texture analyser or Texture analyser TA-XT2 (Source: Rousselot a Vion Company, July 2007). The method is commonly used to measure the gel strength of hydrocolloid especially gelatin sample. In order to determine the composition, element and relative concentration of kappa, iota and lambda carrageenan, it is recommended to use hydrogen-nuclear magnetic resonance (H-NMR), (Prado, 2002; Usov, 1998). It is also recommended to use X-ray diffraction (XRD) in determining the chemical composition and element in the carrageenan.

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APPENDIX A.1

Table of difference weight of dried carrageenan before collapse and after collapse, and yield percentage of carrageenan treated with different alkaline solution

Table 1: Yield percentage of carrageenan treated with KOH solution

Samples	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**
Mass of dried carrageenan (g)	1.9517	1.6095	1.7843	1.8100	1.9320	1.7904	1.8499	1.5273	1.7678
Mass of dried seaweed (g)	10.3666	10.1345	10.0562	10.1164	10.2322	10.3278	10.0041	10.2559	10.1258
Yield percentage (%)	18.8	15.9	17.7	17.9	18.9	17.3	18.5	14.9	17.5

Selected yield for carrageenan treated with KOH solution =17.6%

Table 2: Yield percentage of carrageenan treated with NaOH solution

Samples	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
Mass of dried carrageenan (g)	2.3867	2.8017	2.5611	2.6783	2.2897	2.3456	2.3971	2.7348	2.3569
Mass of dried seaweed (g)	10.0435	10.0293	10.1287	10.0632	10.1016	10.3460	10.0731	10.2548	10.0894
Yield percentage (%)	23.8	27.9	25.3	26.6	22.7	22.7	23.8	26.7	23.4

Selected yield for carrageenan treated with NaOH solution = 23.7%

Table 3: Yield percentage of carrageenan treated with $Ca(OH)_2$ solution

Samples	C1	C2	C3	C4	C5	C6	C7	C8	C9
Mass of dried carrageenan (g)	2.1637	2.2433	2.2982	2.0121	2.1878	2.4326	2.2873	2.0318	2.4007
Mass of dried seaweed (g)	10.0028	10.2901	10.1174	10.1732	10.1635	10.0641	10.4509	10.0332	10.1569
Yield percentage (%)	21.6	21.8	22.7	19.8	21.5	24.2	21.9	20.3	23.6

Selected yield for carrageenan treated with $Ca(OH)_2$ solution = 21.7%

Table 4: Yield percentage of carrageenan treated with no alkaline solution

Samples	D1	D2	D3	D4	D5	D6	D7	D8	D9
Mass of dried carrageenan (g)	2.1770	2.4532	2.3990	2.6071	2.5641	2.5317	2.6709	2.0824	2.3550
Mass of dried seaweed (g)	10.0331	10.1903	10.1456	10.3201	10.2488	10.1121	10.0014	10.0847	10.1936
Yield percentage (%)	21.7	24.1	23.1	25.3	25.0	25.0	26.7	20.6	23.1

Selected yield for carrageenan treated with no alkaline solution = 25.1%

Table 5: Difference weight of dried carrageenan before collapse and after collapse for carrageenan treated with KOH solution

Samples	A1	A2	A3	A4	A5	A6	A7	A8	A9
Mass of dried carrageenan before collapse (g)	5.2441	5.0247	5.0328	5.2984	5.0435	5.6410	5.0873	5.2903	5.2311
Mass of dried carrageenan after collapse (g)	5.2440	5.0243	5.0326	5.2970	5.0434	5.6407	5.0873	5.2897	5.2309
(gel strength) difference weight of dried carrageenan before collapse and after collapse, (mg)	0.1	0.4	0.2	1.4	0.1	0.3	0.0	0.6	0.2

Selected difference weight of dried carrageenan before collapse and after collapse for carrageenan treated with KOH solution = 0.15 mg

Table 6: Difference weight of dried carrageenan before collapse and after collapse for carrageenan treated with NaOH solution

Samples	B1	B2	B3	B4	B5	B6	B7	B8	B9
Mass of dried carrageenan before collapse (g)	5.2587	5.2175	5.3109	5.2781	5.2104	5.6138	5.4295	5.2357	5.0763
Mass of dried carrageenan after collapse (g)	5.2583	5.2170	5.3104	5.2773	5.2098	5.6129	5.4291	5.2339	5.0751
(gel strength) difference weight of dried carrageenan before collapse and after collapse, (mg)	0.4	0.5	0.5	0.8	0.6	0.9	0.4	1.8	1.2

Selected difference weight of dried carrageenan before collapse and after collapse for carrageenan treated with NaOH solution = 0.50 mg

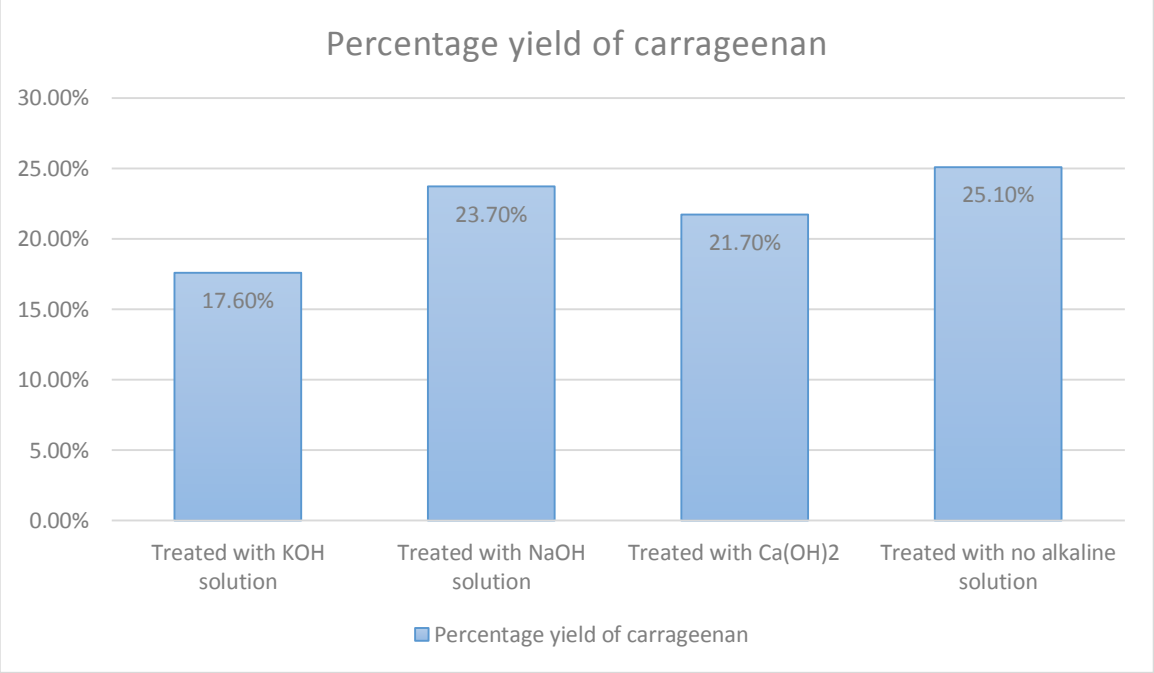
Table 7: Difference weight of dried carrageenan before collapse and after collapse for carrageenan treated with $Ca(OH)_2$ solution

Samples	C1	C2	C3	C4	C5	C6	C7	C8	C9
Mass of dried carrageenan before collapse (g)	5.2041	5.3842	5.0038	5.1059	5.1134	5.1893	5.4217	5.3221	5.4001
Mass of dried carrageenan after collapse (g)	5.2029	5.3833	5.0021	5.1036	5.1121	5.1874	5.4196	5.3199	5.3982
(gel strength) difference weight of dried carrageenan before collapse and after collapse, (mg)	1.2	0.9	1.7	2.3	1.3	1.9	2.1	2.2	1.9

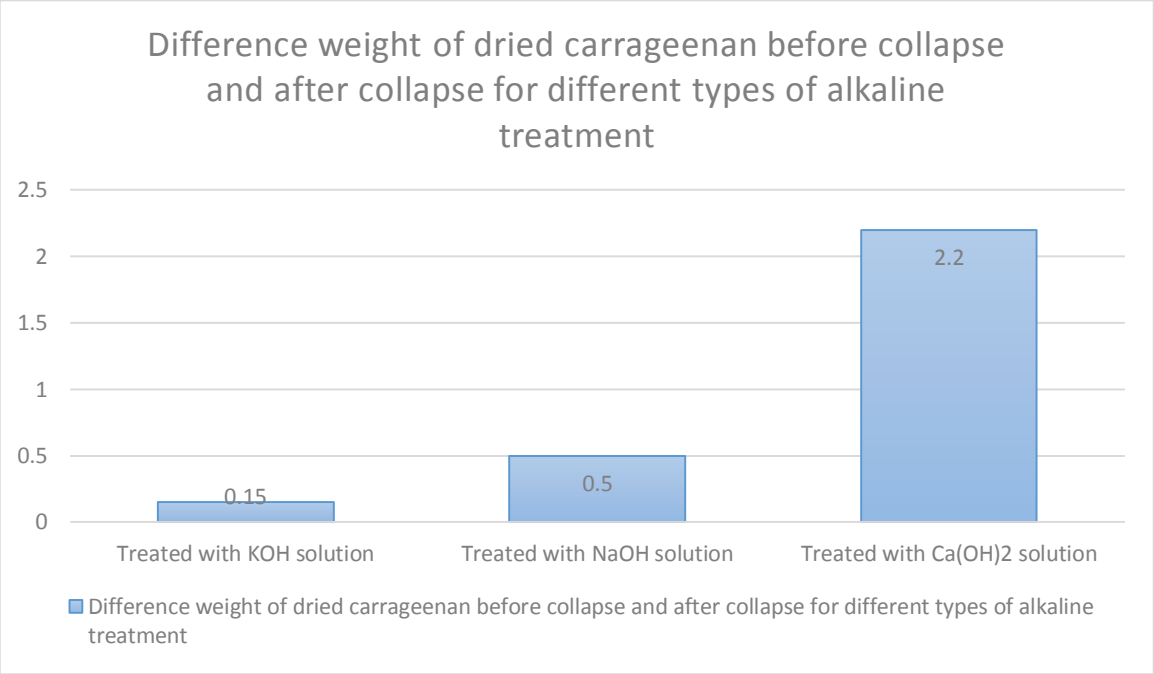
Selected difference weight of dried carrageenan before collapse and after collapse for carrageenan treated with $Ca(OH)_2$ solution = 2.2 mg

APPENDIX A.2

Graph of difference weight of dried carrageenan before collapse and after collapse, and yield percentage of carrageenan treated with different alkaline solution

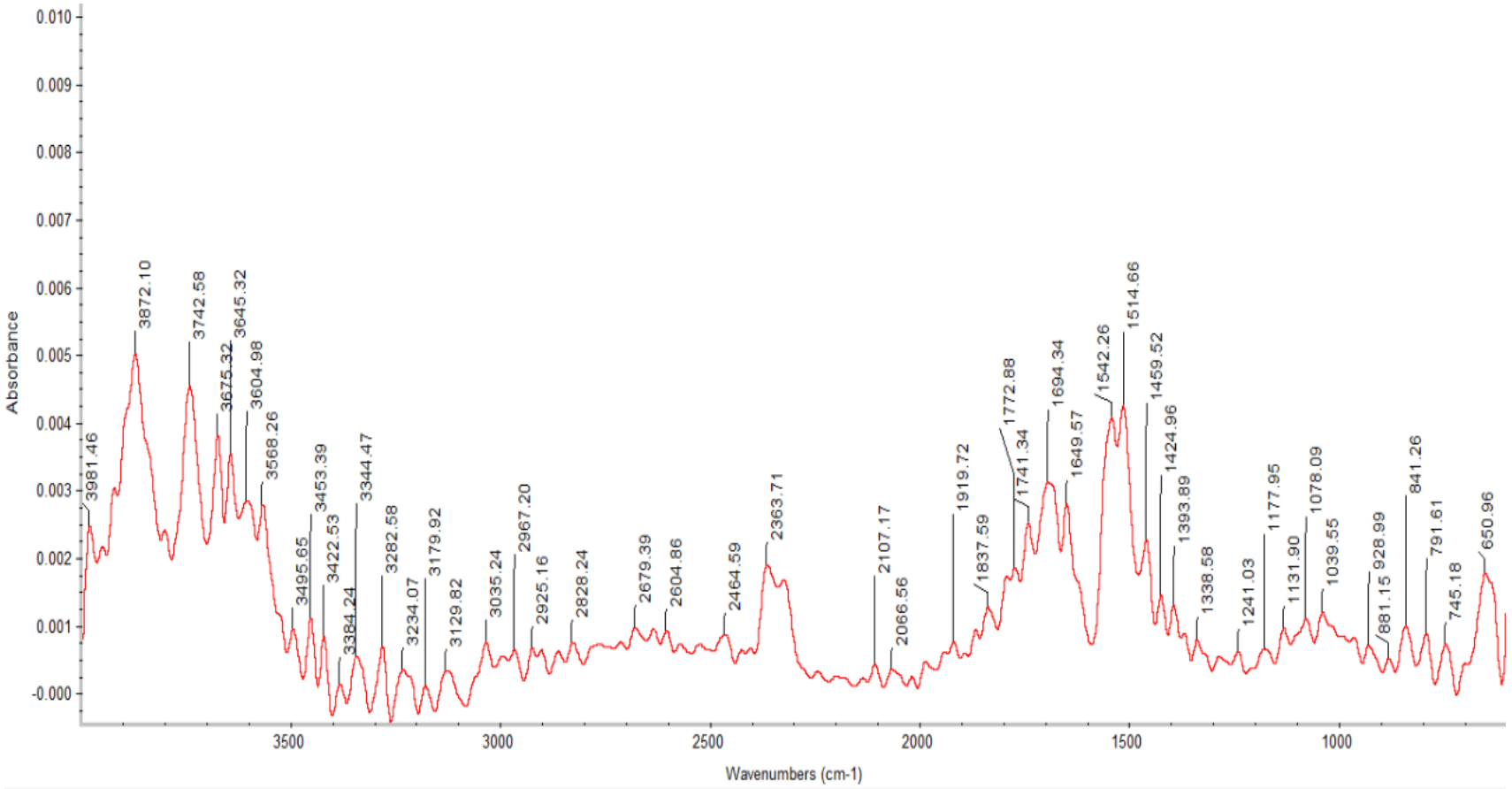


Graph 1: Percentage yield of carrageenan at different alkaline treatment

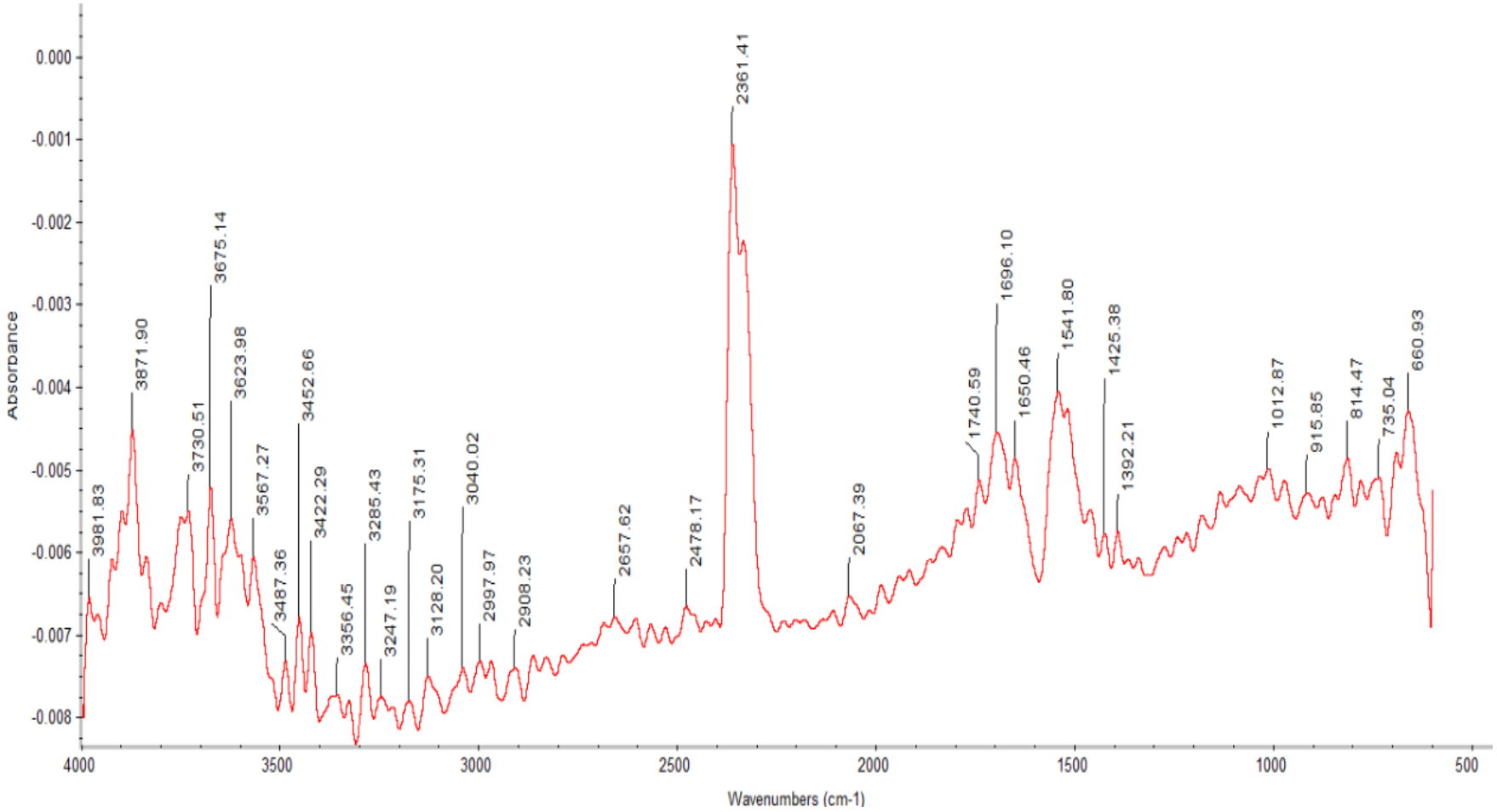


Graph 2: Difference weight of dried carrageenan before and after collapse for different types of alkaline treatment

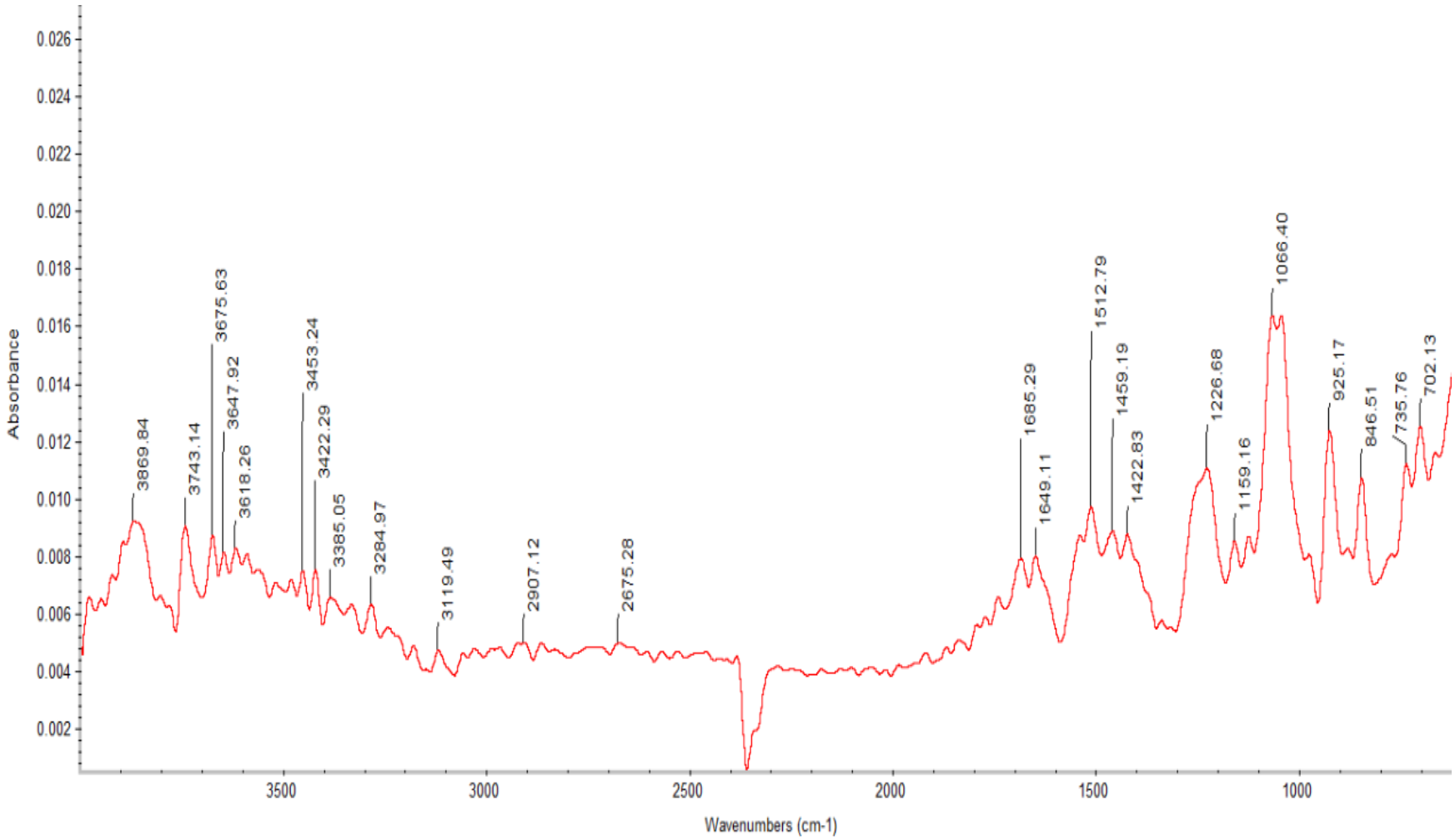
FTIR RESULT, A1



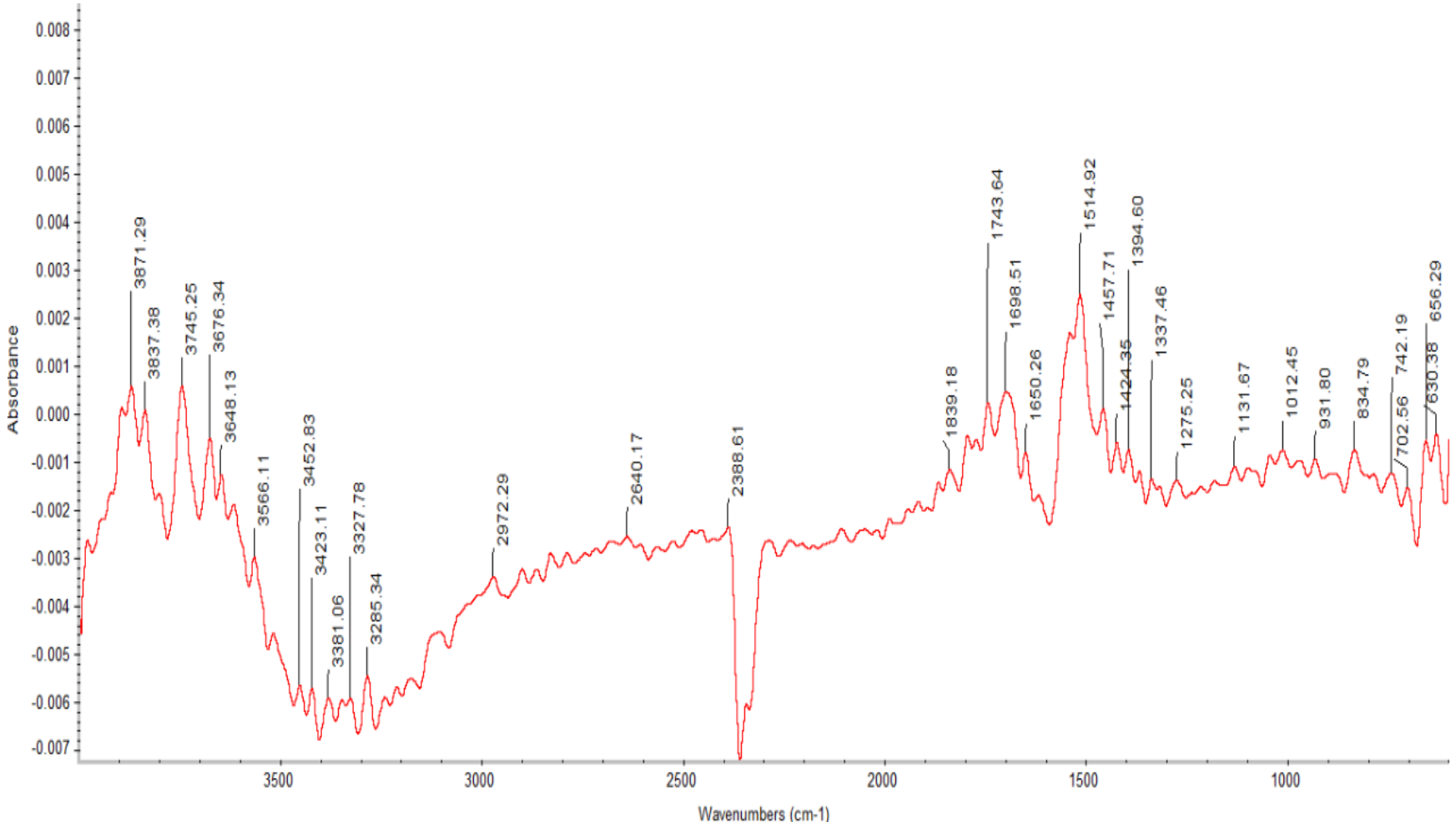
FTIR RESULT, B1



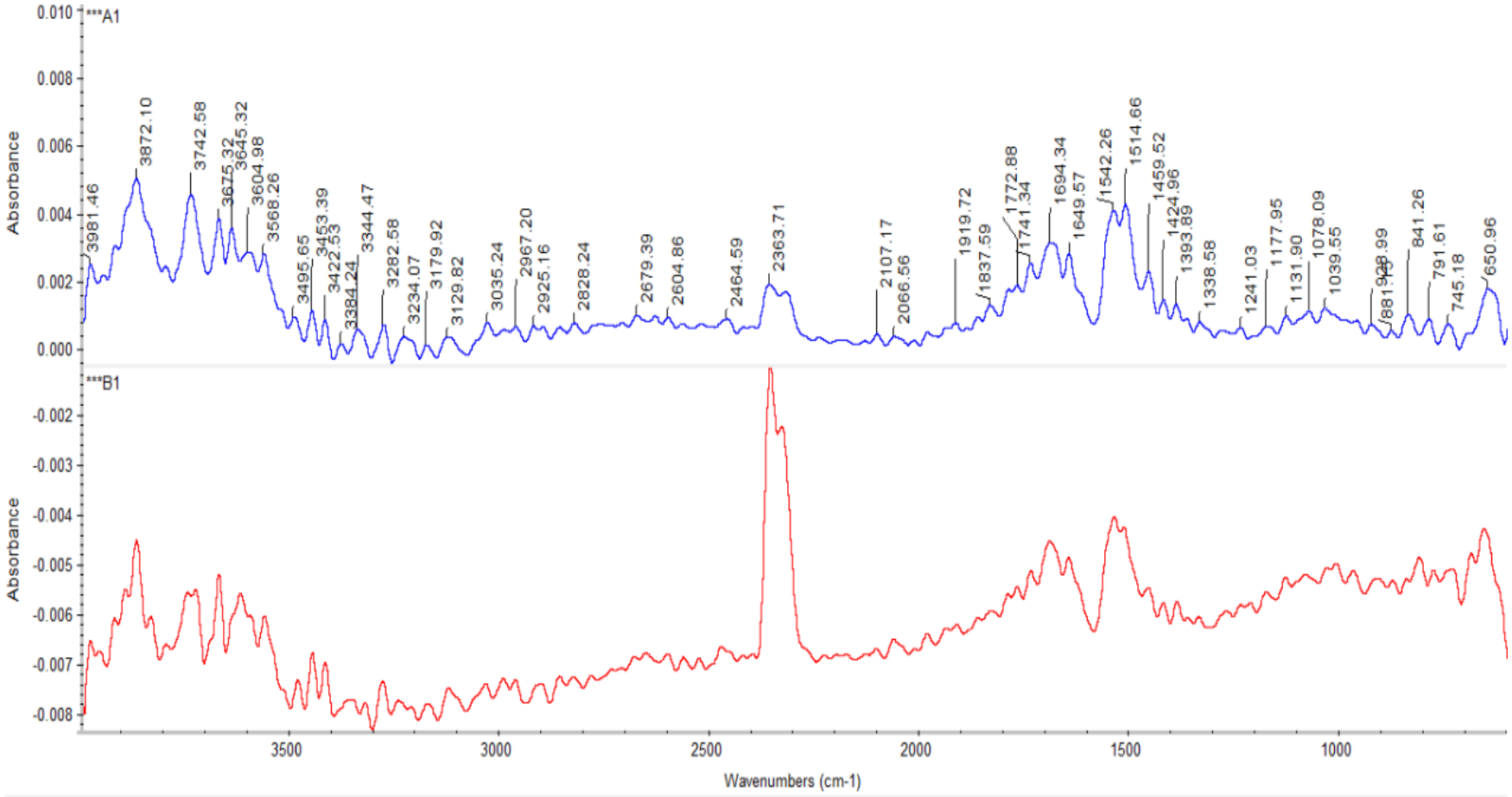
RESULT FTIR, C1



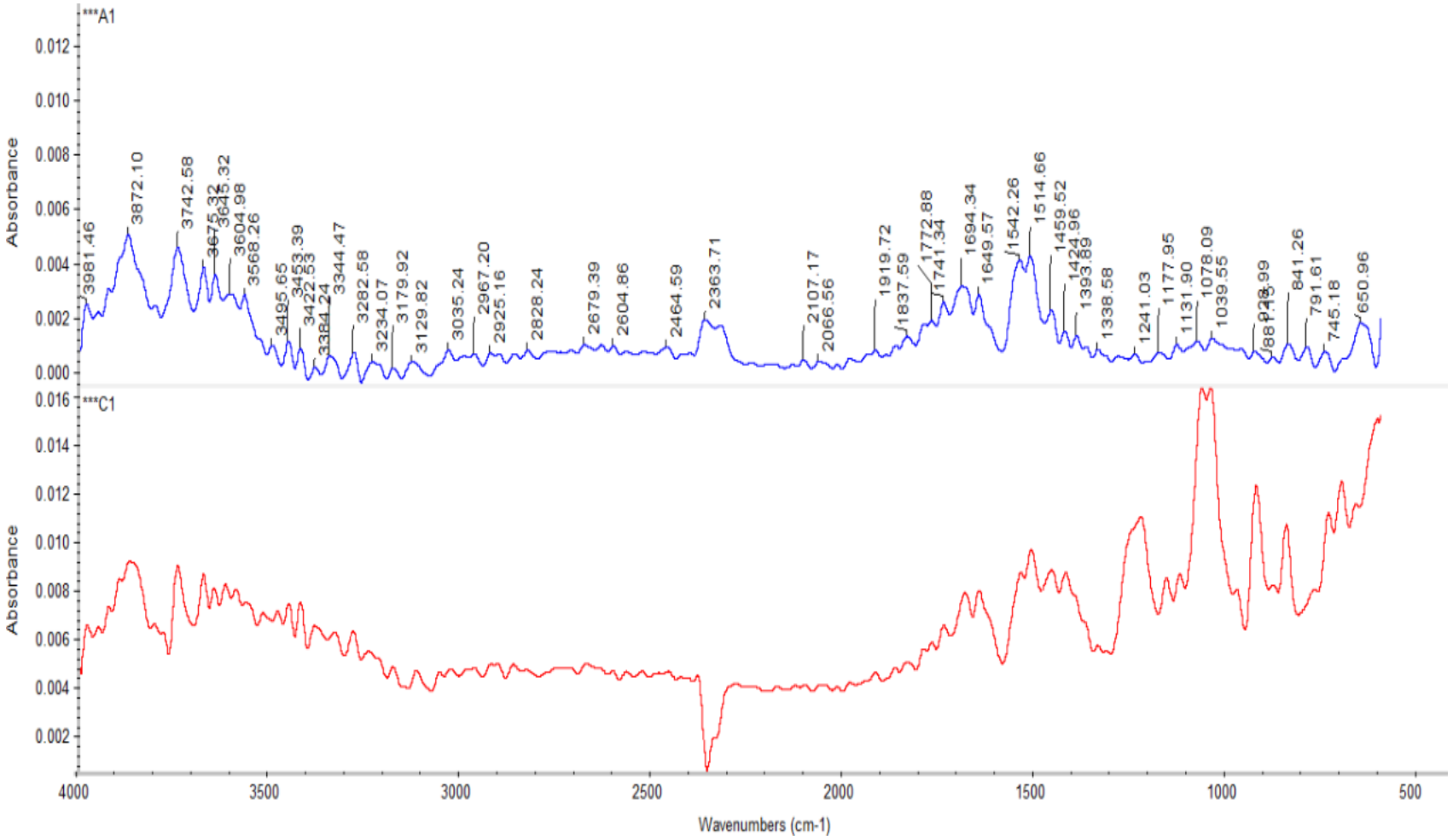
RESULT FTIR, D1



FTIR RESULT, A1 VERSUS B1



FTIR RESULT, A1 VERSUS C1



FTIR RESULT A1 VERSUS D1