COLOUR REMOVAL OF RAW CARRAGEENAN BY USING SODIUM HYPOCHLORITE AS BLEACHING AGENT

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SUPERVISOR'S DECLARATION

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

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Position : Supervisor
Date : 25th January 2013

STUDENT'S DECLARATION

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

Signature :
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Special dedication to my beloved family, especially my parents (Hanen bin Kontan & Siti Roshaidah binti Hj Mohd Fadzil) for their love and care.

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To my friends, that encouraged and fully supports me throughout completing this thesis.

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COLOUR REMOVAL OF RAW CARRAGEENAN BY USING SODIUM HYPOCHLORITE AS BLEACHING AGENT

ABSTRACT

Carrageenans are natural ingredients that represent one of the major texturizing ingredients used by the food industry. However, the extracted carrageenans from seaweeds may have colour such as light brown. These criteria will affect marketed sector because the product are not presented in an interesting ways. Therefore, sodium hypochlorite is one of the effective treatment options and it has potential for removing of colour as bleaching agent. This is due to chlorine is the basis for the most common material used on bleaching. However, the availability of chlorine will give unpleasant odour to the product. This problem can be overcome by addition of sodium thiosulfate into the seaweed to neutralize the sodium hypochlorite. The purpose of this study is to remove colour of carrageenan from seaweed. Amount of sodium hypochlorite are the variable need to be controlled in order to get the optimum condition removing colour of extracted carrageenan from seaweed. When the amount of sodium hypochlorite increased, the time needed to decolourizing carrageenan become more faster. However, by using chlorine bleach (sodium hypochlorite) as a bleaching agent in food processing, this operations must follow permissible regulations to dealing with health effect.

PENYINGKIRAN WARNA KARAGENAN MENGGUNAKAN NATRIUM HIPOKLORIT SEBAGAI AGENT PELUNTUR

ABSTRAK

Karagenan merupakan bahan semulajadi yang terdiri sebagai salah satu jalinan bahan yang digunakan di dalam industri makanan. Walaubagaimanapun, pengeluaran karagenan daripada rumpai lautakan mengeluarkan warna coklat seakan cair. Faktor ini akan memberikan kesan kepada pasaran kerana ia tidak dihasilkan dalam bentuk yang menarik. Oleh itu, Natrium hipoklorit salah satu rawatan yang berkesan dan ia berpotensi untuk mengeluarkan warna kerana ia adalah bahan peluntur. Hal ini kerana klorine merupakan bahan asas yang dikenali digunakan sebagai peluntur. Tetapi kehadiran klorin memberikan kesan bau yang kurang menyenangkan kepada produk. Masalah ini dapat diatasi dengan penambahan Natrium thiosulfat kedalam rumpai laut untuk meneutralkan Natrium hipoklorit. Tujuan pembelajaran ini adalah untuk mengeluarkan warna karagenan daripada rumpai laut. Bila bilangan Natrium hipoklorit bertambah, masa yang diperlukan untuk melunturkan warna karagenan adalah singkat. Walaubagaimanapun, bila mengunakan klorin sebagai bahan peluntur di dalam pemprosesan makanan, operasi ini perlu mengikut kebenaran pertubuhan untuk mengelakkan dari memberikesan kepada kesihatan.

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LIST OF SYMBOLS

°C	Degree celcius
g	Grams
L	Litre
mL	Millilitre
min	Minutes
s	Seconds
%	Percentage

LIST OF ABBREVIATIONS

ABS	Absorbance
FTIR	Fourier transform infrared spectroscopy
HCl	Hydrochloric acid
KOH	Potassium hydroxide
NaOCl	Sodium hypochlorite
$Na_2S_2O_3$	Sodium thiosulfate
UV-Vis	UV-Vis specphotometer

CHAPTER 1

INTRODUCTION

1.1 Introduction

Carrageenan is natural ingredients has been used in food application for decades and are generally regarded as safe (GRAS) (Pereira *et al.,* 2009). It has been used in food industry for additive, thickening, gelling agent and new application of carrageenan in the food industry are in pill and tablets (Distantina *et al.,* 2011).In addition, carrageenan is the third most fundamental hydrocolloid in the world after starch and gelatin (Mustapha *et al.,* 2011). Carrageenan is the main structure of the seaweed which is located in the cell wall and intracellular matrix of the plant tissue (Christopher *et al.,* 1998).

Seaweed can be classified into three basic type of colour which is brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) seaweed. The red seaweed from kappa carrageenan types is used on this research because it is able to form rigid

gels. The red colour of the Rhodophyta (red seaweed) is comes from the present of pigment phycoerythrin which reflects red light and absorb blue light (Hashim and Chu, 2004).

In this research, sodium hypochlorite is selected as bleaching agents due to their special functional properties. When used properly, chlorine bleach such as sodium hypochlorite is the most active chemical to kills the undesirable microorganism. This is due to the fact that, chlorination is widely used in water disinfection and also an effective method chlorinating agent since the beginning 20^{th} century. Furthermore, chlorine and sodium hypochlorite was economic disinfectants to be used for bleaching of seaweed.

1.2 History of Carrageenan

In Ireland, carrageenan was found by a British pharmacist Stanford in 1810 who extracted it from Irish Moss (Chondrus crispus). Then, the name for the extracted seaweed is given as ‘carrageen’ introduced around 1829. The name was changed to carrageenan so as to meet with the ‘-an’ suffix for the name of polysaccharides. The application of Irish Moss was spreading to New England and USA due to migrants fleeing the potato famines of the 18^{th} and 19^{th} centuries. During World War II, a small processing carrageenan occurs in Ireland, New England and

USA because the lack of agar supply from Japan. After the war finished, carrageenan became a major force in the food-additives business and is now the leading seaweed-extract on the worlds markets (The Seaweed Site Website).

Carrageenans represent one of the major texturizing ingredients used by the food industry. Besides carrageenan being used in food industry, agar is the first colloid to be industrialized and it has the same purpose as a gelling agent for food besides as an inert support medium for microbial culture. The traditional process for the production of carrageenan is the alcohol precipitation process. This method was able to produce any type of seaweed as well as produce any type of carrageenan. However, the cost involves in this process is very high. Due to this reason, alcohol precipitation becomes less favourable in the production of carrageenan (CyberColloids Website).

1.3 Problem Statement

Carrageenans are one of other types gelling agent use in food industry. It can be obtained by extraction from seaweed. However, after the extraction,the solution may have unpleasant odour and colour such as light-brown colour. These criteria will affect the marketed sector because the products are not presented in an interesting ways. Therefore, sodium hypochlorite is act as bleaching agents to remove the colour of the carrageenan. However, sodium hypochlorite has smelled of chlorine odour on

the final product. This odour could be overcome by neutralizing the free chlorine with sodium thiosulphate (Kalinowski, 2009).

According to Warburton, the exposure free chlorine gas will cause health effect to the workers. Chlorine has distinct odour that harmful if inhaled, will cause respiratory tract burns, skin burns and eye burns. Beside that, the chlorine gas produced during bleaching process tends to environment pollution. Thus, an environment eco-friendly bleaching of seaweed is required on further research (Haiyan, 2008).

1.4 Objectives

The objectives of this research are

(1) to investigate the effectiveness of sodium hypochlorite as bleaching agent from seaweed.
(2) to study odour removal of free chlorine by using sodium thiosulfate from seaweed.
(3) to produce raw carrageenan with better appearance, low colour and free from unpleasant odour.

1.5 Scope of Study

In order to achieve the objective stated above, the scopes of study in this research are:

(1) to investigate the efficiency of sodium hypochlorite as the bleaching agent with different concentration which is 0, 0.1, 0.2, 0.3 and 0.4 percent of sodium hypochlorite.

(2) to investigate the efficiency of sodium thiosulphate as odour removal of free chlorine with different concentration which is 0, 0.1, 0.2, 0.3 and 0.4 percent sodium thiosulfate.

(3) to determine the effectiveness of sodium hypochlorite with time which is 0 minutes, 15 minutes, 30 minutes, 45 minutes and 60 minutes.

(4) to study the effect of temperature on bleaching which is $22^{O}C$ (room temperature), $30^{O}C$ and $35^{O}C$, $40^{O}C$ and $45\ ^{O}C$

1.6 Rational and Significant

The main purpose of this study is to remove the odour and colour from the raw carrageenan using sodium hypochlorite as bleaching agents. The bleaching agent used in this research was sodium hypochlorite. This is because sodium hypochlorite is able to bleach the seaweed colour. In addition, it is also the best example of chlorine compound that used as disinfectant which is can kills the living microorganisms on the substance (Woo Byun *et al.*, 2006).

Further seaweed processing is required to remove the odour of free chlorine on the seaweed. This chlorine can be overcome by the addition of sodium thiosulfate into the seaweed. This is because sodium thiosulfate is neutralizing agent for the sodium hypochlorite. In medical application, sodium thiosulfate is used for medical therapies as an antidote in the treatment of cyanide toxicity and to avoid ototoxicity in carboplatin recipients (Chetan Vedvyas, 2012).

CHAPTER 2

LITERATURE REVIEW

2.1 Types of Carrageenan

There are many types of carrageenan, it can be identify by their properties and chemical structure. However, the type of carrageenan usually used in food industry comprises of mixture from a lot of species red algae depends on industrial needed (Molecular Gastronomy Network Website). The carrageenan family has three main branches types as kappa (k-), iota (i-) and lambda carrageenan by the number (one, two or three) of sulphate groups per repeat unit of disaccharides (Yuguchi *et al*., 2003).

2.1.1 Iota Carrageenan

Iota carrageenan is thermoreversible gels. It has high molecular weight linear polymer consisting principally of an alternating sequence of 3-linked β-d-galactose 4-sulfate and 4-linked 3,6-anhydro-β-d-galactose 2 sulfate (Ozbek and Pekcan, 2006). The iota carrageenan is predominantly gets from *Eucheuma Spinosum* and *Ahnfeltia concinna.* The iota carrageenan is a gelling agent that especially elastic and flexible in the present of calcium ions (Totalingredients.net Website). On the other hand, carrageenan gels made from iota product are flaccid and compliant (Christopher *et al*, 1998). Figure 2.1 below depicts the structure of iota carrageenan.

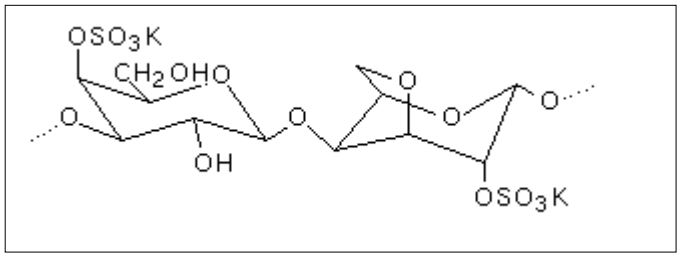

Figure 2.1 Structure of iota carrageenan.

2.1.2 Lambda Carrageenan

It's has structure is based on alternating chain of O-2-sulfated-β-d-galactose-(1→4)-O-2,6-disulfated-α-d-galactose units (Zhou *et al.*, 2006). The lambda carrageenan is produced from the extraction of the *Gigarginacaea* and *Phyllophoracaea* types of red algae. Lambda carrageenan is non-gelling type and forming thick viscous solution (Mustapha *et al.*, 2011). In food application, lambda carrageenans provide an instant smooth and creamy texture. Lambda carrageenan is protein reactive and can be used for soy protein and dairy product purpose (Gum Technology Website). Figure 2.2 below depicts the structure of lambda carrageenan.

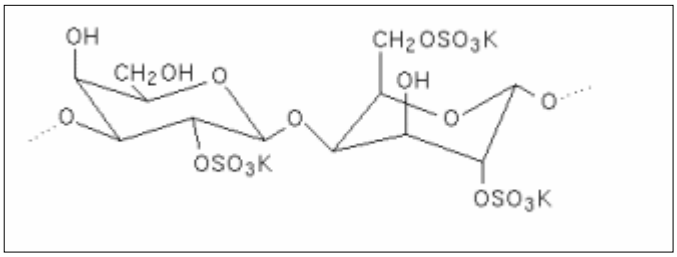

Figure 2.2 Structure of lambda carrageenan.

2.1.3 Kappa Carrageenan

The kappa-carrageenan is linear water soluble polysaccharides extracted from a lot of type of marine red algae with the main structure on an alternating disaccharide repeating unit of a-(1-3)-d-galactose-4-sulphate and h-(1-4) 3,6-anydro-d-galactose (Magione, 2005). It is the commonly used type of carrageenan. This type of carrageenan is used as gelling agent. In the presence of potassium ions, the carrageenan becomes firm and elastic gels. On the other hand, it will become stiff and brittle texture in the presence of calcium ions.

Kappa carrageenan is thermo-reversible means it melts when heated and becomes gels during cooling (Totalingredients.net Website). It has the ability to form gels on cooling hot solution between 40^{O}C and 60^{O}C. It is depends on the amount of cation present on the solution. Kappa-carrageenan is thermally reversible and will be re-gels on cooling. Beside that, it will melt by heating 5 to 20^{O}C above the gelling temperature which is between 65 into 80^{O}C (FMC Corporation (UK) Ltd). Figure 2.3 below depict the structure of kappa carrageenan.

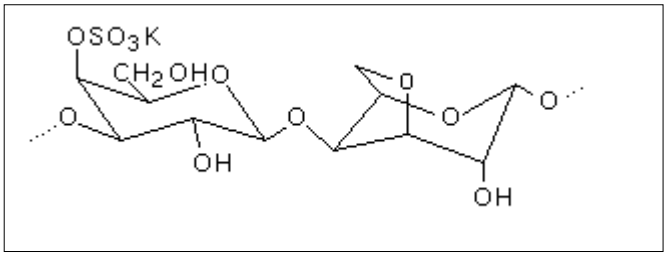

Figure 2.3 Structure of kappa carrageenan.

Carrageenan can be obtained within the cell wall and intracellular matrix of the plant tissue. The percentage of carrageenan content on the seaweed is about 30% to 80% based on seaweed dry weight (Tsai *et al.*, 2010). Kappa-carrageenan can form strong gel make it useful for dairy product. Kappa-carrageenan can be obtained from *Eucheuma cottonii* that produce higher gel strength and more brittle than the kappa from *Chondrus crispus* or *Gigartina sp* (Bost *et al*, 2002).

This seaweed type (kappa-carrageenan) is the largest production worldwide. Nowadays, the *Eucheuma cottonii* is the most important commercial seaweed for carrageenan production (Qhairul Izzreen and Ratnam, 2011). There are no nutritional provided by carrageenan to human body because it cannot be digested. Carrageenan used in food industry because its functional properties that can be used to control moisture, texture and to stabilize food (Mishra *et al.*, 2006). The yield and functional properties of carrageenan such as gel strength contribute its value to food industry (Distantina *et al.*, 2011).

2.1.4 Difference Between Kappa, Iota and Lambda Carrageenan

From an observation, kappa carrageenan and iota carrageenan almost have the same alternating sequence of disaccharide repeat units. The different only on the presence of iota or absence of kappa of the sulphate group on the 2 position of the 4-linked galactopyranosyl unit. Kappa has one negatively charged sulphate groups per every disaccharide repeating unit whereas iota carrageenan have only one. Therefore, kappa-carrageenan with one negative charged sulphate groups shows selectivity for monovalent potassium ions while the iota carrageenan that has two sulphate groups prefers divalent calcium ions. Other research shows, lambda carrageenan that has three negative charged sulphate group prefer with iron (III) ions (Running *et al.,* 2011).

2.2 Bleaching Agent

Bleaching agents are the compound widely used indairy products or industrial purposes. This is due to the fact that, bleaching agents is able to lighten or remove the colour of the compound in order to get the specific needs. Clorox is the example of bleaching agents was very popular real laundry bleach on the market. Bleaching agents also can be used for laundry detergents to remove the dirt at the clothes. In addition, it can be used as clean and sterilize objects and surface. There are twotypes

of bleaching agent that can be classified as oxygen bleach and chlorine bleach (eHow Website).

2.2.1 Oxygen Bleach

The example of oxygen bleach is hydrogen peroxide whereas chlorine bleach is sodium hypochlorite. Hydrogen peroxide used for bleaching wood pulp and paper (and blondes) that expensive and unstable. At concentrated hydrogen peroxide, it is very corrosive and will cause fire risk. Moreover, 1% solution in bleaching the carrageenan in one hour did not give much effect on decolouring laboratory-made carrageenan (Kalinowski, 2009).

2.2.2 Chlorine Bleach

Sodium hypochlorite is one of the examples of chlorine bleach. It is cheaper and safer than hydrogen peroxide. Furthermore, the result in laboratory-made carrageenan by using 1% of sodium hypochlorite for one hour gives better decolouring result than hydrogen peroxide. Nevertheless, it will produce strong smell of chlorine. This problem can be overcome neutralizing the solution with sodium thiosulfate (Kalinowski, 2009). In addition, sodium hypochlorite are able to kills the undesirable microorganism. This germ killing effect is due to the available chlorine

in the hypochlorite. Donald Shclimme said using the chlorine as bleaching agents economical, readily soluble in water and no adverse effects on the food.

Sodium hypochlorite tends to antimicrobial activity besides has the ability to dissolve organic matter. In addition, it is toxic chemical that can cause health problem on vital tissues. This damage will cause haemolysis, skin ulceration and necrosis. This is due to the fact that, sodium hypochlorite that react with natural organic matter will produce trihalomethanes and halocetic acids which can cause animal carcinogens and suspected human carcinogens (Sandeep Singh, 2012).

The Food and Drug Administration (FDA) allows the use of sodium hypochlorite as sanitizing agents for the food contact surfaces of food processing. The regulation (21 CFR Part 173) gives two conditions when using hypochlorite solution in washing procedure. Firstly, the concentration of sanitizer in the wash water must not exceed 2000 ppm hypochlorite. Secondly, the produce must be rinsed with potable water following the chlorine treatment (Food technology fact sheet).

2.3 Sodium Thiosulfate

Sodium thiosulfate is colourless crystalline chemical substances that act as neutralising agent for chlorine (dechlorinator). Sodium thiosulfate is consider as safe substance and permit to be used on food according to Food and Drug Administration (FDA). Beside that, it is allowable by Environmental Protection Agency (EPA). Table below shows neutralizing agents for common disinfectants.

Table 2.1 Neutralizing Agents for Common Disinfectants.

Disinfectant	Neutralizing Agent
Alcohols	Dilution or polysorbate 80
Glutaraldehyde	Glycine and sodium bisulfite
Sodium hypochlorite	Sodium thiosulfate
Chlorhexidine	Polysorbate 80 and lecithin
Mercuric chloride and other mercurials	Thioglycolic acid
Quaternary ammonium compounds	Polysorbate 80 and lecithin
Phenolic compounds	Dilution or polysorbate 80 and lecithin

For neutralizing of chlorine solutions, the amount of sodium thiosulfate required approximately 2 to 7 part to neutralize one part of chlorine is generally suggested. In other words, to neutralize 1 liter of a 200 ppm chlorine solution, approximately 0.4 - 1.4 grams of sodium thiosulfate required (Western Chemical Inc Website).

2.4 Potassium Hydroxide

Potassium hydroxide, KOH is non-organic compound which is generally called caustic potash. Potassium hydroxide is colourless solid that have strong base beside sodium hydroxide. It has either white or yellow colour which is reactive to water, corrosive and harmful if swallowed. Therefore, potassium hydroxide must be handling with care (Nuansa Kimia Sejati Website, 2012). The concentration of sodium hydroxide will influence the yield and gels strength of carrageenan product. At low potassium hydroxide concentration, the gel strength is lower compared to high concentrated carrageenan. This is because potassium hydroxide will exhibit for the formation of gels strength of seaweed during extraction process (Mustapha *et al.*, 2011). Table below shows the result of potassium hydroxide on sulphate and gel strength.

Table 4.2 Some parameter results from extraction of carrageenan.

Carrageenan type	Yield, %	Sulphate, %	Gel strength, g/cm^3
Sigma solvent	-	18.62	223.63
Distilled water	46.43	15.80	Not determined
0.1N KOH	44.43	16.15	69.8
0.3N KOH	38.22	14.24	73.89
0.5N KOH	37.02	11.45	127.3

Based on the table above, the sulphate content also influences the gel strength of potassium hydroxide. When the concentration of potassium hydroxide increased, the sulphate percent will decrease while the gel strength is increased. The reduction of sulphate content by potassium hydroxide shows there is carrageenan reaction which converted precursor carrageenan into kappa carrageenan (Distantia *et al.*, 2011).

2.5 Neutralization of Seaweed

Neutralization of seaweed can be performed by using dilute acid such as hydrochloric acid (HCl) or by using fresh water. The time required for neutralization of seaweed by using hydrochloric acid is faster than fresh water in order to reduce the pH of seaweed. On the other hand, unnecessary depolymerisation of carrageenan occurs that can cause health effect such as carcinogens which is inducing cancer in human. Neutralization of seaweed is vital to eliminate any significant amount of residual potassium hydroxide (KOH) and residues from carrageenan containing seaweed (Kalinowski, 2009).

2.6Application

Natural carrageenans are the mixtures from different sulphated polysaccharides. This unique properties makes carrageenan applicable and valuable for industry to make an improvement in research and development of seaweed. The major applications of carrageenan are in food industry especially on dairy product. For instance, kappa carrageenans are applied in ice cream to prevent the separation of whey (milk serum).In addition, kappa carrageenan will control the texture and ice crystal growth of the ice cream. This is due to the fact that, kappa carrageenan can be used as gelling and binding agent in various dairy application (Villanueva, 2004).

Beside that, kappa carrageenan can be used on immobilized biocatalysts. Kappa carrageenans provide the strong gels. For example the use of enzyme for the conversion of glucose to fructose and the product is applied in food. Moreover, kappa carrageenan also can be used in the continuous production of yoghurt. This product can be obtained by immobilizing the enzyme by trapping them in a material that will still allow penetration by the substance to be changed (Kalinowski, 2007).

CHAPTER 3

METHODOLOGY

3.1 Equipment and Apparatus

3.1.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an analysis practice that offer information regarding the molecular structure and chemical bonding of materials. This analysis can be determined by the wavelength of the sample readings against absorbance or transmittance on the graph. The FTIR works by vibration of chemical bond molecule at certain frequencies.

Figure 3.1 Fourier transform infrared spectroscopy (FTIR)

3.1.2 UV-Vis Specphotometer (UV-Vis)

The equipment that used is U-1800 by Hitachi. UV-Vis specphotometer can measure the wavelength and intensity of absorption of near-ultraviolet and visible light by a sample. The wavelength used on this research is 300 nm.

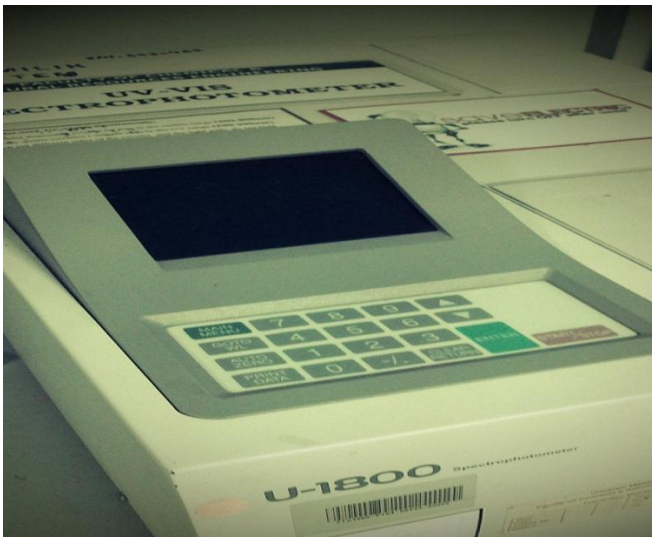

Figure 3.2 UV-Vis Specphotometer

3.1.3 pH Meter

pH meter is used to measure the pH of a liquid sample. It can measure either acid or alkali sample. The pH meter was calibrated first using standard buffer solution.

Figure 3.3 pH meter

3.1.4 Spray Drying

Spray drying is used for producing a dry carrageenan powder from a liquid by rapidly drying with a hot gas. The temperature used to produce the carrageenan is 200^{O}C and the pump setting used is 8.

Figure 3.4 Spray drying

Beside that, the other equipment used in this research is thermometer, magnetic stirrer, analytical balance, beaker, conical flask, glass rod, spatula, hot plate, micro pipett, cuvett, filter, measuring cylinder, knife and dropper.

3.2 Materials

The chemicals or materials used in this research was fresh water, distilled water, 12% of potassium hydroxide (KOH), sodium hypochloride (NaClO), sodium thiosulphate ($Na_2S_2O_3$).

3.3 Flow chart

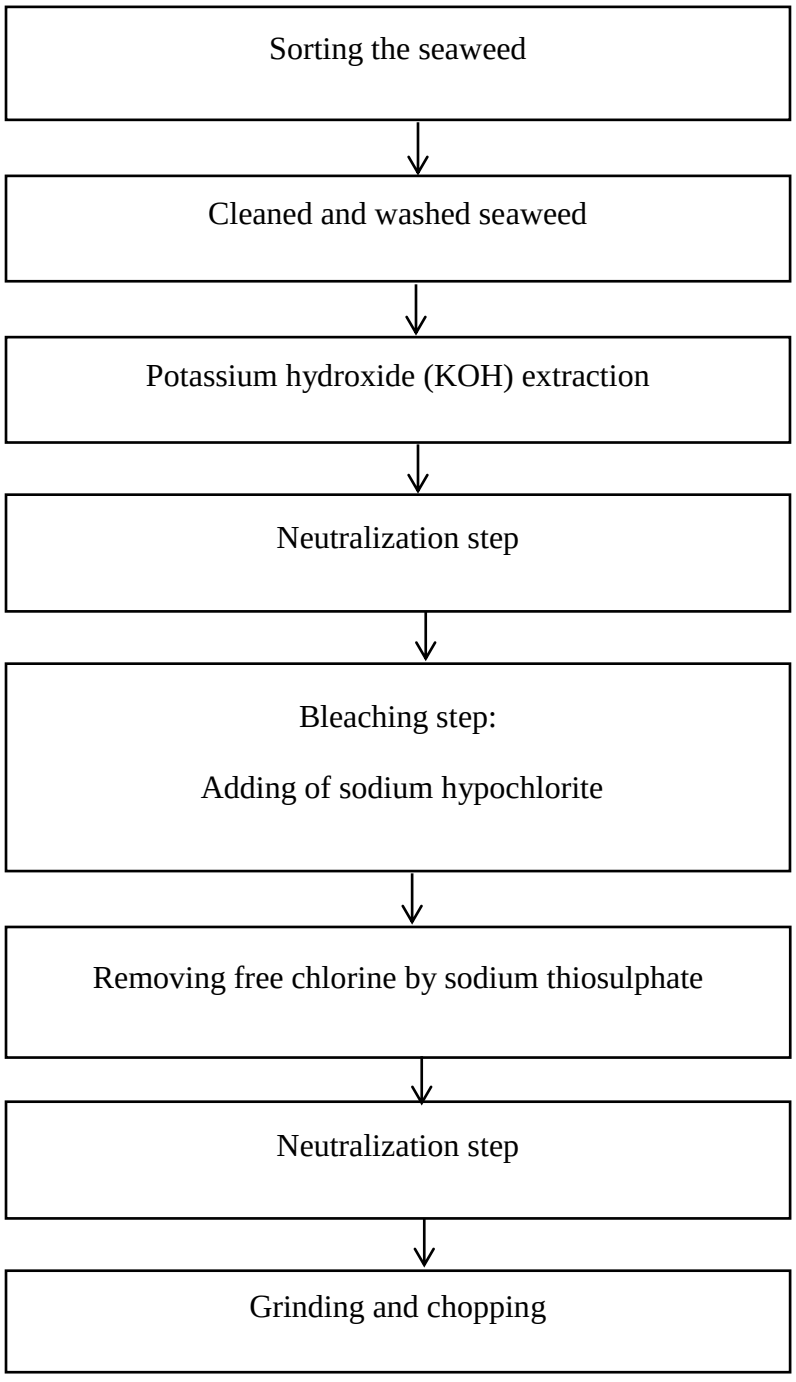

Figure 3.4 Flow chart removal of colour of raw carrageenan using sodium hypochlorite as bleaching agent.

3.4 Method

3.4.1 Sorting Stage

The surface impurities such as sand or other foreign matter on dried seaweed was removed. After that, the seaweed was washed with fresh water and weights the seaweed by analytical balance for 120g.

3.4.2 Extraction Process

120 g of seaweed was poured into the beaker. Then, 12% of 2000 ml of potassium hydroxide (KOH) solution was mixed with the seaweed for extraction process. The mixture was heated at temperature $75^{O}C$ (during this stage the minimum temperature are $60^{O}C$ and maximum temperature is $80^{O}C$) for two hours. During this extraction time, the pH value for the KOH solution is about 13 to 14 which is very corrosive and must be handled with supervision.

After the seaweed drained, the seaweed was proceeds on neutralization stage by using the flowing of fresh water. It was carried out on open system and there are no heat given. The time required during this neutralization step is 150 minutes. The use of fresh water is to reduce the pH of the seaweed and to clean the seaweed from KOH solution.

3.4.3 Introduction of Bleaching Step

For bleaching step, the sodium hypochlorite solution was poured into the beaker that contains of extracted seaweed. Then, the mixture was left for bleaching reaction. The seaweed was filtered and washes it with fresh water. To remove chlorine odour, sodium thiosulphate solution was added into the beaker. After bleaching step was completed, the seaweed was washed with fresh water to neutralize the seaweed.

There are five factors that need to investigate in order to get the optimum condition of seaweed which is the effect of time on bleaching, the effect odourless on sodium thiosulfate concentration, the effect on bleaching concentration without addition of sodium thiosulfate, the effect on bleaching concentration with additionof sodium thiosulfate and lastly was the effect of temperature on bleaching.

3.4.3.1 The Effect of Time on Bleaching

300ml of 0.2% sodium hypochlorite was poured into the beaker containing 50g of seaweed. Then, the mixture was left at room temperature with varies of time which is 0 minutes, 15 minutes, 30 minutes, 45 minutes and 60 minutes. After that, the seaweed were filtered and poured into another beaker. The seaweed was washed with fresh water for one hour to neutralize it. Lastly, the seaweed were analysed by using UV-Vis specphotometer with wavelength 300nm to determine the colour

intensity and Fourier Transform Infrared Spectroscopy (FTIR) to determine the structure of bleached seaweed. The result was recorded in Appendix B.1.

3.4.3.2 The Effect Odourless on Sodium Thiosulfate Concentration

In this step, six samples were run to neutralize the chlorine odour by sodium thiosulfate. 300ml of 0.2% sodium hypochlorite was poured into the beaker containing 50 g of seaweed. The sample was left for 30 minutes on room temperature. After the seaweed were filtered and washed with fresh water, different amount of sodium thiosulfate is poured into the seaweed. The percentage of sodium thiosulfate used was 0, 0.1, 0.2, 0.3 and 0.4 percent sodium thiosulfate (300ml solution). After that, the sample was run by using spray drying to produce carrageenan powder. Lastly, the sample was identified by 10 panels to judge the odour of chlorine from seaweed as shown on Appendix B.2.

3.4.3.3 The Effect on Bleaching Without Addition of Sodium Thiosulfate

In this step, five samples were run to determine the effect of sodium hypochlorite on bleaching. The concentrations of sodium hypochlorite used were 0, 0.1, 0.2, 0.3 and 0.4 percent for 300ml solution. The sample was left at room temperature and bleached for 30 minutes. Then, the seaweed was washed with fresh water for one hour. Lastly, the seaweed were analysed by using UV-Vis specphotometer with wavelength 300nm to determine the colour intensity and

Fourier Transform Infrared Spectroscopy (FTIR) to determine the structure of bleached seaweed. The result was recorded in Appendix B.3.

3.4.3.4 The Effect on Bleaching With Addition of Sodium Thiosulfate

This step almost same as method stated above but there are an addition of sodium thiosulfate for this reaction. There are 5 samples were run which is 0, 0.1, 0.2, 0.3 and 0.4 percent sodium hypochlorite (300ml solution). The sample was left at room temperature and bleached for 30 minutes. After that, to remove the odour of chlorine from sodium hypochlorite, the sodium thiosulfate was added into the beaker containing seaweed after washes with fresh water. The 300ml solution of sodium thiosulfate used is 0, 0.1, 0.2, 0.3 and 0.4 percent respectively.

After 30 minutes, the seaweed was washed with fresh water for one hour. Then, the seaweed were analysed by using UV-Vis specphotometer with wavelength 300nm to determine the colour intensity and Fourier Transform Infrared Spectroscopy (FTIR) to determine the structure of bleached seaweed. The result was recorded in Appendix B.4.

3.4.3.5 The Effect of Temperature on Bleaching

300ml of 0.2% sodium hypochlorite was poured into the beaker containing 50g of seaweed. The temperature used in this method is 22^{O}C (room temperature), 30^{O}C and 35^{O}C, 40^{O}C and 45^{O}C and bleached for 30 minutes. Finally, the seaweed were analysed by using UV-Vis specphotometer with wavelength 300nm to determine the colour intensity and Fourier Transform Infrared Spectroscopy (FTIR) to determine the structure of bleached seaweed. The result was recorded in Appendix B.5.

3.4.4 Preparation Sample for FTIR and UV-Vis Analysis

After bleaching step, the seaweed was undergoing FTIR and UV-Vis analysis. The bleached seaweed was chopped into the small pieces and put the seaweed into the beaker. Then, 1000ml of water was poured into the beaker containing seaweed. The seaweed was heated at temperature between 65 ^{O}C to 80^{O}C in order to dilute the seaweed.The solution stirred by using magnetic stirrer to ensure the mixture well mixed. After one hour, 10 ml of the samples were put at test tube for analysis of UV-Vis and the left solution is used to spray drying. The parameter used to spray the carrageenan solution is at 200^{O}C and pump setting used is 8. Then, the sample was left for one hour to get the carrageenan powder. The carrageenan powder was used for FTIR analysis to determine the structure of carrageenan present in the sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Extraction Step

The result of pH for the extraction of carrageenan from seaweed is shown in Table 4.1. KOH reading is very high during the extraction which is 13.97 and pH of seaweed after the extraction is 13.53. This seaweed was extracted by 12% of KOH solution for about two hour.

Table 4.1 pH of seaweed and KOH solution after extraction

pH KOH	13.97
pH of seaweed after extraction	13.53
pH of seaweed after neutralization	9.89

In the present of KOH solution on the seaweed, it will affect the gels strength of carrageenans. This is because KOH solution will reduce the number of sulphate content on the seaweed. Distantina *et al.* (2011) state that, the reduction of sulphate content by KOH shows there was a carrageenan reaction which converted precursor carrageenan into kappa carrageenan. The cyclization process involves the release of sulphate groups and strengthens the structure of kappa carrageenan. Carrageenans are located in the cell wall and at the intracellular matrix of seaweed. The cyclization of carrageenan can be depicts as figure 4.1.

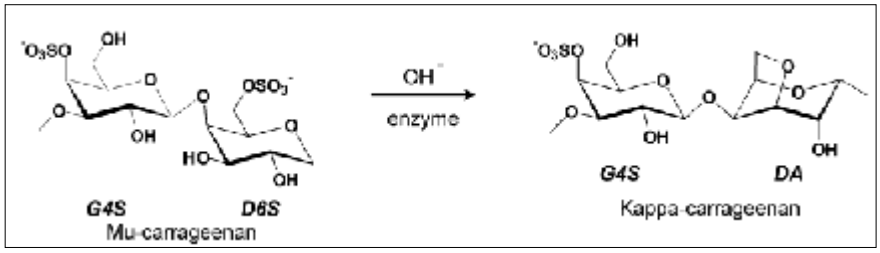

Figure 4.1 Cyclization reaction with hydroxide to generate kappa carrageenan

There are two chemical alterations occurs when the seaweed was cooked with the KOH solution. The first alteration is desulfation of carrageenan. This process occurs when the sulphate group is bonded to the 6-position of the galactose units of carrageenan of a carrageenan polymer molecule is removed by the K^+ ions to form KSO_4 (acid Potassium Sulphate) in solution. In addition, cooking with adequate KOH solution weakens the tertiary sulphate-galactose bonds to ensure K^+ ions remove the sulphate group from the galactose by creating potassium sulfate salt in the solution.

The second reaction step is a dehydration of desulfated product to create the reoccurring 3,6 anhydrous galactose polymers. The OH- ions from the KOH solution combine with the tertiary and secondary bonded H^+ groups at the 3 and 6 positions to form anhydrous kappa carrageenan polymer with water (Kalinowski, 2007).

4.2 Bleaching Reaction

4.2.1 ABS Reading

Figure 4.2 shows the ABS value is decreases across time. In this method, the percent sodium hypochlorite used is 0.2%. The graph above shows that at time 30 minute and above, the ABS shows almost same against time. Therefore, it can be concluded that, 0.2% of available chlorine in sodium hypochlorite is the optimum condition for 30 minutes bleaching. This is because hypochlorite ion (OCl^-) is an active compound in the sodium hypochlorite (NaOCl). NaOCl oxidizes and breaks double bonds between the carbon and oxygen atoms of coloured substances. This results the compound losing the capability to absorb natural light and then appear colourless (Food additives, 2012).

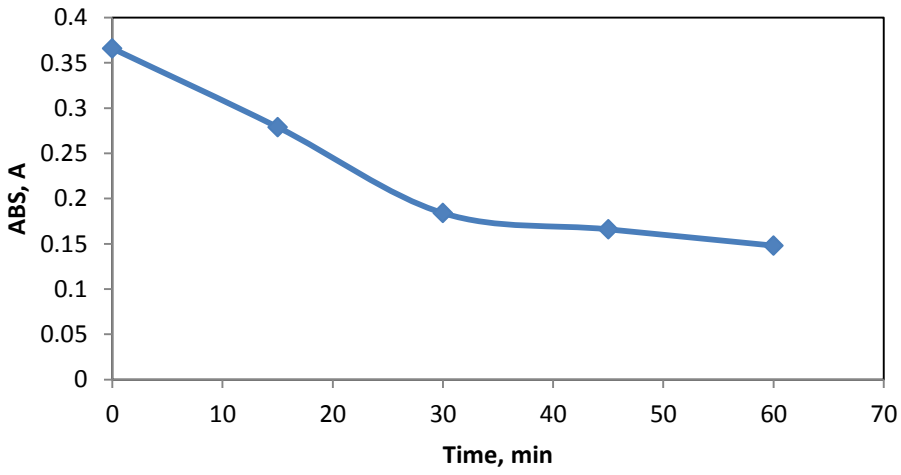

Figure 4.2 Effect of time on bleaching of carrageenan

Figure 4.3 shows the ABS value is decreases on both effect on bleaching concentration without and with sodium thiosulfate ($Na_2S_2O_3$). The ABS value is decreases with increasing NaOCl concentration in both reactions. Increasing concentration of NaOCl will increase the hypochlorite ion (OCl^-) in the solution thus faster the bleaching process. $Na_2S_2O_3$ is colourless substance and it not gives much effect on bleaching of seaweed. This is because $Na_2S_2O_3$ was reacting as to remove of chlorine odour on the carrageenans of seaweed.

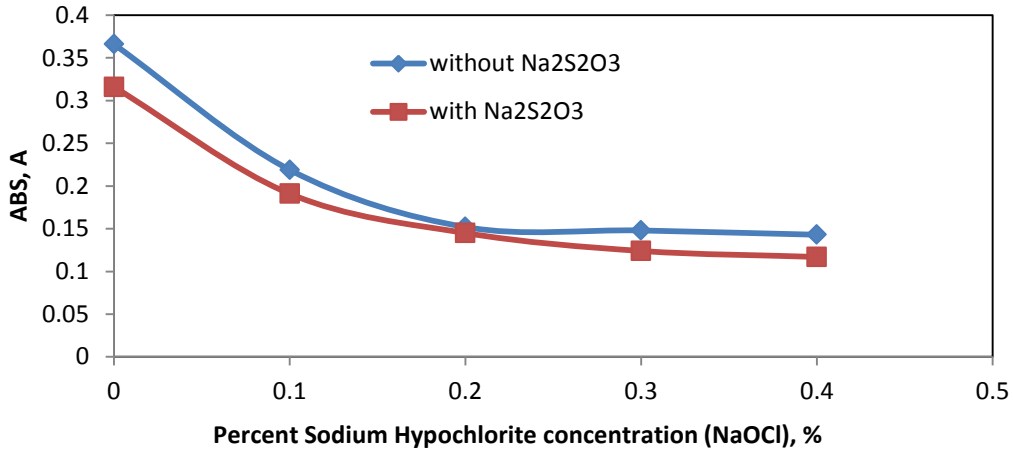

Figure 4.3 A graph of the effect on bleaching concentration without and with sodium thiosulfate.

Figure 4.4 shows the ABS value at different operating temperature on bleaching of carrageenans. There are slight different values on this reaction. Therefore, by using room temperature to bleach the carrageenan is more valuable and economic. By using high temperature during bleaching of seaweed will decrease the carrageenan content on the seaweed. This is because, the carrageenan is easily diffusing in the solution and weaken the gel strength. This is due to the fact that, carrageenan gels will increase in the presence of KOH solution.

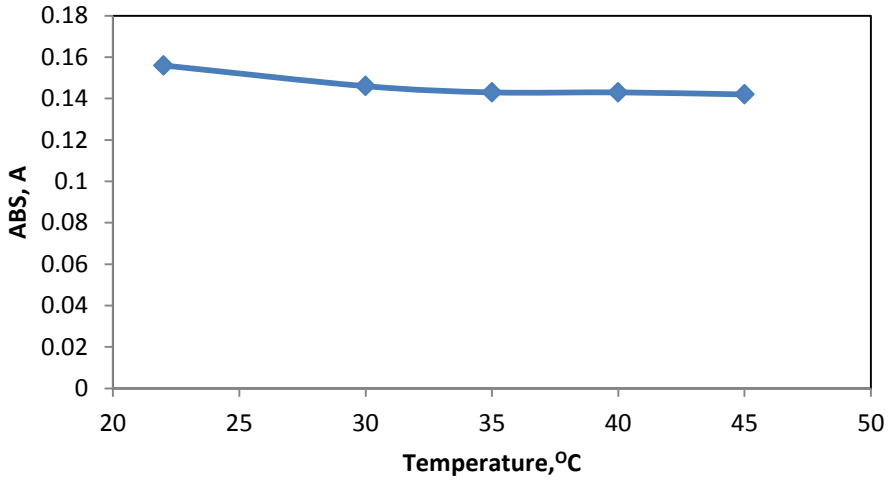

Figure 4.4 A graph of bleaching of carrageenan at different temperature

4.2.2 Odour Detection Threshold

The objective of odour detection threshold method was used to determine the odour of chlorine in the carrageenan powder form. The result of odour was responded by 10 panels and the results are shown in Appendix B.2. The chlorine odour comes from the chemical sodium hypochlorite used. Therefore, the concentration of sodium hypochlorite used is 0.2%. Theresult from the panels shows that, there are no odours detected when 0.2% sodium thiosulfate was used. In addition, the panels judged faint odour and very faint odour at 0% and 0.1% concentration sodium thiosulfate used. This is because dilute concentration of sodium hypochlorite used in this research. Therefore, the odour respond from the panels does not follow equation below.

$$2Na_2S_2O_3 + NaOCl + H_2O \rightarrow Na_2S_4O_6 + NaCl + 2NaOH \quad (4.1)$$

Equation 4.1 shows that, two mol of sodium thiosulfate react with one mol of sodium hypochlorite and water to produce sodium tetrathionate, sodium chlorite and sodium hydroxide. Thus, two mole of sodium thiosulfate is needed to neutralize one mole of sodium hypochlorite. The odour responded from panel against the equation above due to the panels cannot detect the present of chlorine odour at the carrageenan powder. This is due to only small amount of sodium hypochlorite has been used. In addition, another factor is the dissociation of chlorine during neutralizing of seaweed by water. When the chlorine is dissolved in water, the

chlorine molecules combine with water in a reaction called hydrolysis. The hydrolysis reaction produces hypochlorous acid (HOCl) (Mauro Herrera, 2012). The reaction was described as equation (4.2) below.

$$H_2O + Cl_2 \rightarrow HOCl + Cl^- + H^+ \qquad (4.2)$$

4.2.3 FTIR Spectra of Carrageenan

The functional groups of *Eucheuma cottonii* carrageenan that produced from extracted 12% of potassium hydroxide solution was determined by FTIR. The spectra are obtaining from preparing carrageenans powder. The spectra could explained that the extracted carrageenan and after treatment with sodium hypochlorite and sodium thiosulfate is almost same. Figure 4.5 below shows the initial carrageenan or pure carrageenan spectra.

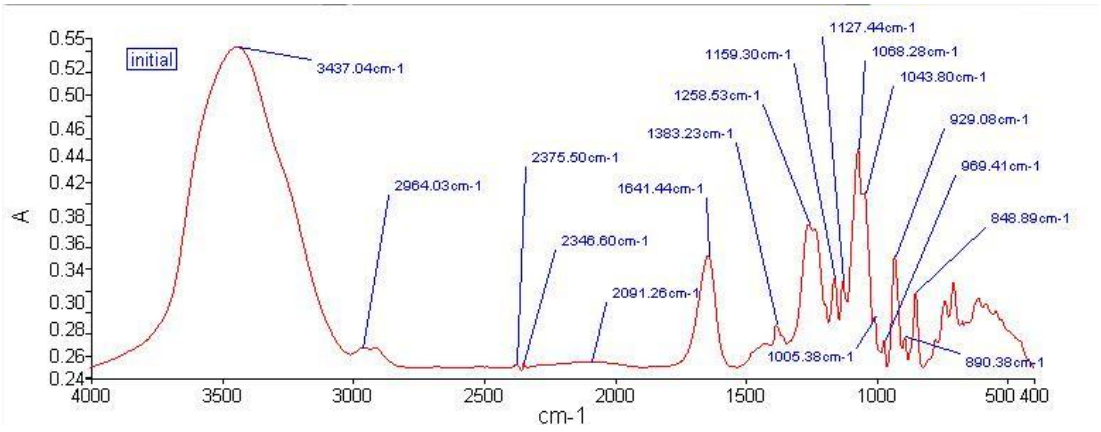

Figure 4.5 FTIR spectra of carrageenan at initial condition

According to JECFA (2012) the study of carrageenan by FTIR spectroscopy shows the presence of very strong absorption band in 1210-1260 cm^{-1} region (due to the S=O of sulphate esters) and 1010-1080 cm^{-1} (glycosidic linkage) in all carrageenan type. From figure above, sulphate esters shows the very strong absorption bands in the 1258.53 cm^{-1} region whereas glycosidic linkage shows absorbance bands at 1043.80 cm^{-1}. According to Figure 2.3, kappa carrageenan has alternating disaccharide repeating unit of a-(1-3)-d-galactose-4-sulphate and h-(1-4) 3,6-anydro-d-galactose. D-galactose-4-sulfate can be identified at band 848.89 cm^{-1} and 3,6-anhydro-d-galactose can be identified at 929.08 cm^{-1}. Figure 4.6 shows the band of respective structure at the initial carrageenan.

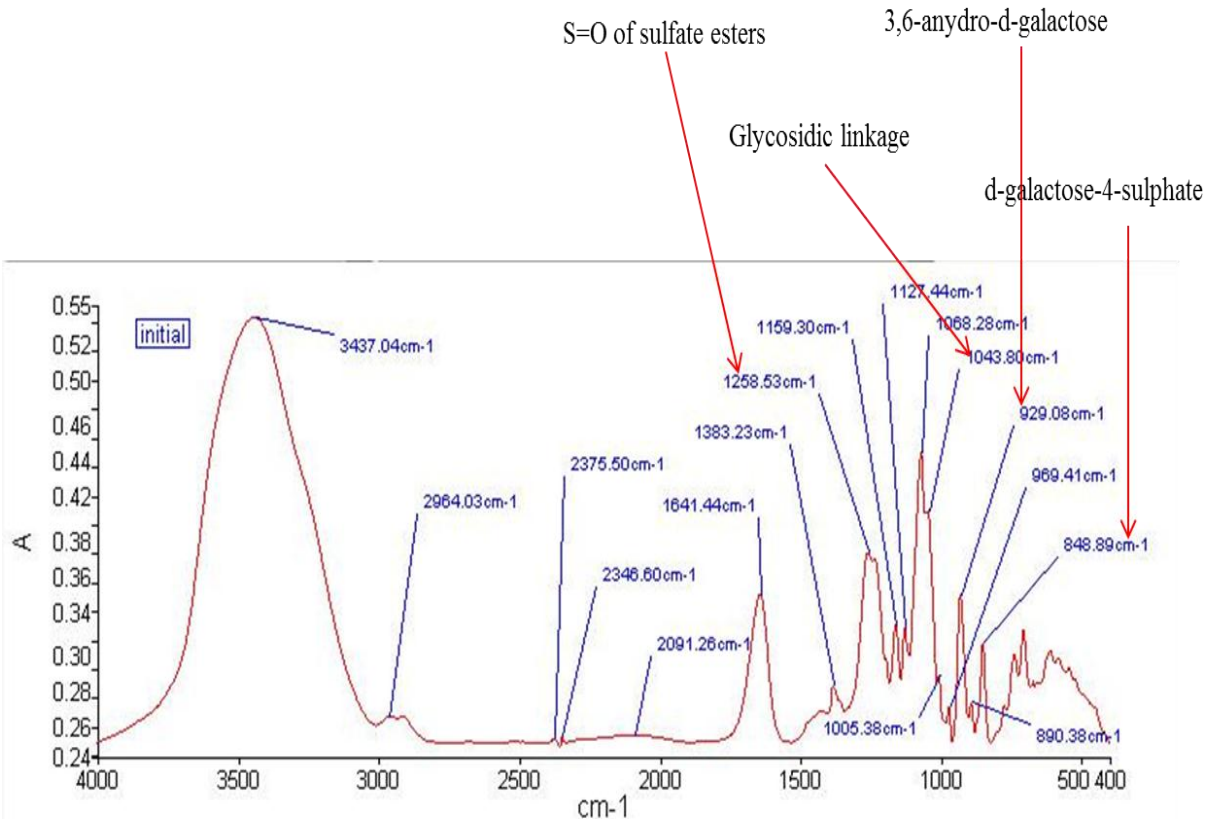

Figure 4.6 FTIR spectra of carrageenan at initial condition with the identified functional group of carrageenan respectively.

Figure 4.7 and figure 4.8 show the FTIR analysis spectra for 30 minutes bleaching by using sodium hypochlorite. The different between these two graphs is Figure 4.7 without the addition of sodium thiosulfate into the seaweed whereas Figure 4.8 is with the addition of sodium thiosulfate. Both graphs show the strong absorption band at 848 cm^{-1} and 929 cm^{-1} indicating the presence of kappa carrageenans structures. Therefore it can be concluded that with addition of sodium thiosulfate and sodium hypochlorite into the seaweeds for bleaching reaction does not give effect on the carrageenan structure. This statement is proved from the analysis by FTIR from Figure 4.5, Figure 4.7 and Figure 4.8 respectively.

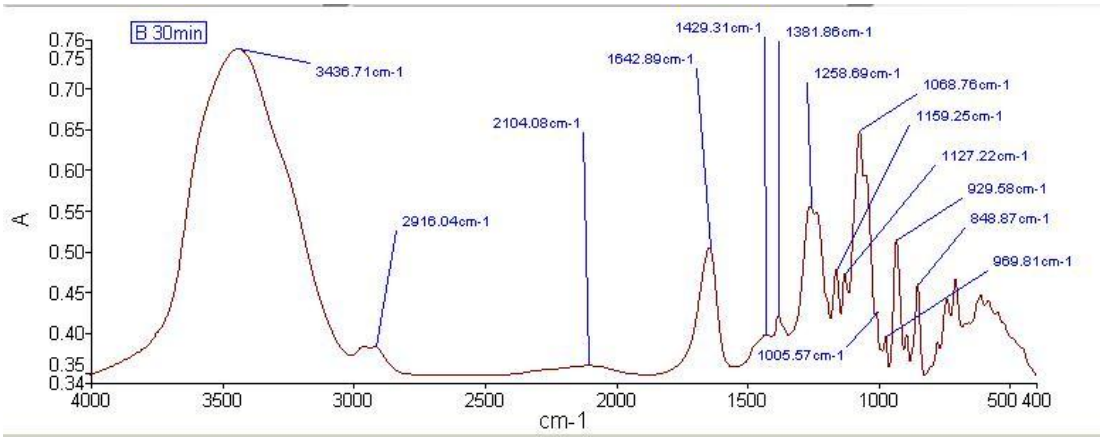

Figure 4.7 FTIR spectra of carrageenan without the addition of sodium thiosulfate

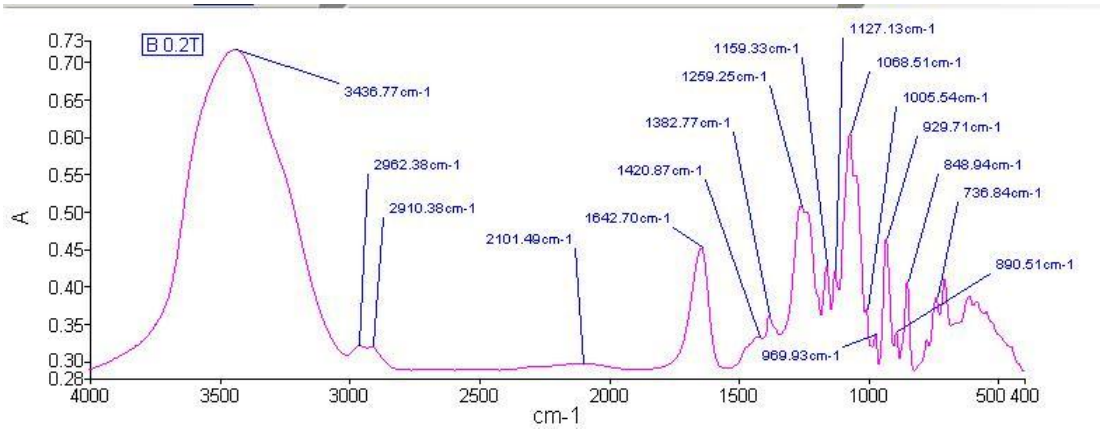

Figure 4.8 FTIR spectra of carrageenan with addition of sodium thiosulfate

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on the result obtained and analysis that have been done by using FTIR and UV-Vis, it can be concluded that removal of seaweed colour was achieved. This has been proved that when the concentration of sodium hypochlorite increased, the bleaching of carrageenans become faster. This is because the amount of hypochlorite ion (OCl^-) produced in the solution is more thus increased the time of bleaching process. The result shows that, the optimum condition in removing the colour of carrageenan is by using 0.2% of sodium hypochlorite in 30 minutes.

Moreover, the temperature does not give much effect on bleaching of carrageenans. This is because the ABS value is almost similar after bleaching the seaweed at different temperature. On the other hand, the carrageenan content at the seaweed will decrease because in the absence of potassium hydroxide, the

carrageenan gels weaken. The odour of chlorine can be removed by using the same amount of both sodium hypochlorite and sodium thiosulfate which is 0.2% respectively. This is due to the reaction between the thiosulfate ions that neutralize the hypochlorite ions in the solution.

Furthermore, the analysis on the structure of carrageenans shows the positive result by FTIR spectra. The carrageenas shows the strong bands between 1500 cm^{-1} and 600cm^{-1}. Hence, the presence of carrageenans in the seaweed were proved by using FTIR spectra when there was addition of other chemical on seaweed for bleaching purposes such as sodium hypochlorite and sodium thiosulfate.

5.2 Recommendation

Removal of colour and odour of carrageenan from extracted seaweed is important studies that need to be further improved. This is because carrageenan is natural polysaccharides that can be applied to various kind of compound or product. The excellent of treatment should be address on some criteria such as economic. For example, type of chemical used for the production semi refined carrageenan. The technology used also much easily followed and simple design.

According to McHugh (2003), the seaweed powder is coloured because it has a high bacteria in the carrageenans content and this is not suitable for human consumption. Thus, in order to determine the amount of bacteria on the seaweed, further research must be continued by determining the presence or total of bacteria in the seaweed. This further research is vital in order to know the type of bacteria present in the seaweed and the suitable method to eliminate the bacteria.

In order to get the better result, the analysis on the structure of carrageenan must be run into a calibration or training set. This method is made by performing a preliminary principal component analysis (PCA) on the overall data set. From this method, this makes the calibration and test set to present more variability of carrageenans. Therefore, PCA will lead for successions in quality of carrageenan are improved.

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APPENDIX

APPENDIX A Calculation for weight of KOH and $Na_2S_2O_3$

The weight of 12% KOH in 2000mL of water

$$\frac{12}{100} \text{X } 2000\text{ml} = 240\text{g of KOH}$$

The weight of 0.2% $Na_2S_2O_3$ in 300mL of water

$$\frac{0.2}{100} \text{X } 300\text{ml} = 0.6\text{g of } Na_2S_2O_3$$

APPENDIX B Experimental data

1. The effect of time on bleaching

Table B.1 UV Vis (Absorbance) and pH reading on the effect of bleaching with different time.

Time, minutes	Absorbance (ABS), A	pH
0	0.366	9.89
15	0.279	9.74
30	0.184	9.65
45	0.166	9.63
60	0.148	9.53

FTIR Reading

Time: 0 minutes

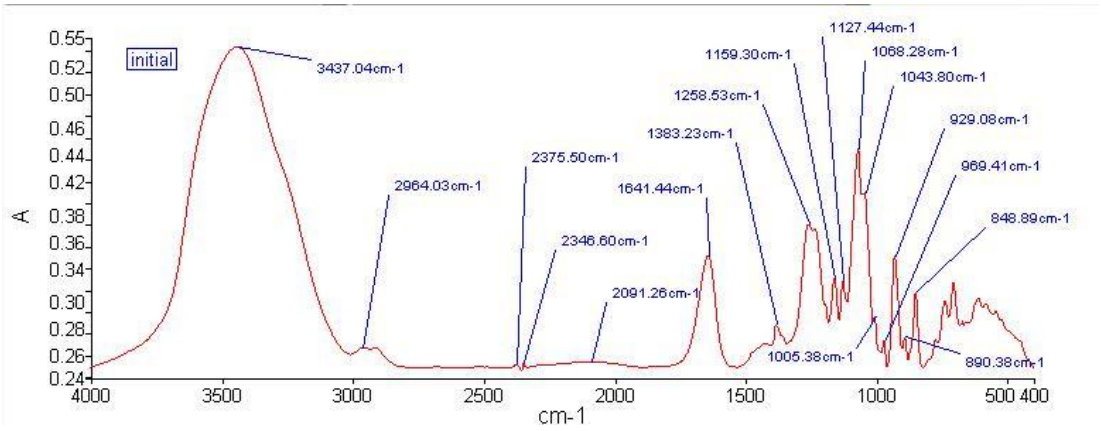

Figure B.1.1 FTIR spectra of carrageenan bleaching at 0 minutes

Time: 15 minutes

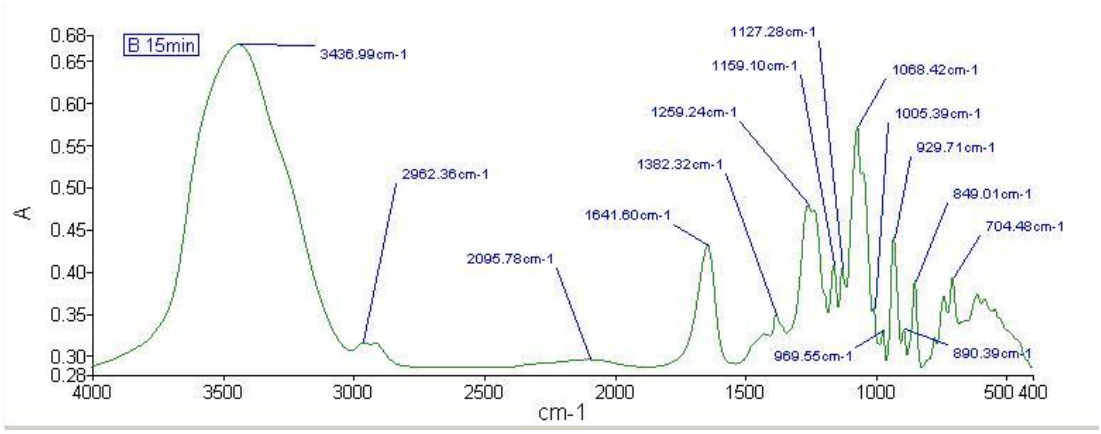

Figure B.1.2 FTIR spectra of carrageenan bleaching at 15 minutes

Time: 30 minutes

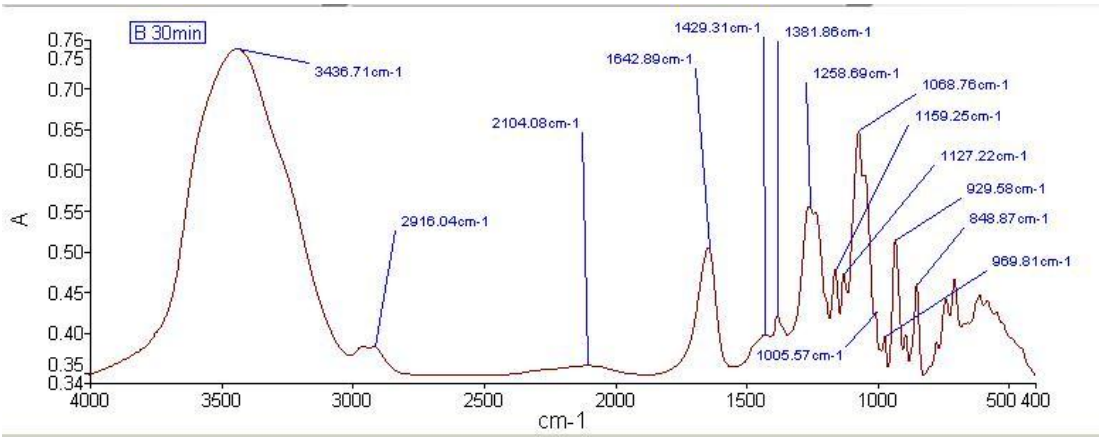

Figure B.1.3 FTIR spectra of carrageenan bleaching at 30 minutes

Time: 45 minutes

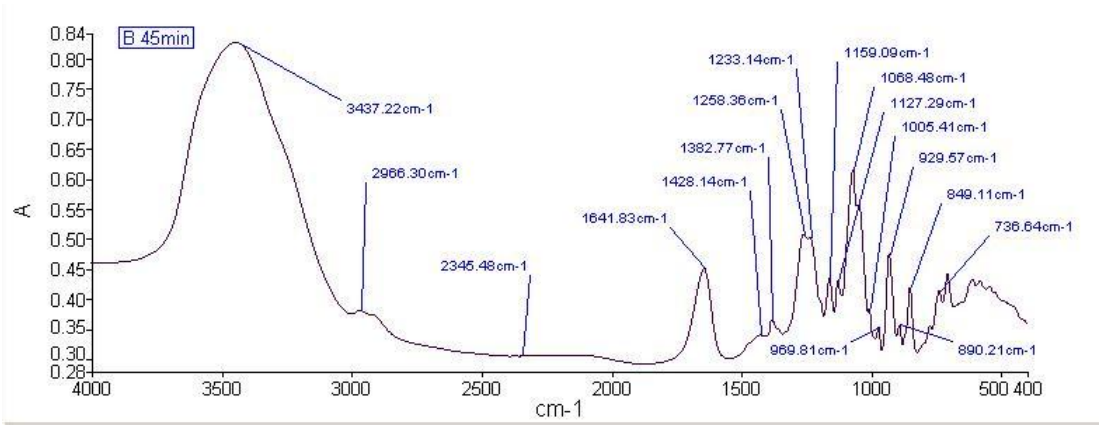

Figure B.1.4 FTIR spectra of carrageenan bleaching at 45 minutes

Time: 60 minutes

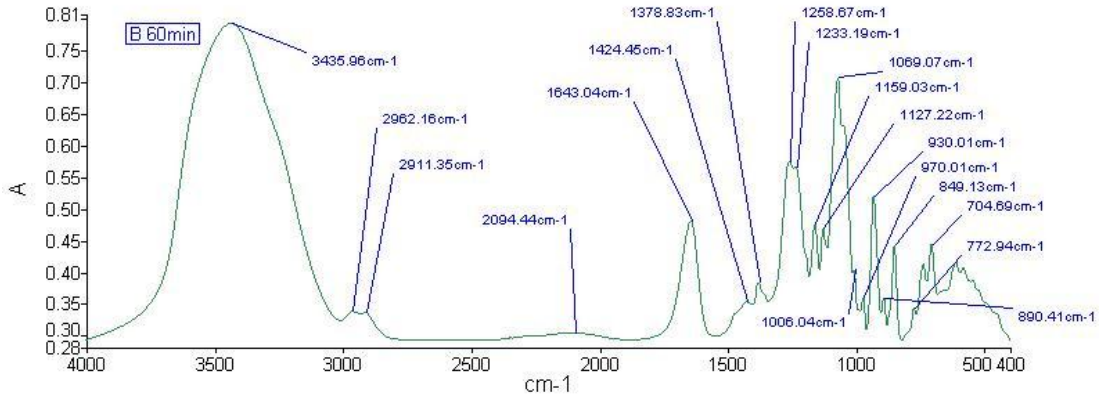

Figure B.1.5 FTIR spectra of carrageenan bleaching at 60 minutes

2. The effect odourless by sodium thiosulfate concentration

Table B.2 Detection on chlorine odour based on 10 panel.

Sodium thiosulfate (%)	0	0.1	0.2	0.3	0.4
Panel					
1	Very Faint odour	Very Faint odour	No odour	No odour	No odour
2	Very Faint odour	Very Faint odour	No odour	No odour	No odour
3	Faint odour	Very Faint odour	No odour	No odour	No odour
4	Faint odour	Faint odour	No odour	No odour	No odour
5	Faint odour	Very faint odour	No odour	No odour	No odour
6	Faint odour	No odour	No odour	No odour	No odour
7	Faint odour	Very faint odour	No odour	No odour	No odour
8	Very faint odour	No odour	No odour	No odour	No odour
9	Faint odour	Very faint odour	No odour	No odour	No odour
10	Very faint odour	No odour	No odour	No odour	No odour

3. The effect on bleaching without sodium thiosulfate

Table B.3 UV Vis (absorbance) and pH reading on the effect of bleaching concentration without sodium thiosulfate.

Concentration, %	Absorbance (ABS), A	pH
0.00	0.366	9.89
0.05	0.219	9.81
0.10	0.152	9.77
0.15	0.148	9.74
0.20	0.143	9.63

4. The effect on bleaching with sodium thiosulfate

Table B.4 UV Vis (Absorbance, A) and pH reading on the effect of bleaching with sodium thiosulfate.

Concentration, %	Absorbance (ABS), A	pH
0.00	0.316	9.89
0.05	0.191	9.72
0.10	0.145	9.63
0.15	0.124	9.54
0.20	0.117	9.52

FTIR Reading

Percent concentration:

Sodium hypochlorite:0.00%

Sodium thiosulfate: 0.00%

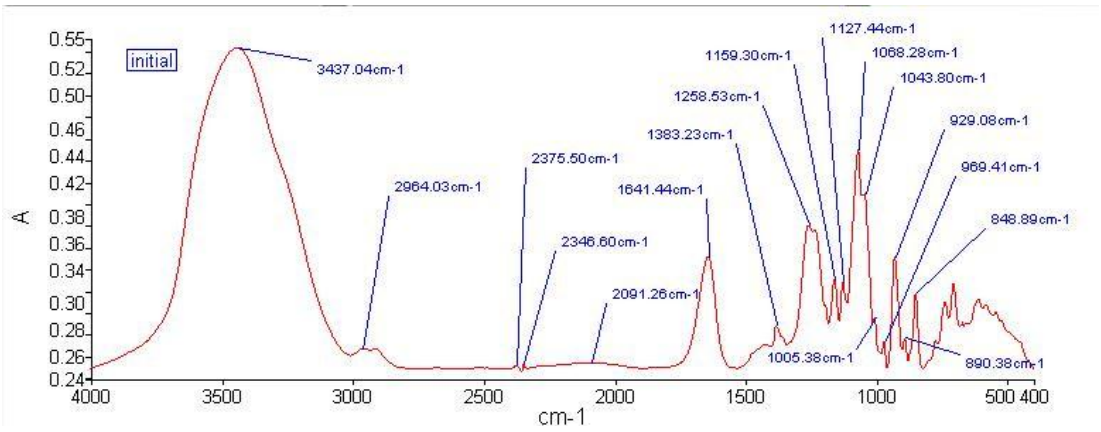

Figure B.4.1 FTIR spectra of carrageenan (Sodium hypochlorite 0%, sodium thiosulfate 0%)

Percent concentration:

Sodium hypochlorite: 0.1%

Sodium thiosulfate: 0.1%

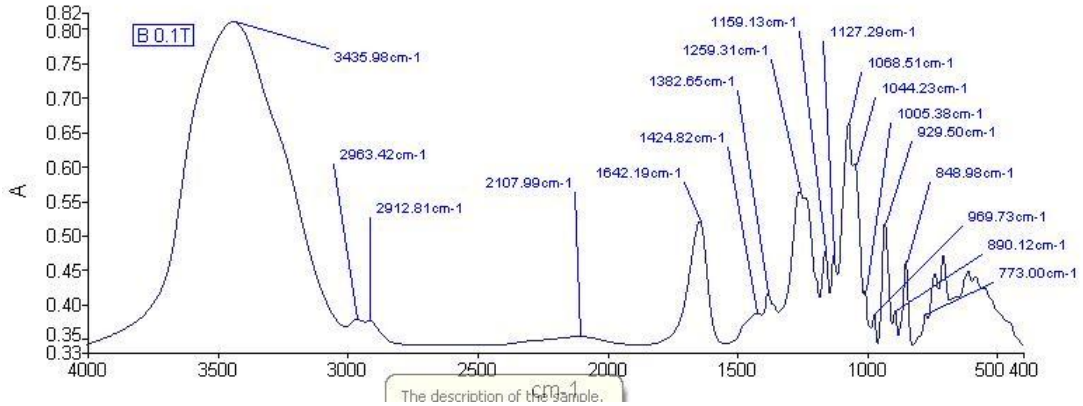

Figure B.4.2 FTIR spectra of carrageenan (Sodium hypochlorite 0.1%, sodium thiosulfate 0.1%)

Percent concentration:

Sodium hypochlorite: 0.2%

Sodium thiosulfate: 0.2%

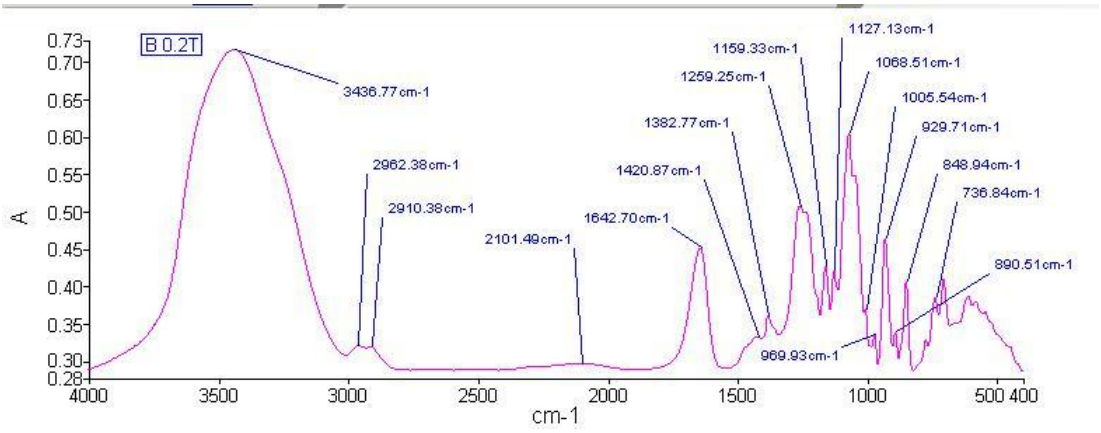

Figure B.4.3 FTIR spectra of carrageenan (Sodium hypochlorite 0.2%, sodium thiosulfate 0.2%)

Percent concentration:

Sodium hypochlorite: 0.3%

Sodium thiosulfate: 0.3%

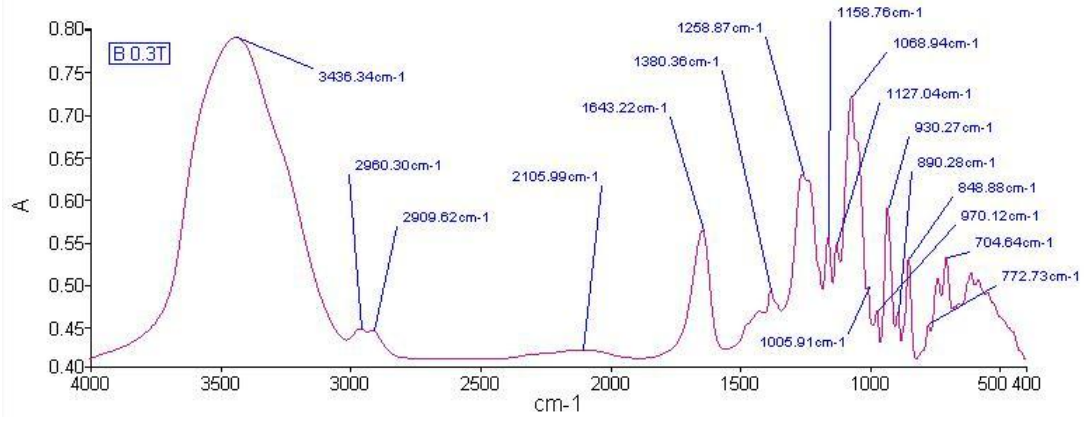

Figure B.4.4 FTIR spectra of carrageenan (Sodium hypochlorite 0.3%, sodium thiosulfate 0.3%)

Percent concentration:

Sodium hypochlorite: 0.4%

Sodium thiosulfate: 0.4%

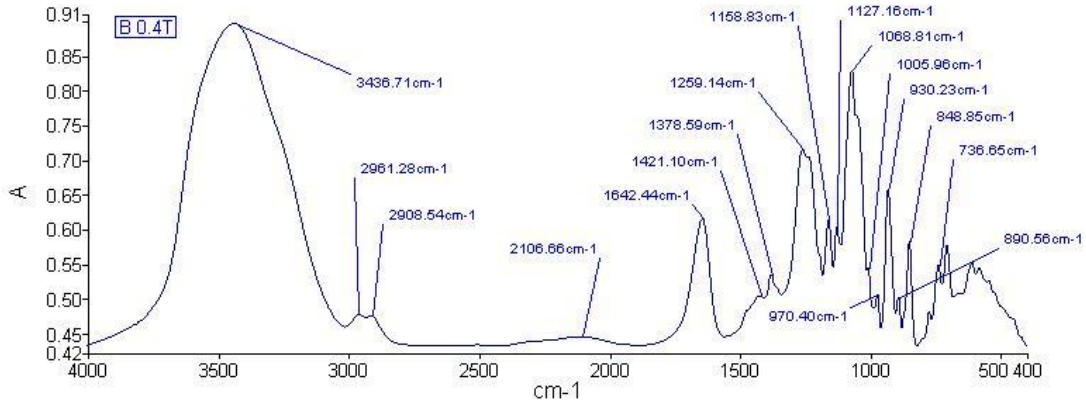

Figure B.4.5 FTIR spectra of carrageenan (Sodium hypochlorite 0.40%, sodium thiosulfate 0.40%)

5. The effect of temperature on bleaching

Table B.5 UV Vis (Absorbance) and pH reading at different temperature.

Temperature, ^{O}C	Absorbance (ABS), A	pH
22 (Room temperature)	0.136	9.63
30	0.129	9.62
35	0.124	9.44
40	0.127	9.09
45	0.122	8.84

APPENDIX C Figure for procedure analysis

Figure C.1 Seaweed: *Eucheuma cottonii*

Figure C.2 Extraction of carrageenan

Figure C.3 Seaweed after extraction

Figure C.4 Seaweed during bleaching process

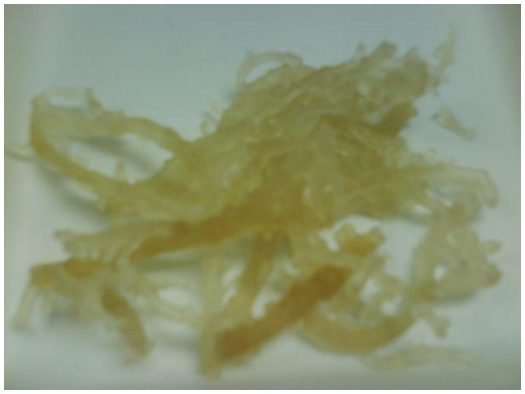

Figure C.5 Seaweed before bleaching

Figure C.6 Seaweed after bleaching

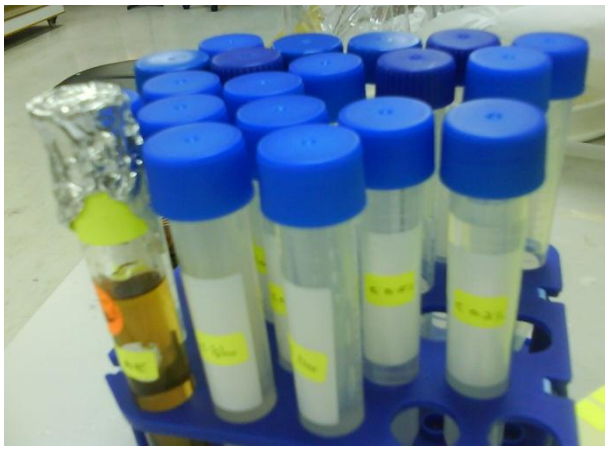

Figure C.7 Sample for analysis

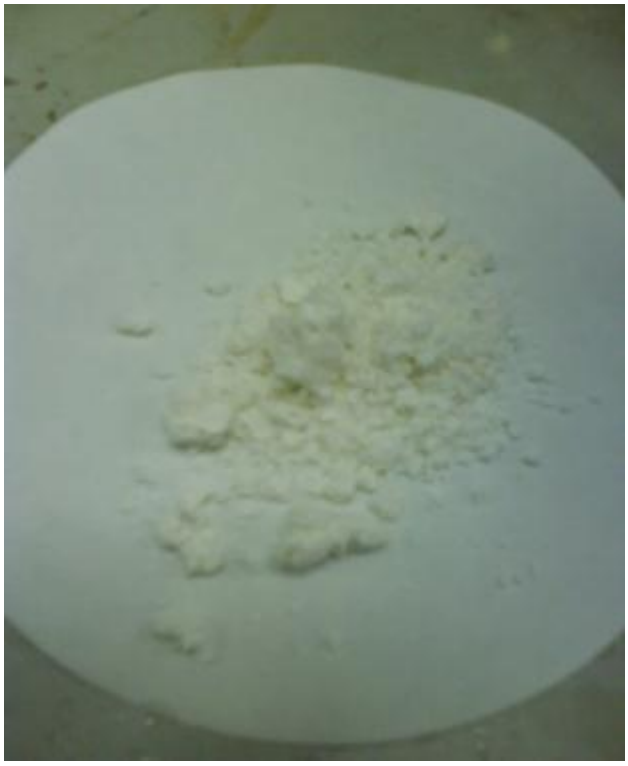

Figure C.8 Carrageenans powder