EXTRACTION AND ISOLATION OF KAPPA CARRAGEENAN FROM RED SEAWEEDS

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ABSTRACT

This works present the extraction and isolation process of kappa carrageenan from red seaweed. Product from this research can be use in pharmaceutical industry for production of capsule as it give advantages in aspect of economical, health and cultural. To extract and isolate kappa carrageenan, alkali treatment and alcohol precipitation was involved. In the alkali treatment of extraction process, three variables (i.e. temperature, concentration and time) have been investigated whereas in isolation process, isopropanol was used to study the separation of kappa carrageenan through precipitation. Based on experimental analysis, alkali treatments influence the yield, rheological and physichochemical properties of kappa carrageenan. Increasing potassium hydroxide (KOH) concentration decreased the yield and viscosity of kappa carrageenan due to degradation of polysaccharides. Temperature and time gave insignificant effect to the properties of extracted carrageenan. From the experimental result, the extraction and isolation of kappa carrageenan has been successfully conducted. Therefore, the range of concentration, temperature and time used in this analysis is acceptable to extracte and isolate kappa carrageenan.

ABSTRAK

Kajian ini berkaitan dengan proses pengekstrakan dan pengasingan kappa carrageenan daripada rumpai laut merah. Hasil produk daripada kajian ini boleh digunakan dalam industri farmaseutikal bagi pengeluaran kapsul kerana ia memberikan kelebihan dari aspek ekonomi, kesihatan dan budaya. Untuk menghasilkan kappa carrageenan, rawatan alkali dan mendakan alkohol terlibat. Dalam rawatan alkali, suhu, kepekatan dan masa adalah berbeza bagi setiap proses pengekstrakan. Bagi proses pengasingan kappa carrageenan, isopropanol telah digunakan bagi mengkaji pengasingan kappa carrageenan melalui mendakan alkohol. Berdasarkan analisis eksperimen, rawatan alkali mempengaruhi kadar penghasilan, sifat reologi dan sifat physichochemical kappa carrageenan. Peningkatan terhadap kepekatan KOH akan membawa kepada pengurangan kadar hasil dan kelikatan kappa carrageenan yang disebabkan oleh degradasi polisakarida. Suhu dan masa tidak banyak memberi kesan kepada sifat-sifat carrageenan yang diekstrak. Kepekatan , suhu dan masa yang digunakan dalam analisis ini boleh diterima untuk pengekstrakan dan pengasingan kappa carrageenan.

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LIST OF ABBREVIATIONS

BSE	Bovine spongiform encephalopathy
HPMC	Hydroxylpropyl methylcellulose
KOH	Potassium Hydroxide
WHC	Water Holding Capacity
kC	Kappa carrageenan

1 INTRODUCTION

1.1 Motivation and statement of problem

Medicinal agent within a hard or soft soluble container in form of pharmaceutical dosage is defined as capsules (Karteek and Krishna, 2011).Capsules can also be defined as container or shell that encapsulated the medicinal substance. It is odorless, tasteless and easily to be swallow. Capsule is very important in medical and pharmaceutical industry. It is widely used as medicine and supplement. Karteek and Krishna (2011) also stated that capsules as the most suitable method for medication consumption. Insides capsules it normally comprise of drug, oil, powdered and active ingredient. Different type of ingredient has different types of shell. Capsules shell can be classify into two types which is hard-shelled capsules and soft shell capsules. Both are easy to be absorbed and dissolved by the stomach lining and small intestine. Most of capsule found in form of hard gelatin shelled and it usually capsulate granular fillings and powders while for oil and bioactive gradient soft capsule shell was used. In 1834, Mothes and Dublanc was the first to patent single-piece gelatin capsule that are made from gelatin solution and in 1846 two-piece hard shell capsule from the mixture of starch, sucrose with gelatin was first patent by Lehuby (Chiwele et al., 2000). During the 1950s commercial production of the capsule started in Europe. According to Al-Tabakha (2010) and Chiwele et al. (2000) gelatin are largest source for the production of capsule in market. Gelatin is product found in white connective tissue, animal skin and bones by the process of partial hydrolysis of collagen (Morrison et al., 1999).Example of animal that are used as the source of gelatin is pigs, cattle, horses and chicken. Extraction of gelatin from the animal sources involved acid and alkali treatment. Gelatin is widely used as substances in the production capsules due to its "melts in the mouth" property, thermal reversible gel, tailor made application, and easy to use. (Karim and Bhat, 2008; Chiwele et al., 2000).

Go'mez-Guille'n et al. (2009) claimed the most abounding source of gelatin are pig skin (46%), bovine hide (29.4%), pork and cattle bones (23.1%) and other which include fish gelatin (1.5%).Production of gelatin from the source of animals gives some problems to the certain consumers especially for 2.1 billions Muslim people (World Muslim Population, 2012) and vegetarian population in the world due to religious, cultural and

personnel issue. In the market, source of capsule shell is doubtful. For Muslim, it is forbidden to consume unslaughtered animal and pork product (Go'mez-Guille'n et al., 2009) while for vegetarian people it violates from their principles which is to avoid product that are animal based. Moreover, gelatin can contribute to some disease, (i.e. bovine disease, kidney or liver disease and gastrointestinal symptoms) and side effect (i.e. allergy and toxin exposure). H. W. Murphy from Eli Lilly and company was the first to patent for gelatin capsule alternative buy using methyl cellulose as the source in 1950 (Al-Tabakha, 2010).Other than methyl cellulose, there are some other sources that are potential to be a substituent for gelatin. According to Murano (1998) claimed that carrageenan, agar, alginate can be used to replace gelatin. All of this is plant based capsules. Both agar and carrageenan can be extracted from red seaweed species while alginates can be extracted from brown seaweeds. The feasibility to use carrageenan, agar and alginates as the source of capsules will give a positive influence in our economic especially in Sabah (i.e. Semporna, Tawau and Lahad Datu). This is due to abundant production of seaweed (Sade et al., 2006) especially in Tawau, that near Philippines which the world largest seaweed producing areas (Chan et al., 2011). Therefore, the production of capsule shell from carrageenan will be one of the alternatives vegan capsules for the Muslim and vegetarian consumer besides developing the seaweed processing and help people to cultivate socio-economy development in rural area especially in Sabah.

Previously there are some researches that are conduct to study the affect of time, temperature and concentration on the properties or characteristic of extracted carrageenan (i.e. Distantina et al., 2011 and Phycol, 2008). Therefore, in this research the main aim is to analyze some parameter that will affect the extracted kappa carrageenan properties (i.e. concentration and temperature). According to Distantina et al. (2011) extraction solvent (alkali) will affect the yield and extracted carrageenan properties while according to Phycol (2008) temperature will affect the molecular properties and dynamic viscoelasticity. Therefore for our work will concentrate on extraction and isolation of kappa carrageenan by modifying the parameter of concentration and temperature for alkaline treatment and it will be test on their properties.

1.2 Objectives

The following are the objectives of this research:

- To establish the technique of extraction and isolation of kappa carrageenan from the red seaweed complex solution.
- To characterize the rheological characteristic and physicochemical properties of extracted kappa carrageenan.

1.3 Scope of this research

The following are the scope of this research:

i) Optimization of alkaline treatment for extraction process by manipulating parameter of temperature, time and concentration.

ii) Rheological analysis of extracted and isolated kappa carrageenan

iii) Physicochemical analysis of extracted and isolated kappa carrageenan.

1.4 Main contribution of this work

The following are the contributions

i) Develop pharmaceutical industry by provide more option on halal and plant-based capsule in the market.

ii) Developing the seaweed processing and help people to cultivate socio-economy development in rural area especially in Sabah.

iii) Understanding the extraction and isolation process of kappa carrageenan.

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides a description of the general design of production of capsule shell from kappa carrageenan that can be extracted and isolated from the red seaweed. A general description on the gelatine, capsules, gelatine substituent, seaweed, carrageenan, kappa carrageenan, as well as the extraction and precipitation process are presented. This chapter also provides a brief discussion of the parameter that affects the rheological and physicochemical characteristic of extracted carrageenan. A summary of the previous experimental work on gelatine substituent and extraction in seaweed is also presented. A brief discussion on the extraction and isolation process is also provided.

Chapter 3 gives a review of the extraction and isolation process as well as the characterization of kappa carrageenan. Different concentration, temperature and time are applied for extraction process and the yield, rheological and physicochemical analysis of 18 different samples was compared. The results for the yield analysis, rheological analysis, water holding capacity and swelling are presented and compared.

Chapter 4 is a detailed description on how the concentration, time and temperature affect the characteristic of extracted and isolated kappa carrageenan.

Chapter 7 draws together a summary of the thesis and outlines the future work which might be derived from this work.

2 LITERATURE REVIEW

2.1 Overview

This chapter presents the experimental studies of extraction process of kappa carrageenan from the red seaweed in 0.03M, 0.05M and 0.1 M of potassium hydroxide (KOH) solution with different temperature and time. It was found that low concentration of KOH with long time of extraction process give the highest yield of carrageenan. It is possible to correlate the effect of time, temperature and concentration with the yield of kappa carrageenan.

2.2 Gelatin

Gelatin is defined as mixture of protein and peptides that is derived from the collagen which can be found in skin, white connective tissues and bones of animals by acid, alkali treatment and enzymatic hydrolysis of collagen (Light & Bailey, 1982). Gelatin comprise of 50.5% carbon, 25.2 % oxygen, 6.8% hydrogen and 17% nitrogen (Smith, 1921). Glycine, proline and hydroxyproline are the typical sequence of amino acid composition that are mostly found in the gelatin. (Gilsenan and Ross-Murphy, 2000; Russell et al., 2007). Choi & Regenstein (2000) estimated that about 200,000 metric tones per year gelatin is used in the world. Figure 2-1 below shows the structural unit of gelatine.

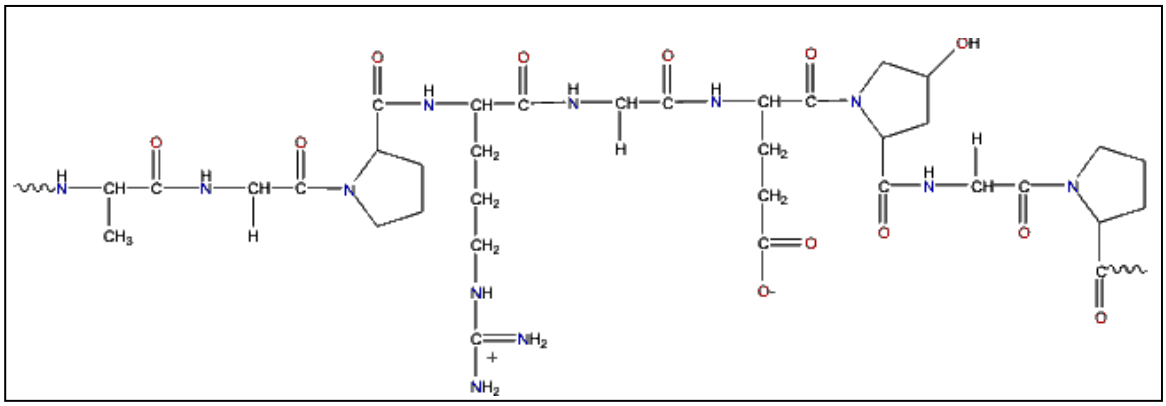

Figure 2-1: Structural unit of gelatin
(Martin Chaplin, 2012)

Gelatin is faintly yellow in colored, odorless, flavorless and form brittle solid when it dried. Pre-treatment and extraction process are two important steps in the production of gelatine as both affect the degree of conversion of collagen into gelatin (Johnston-

Banks, 1990).Different source of protein and pre-treatment process, resulting in different type of gelatin which is type A and type B. Acid treatment (caustic soda solution and dilute mineral acid) will produce type A that are mostly comes from pig skin (porcine) while in alkaline treatment (lime and water) will produce type B gelatin that are usually from the cattle hides sources (bovine). According to Raja et al. (2011) bovine skin gelatin has lower bloom strength than porcine skin gelatin as it has lower content of proline and glycine and lower degree of cross linking. In pre- treatment process hair, salt and other impurities were removed as it influenced physiochemical properties of gelatin. Besides that, this process will produce decent swelling and collagen solubilisation due to the breaking of non covalent bond in the protein structure (Stainsby, 1987). In extraction process, pH, temperature and time will be varies to produce the optimum gelatin product. The commercial qualities of gelatin depend on the gel strength, thermal stability and viscosity (Sanaei et al., 2013; Gómez-Guillén et al., 2011). This natural product have many application in food, pharmaceutical, medical, cosmetic and photographic industries (Bigi, Borghu, Fichera, Panzavolta & Roveri, 2000; Pranoto, Lee, & Park, 2007) and it essentially based on visco-elastic and gel forming properties (Gómez-Guillén et al., 2011). Figure 2-2 show the manufacturing process of type A and type B gelatine.

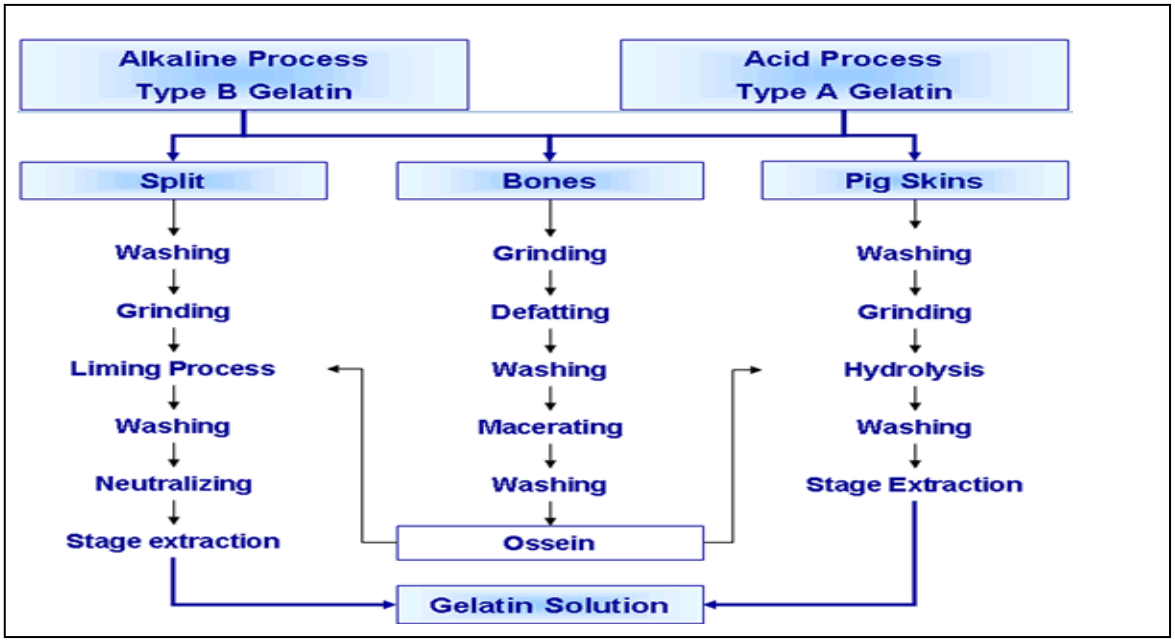

Figure 2-2: Manufacturing process of gelatin

(GEA Filtration, 2013)

Gelatin is widely used in food industry and it also much used in cosmetic, pharmaceutical, photographic and technical applications. In food industry gelatin is used as colloid stabilizers, thickening and foaming agents (Gómez-Guillén et al., 2011) in confectionary like marshmallow, gummi bears and yogurt. In production of yogurt, gelatin creates a creamy texture and fruity taste as it able to bind with the favourable fruit juices. Besides that, it also can be used as food packaging material as it edible, biodegradable and can be recycled (Kerry et al., 2012). In pharmaceutical area, it is used as coatings or edible film for capsule (Park et al., 2008; Sobral, Menegalli, Hubinger, & Roques, 2001).Pork skin, cattle hides, cattle bones, poultry and fish were states as the raw material for gelatin (Gelatin Manufacture Institute of America, 2012). Gelatin World Market (2003) stated that pig skin contribute 42.4 % in production of gelatin while bones and bovine hides contribute to 29.3% and 27.6%. Go'mez-Guille'n et al. (2009) claimed the most abounding source of gelatin is from pig skin (46%). This is due to abundance of this raw material. Gelatin from the pig skin was used since 1930s. Jamaludin et al. (2011) claimed that pig skin provides the best quality of gelatin compared to the other sources. Generally this source can be found at low cost and it is easy to find (Kerry et al., 2012). According to Gelatin Manufacture Institute of America (2012) pork skin is used due to economic factor. This raw material can minimized the waste water generation and less time required for the pre-treatment process. Gelatin is different from other hydrocolloid as in gelation process cations or soluble solids and pH have almost no affect in the process.

2.3 Capsule

A capsule is a solid dosage form which consist of medicinal and inert substances contained within a shell or container that are made from gelatin (Mohan et al., 2013). Filling inside capsule consist of substance that help to relieve or reduce pain as it absorb to the blood stream. Generally, capsule is used to hide the unpleasant taste and odor, help in drug delivery system and administration of the medicinal substance. Besides that, it is economical, easy to handle or carry and has attractive appearance. There are two types of capsule which is soft and hard shell. Figure 2-3 and Figure 2-4 shows the manufacturing of two type of capsule shell that depends on the formulation.

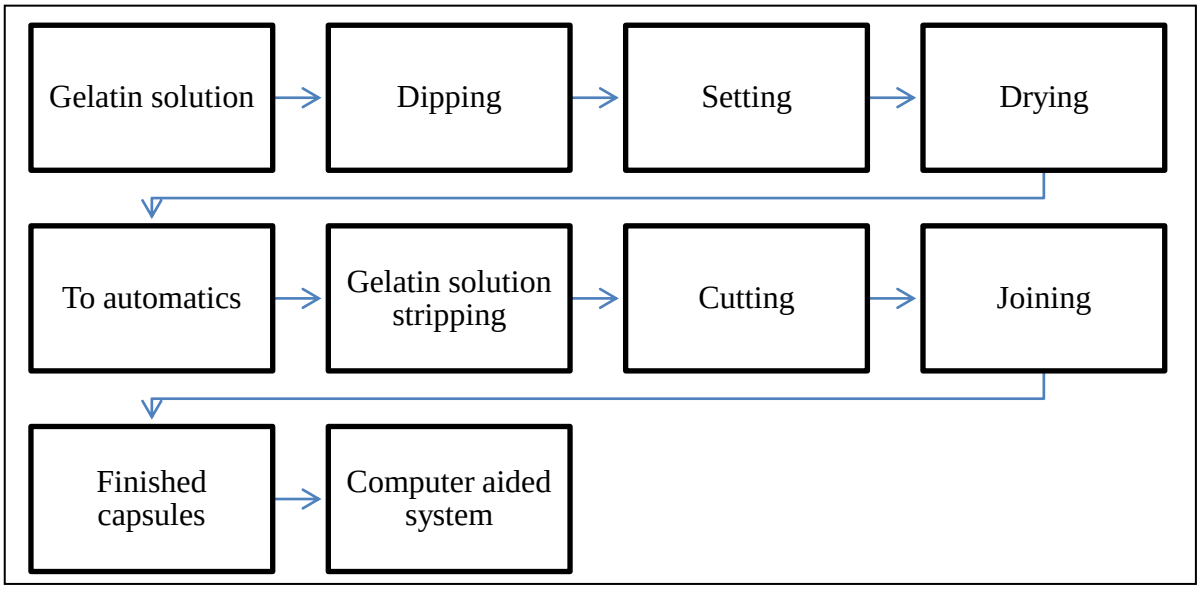

Figure 2-3: Manufacturing of hard gelatin capsule shell
(Mohan et al., 2013)

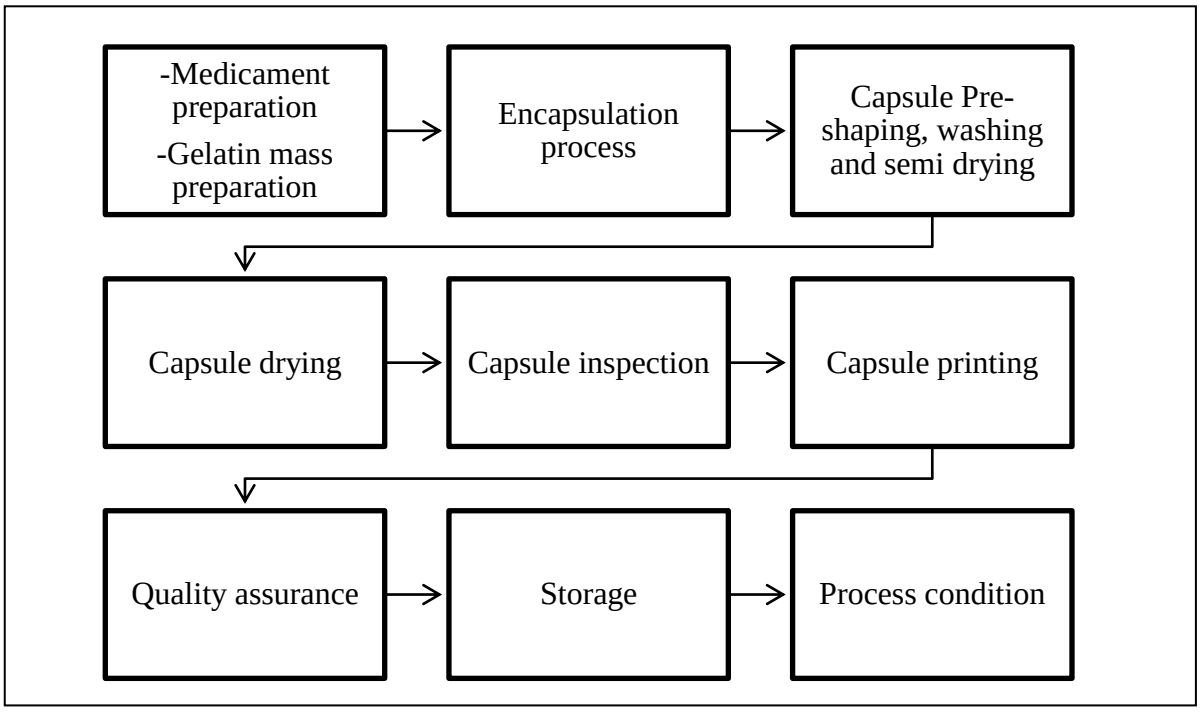

Figure 2-4: Manufacturing of soft gelatin capsule shell

Mohan et al. (2013)

Bhatt and Agarwal (2007) stated that in production of both capsule, there are commonly raw material used which is gelatin, colorants, water and other optional material (i.e. preservatives and process aid). Different type or sources of gelatin produce different

type of capsule (hard and soft). Table 2-1 compare the characteristic of capsule due to the source of gelatin.

Table 2-1: Characteristic of capsule based on the source of gelatin
(Agarwal & Bhatt, 2007)

Source of gelatin	Characteristic of capsule
Bone	• Tough • Firm film • Brittle • Tend to be hazy
Pork skin	• Plasticity • Reducing cloudiness • Clarity to the blend
Blends of pork skin and bone	• Relatively high strength • Normally used for hard shell capsule

Dry, powdered ingredients or miniature pellets regularly within hard-shelled capsules while active ingredients, oils and suspension normally in soft-shelled capsules (Karteek et al., 2011). Stegemann & Bornem (2002) state that the best type of hard gelatin capsule was filled with simple powder filling. The comparison between hard shell capsule and soft shell capsule is shown in Table 2-2.

Table 2-2: Comparison of hard shell capsules and soft shell capsules
(Mohan et al., 2013)

	Hard shell capsules	Soft shell capsules
Filling materials	• Dry ,powder or miniature pellets • Easy to have mixtures	• Active ingredient, oil • Not adaptable to incorporate mixtures
Stability	• Brittle or get sticky • Humidity control required	• Stable • Less impurities
Advantages	• Easy to be swallow • Can be encapsulated in various size • Colored can be added • Cover unpleasant taste, odor and	• Easy to be swallow • Can be encapsulated in various sizes, shapes (i.e. oblong,spherical) and colour • Longer shelf life

	appearance • Rate of drug release can be alter	• Tamper proof for one piece capsule
Disadvantages	• More expensive • Easily tampered	• Easily tampered • Required additional quality control measure

Traditionally, main constituent in capsules made from gelatin that primarily comes from animal (bovine, pork) or non gelatin materials (starch, HPMC) that is usually derives from plant. Bhatt and Agrawal, (2007) stated that gelatin is use as the major component in the production of capsules due flexibility, strength, toxicity and solubility. In recent years, chicken, fish, agar, carrageenan, starch and HPMC are investigated to be the alternative sources for the animal gelatin in production of capsule (Badii & Howell, 2006; Badii et al., 2013; Gudmundsson & Hafsteinsson, 1997).

2.4 Gelatin Substituent

Go'mez-Guille'n et al. (2009) claimed the most abounding source of gelatin is from pig skin. This arise some concern as gelatin that is animal based use in the production of capsules. For Muslims, Jews and Hindus it is religious reason for not accepting gelatin from pig sources (Haug et al., 2013) and beef gelatin is acceptable if it has been slaughtered according to religious rules and requirements (Badii & Howell, 2006). For vegetarian population, gelatin is violates from their personal preferences as they need to avoid animal based product. In 1980s, Bovine spongiform encephalopathy (BSE) or mad cow diseases motivate the searching of gelatin alternative (Sabron et al., 2013) as it linked with Creutzfeldt-Jakob's Disease (CJD) which cause 10 young people died in March 1996 in United Kingdom (Campbell, 2006).Besides that, gelatin can also cause some allergic to some consumers. In the production of capsules, gelatin gives some disadvantages as it has high moisture content (Bhatt and Agrawal, 2007) , less solubility as it cross linking with aldehyde group (Carstensen & Rhodes, 1993 ; Digenes et al., 1994) and have low relative humidity (Chiwele et al., 2000). There is some research that has been done to find the alternative source for gelatin from the pig skin from mammalian species for example like chicken skin (Badii et al., 2013), fish skin and bone residue (Sanaei et al., 2013; Bhat and Karim , 2009). Badii et al. (2013) stated that chicken's gelatin has higher bloom strength than bovine gelatin due to the characteristic of collagen, amino acid contents, molecular weight distribution and extraction process.

Chicken gelatin has higher gel strength than fish gelatin due to lower content of proline and hydroxyproline that contribute to the stability of collagen chain structure (Badii & Howell, 2006; Fernandez-Diaz et al, 2003). Besides that, chicken gelatin is more heat stable and shows the same chemical composition to the pig gelatin. In the research also stated that chicken gelatine has better physicochemical properties. Optimum condition for the extraction of gelatin from the bones of catfish (*Clarias gariepinus)* has been study by Sanaei et al. (2013). In the research, affect of time, temperature and concentration on extraction process has been study and it is found that catfish bone contribute to higher gelatin yield compared to other fish species such as hake 6.5%, cod 7.2% (Gómez-Guillén et al., 2001) and red tilapia 7.8%, black tilapia 5.4% (Jamilah and Harvinder, 2002) but it has slightly lower gel strength than bovine gelatine due to lower gel forming capability (Sanaei et al., 2013). In Malaysia there is research that studies the use of different marine fish species (i.e. kerapu, kembung, and jenahak) as gelatin substituent. This research concludes that different type of fish resulted in different physicochemical characteristic due to the differences in amino acid composition, difference in molecular weight distribution, and living environment (Irwandi et al., 2009). For vegetarian population it is importance for them to choose plant based product. Therefore gelatin free capsules become a great interest in pharmaceutical industry in aspect of economical, health and cultural as some of their consumers comprise of vegetarian people and some religious group. This cause many patents being filed, primarily in the pharmaceutical area (Gennadios, McHugh, Weller, & Krochta, 1994; Park et al., 2008; Sobral, Menegalli, Hubinger & Roques, 2001; Torres, 1994). Some company in United States of America (USA) for example likes Jarrow Formulas, Planetary Herbals and Progressive Labs (Al-Tabakha, 2010) has used Hydroxylpropyl methylcellulose (HPMC) as material in production of their capsule. HPMC that is also known as hypromellose is natural multifunctional polymer cellulose that is usually used as thickening agent and emulsifiers. Others than HPMC, potato starch (Mohan et al., 2013), carrageenan, agar, alginate (Murano, 1998) are one of the potential alternative for gelatin. Bae et al. (2007) compared the mechanical and physical properties of hard starch capsules from the sweet potato, waterchesnut and mugbean with HPMC and gelatine and this vegetables capsules is shows relatively good mechanical and physical properties. It also does not need gelling agent as the starch with high amylose is use as the raw material in the production of hard capsules. Carrageenan, agar, alginates can be extracted from different type of seaweed. In medicinal and pharmaceutical area,

alginates are used as wound healing material by activate the process of coagulation, agar is used in formulation of capsule to carry by increasing the drug release in the body (Paola, 2010) and carrageenan especially kappa type was used in the formation of gelatin free capsules.

2.5 Seaweed

Seaweed which also referred as sea vegetables is used since ancient times as medicine, food and fertilizers. In Japan, seaweed is used local cuisine as one of source of dietary (i.e. nori, kombu, sea grapes) that is beneficial to health. It is used as wrapping for sushi or onigiri and it can be cook as soup. This seaweed or algae are rich in vitamin (i.e. vitamin C, Vitamin E, vitamin B_{12}), mineral, polysaccharides and amino acid. It helps in regulating cholesterol level, blood sugar level, hormone balance during menopause and wound healing (Bocanegra et al., 2009; Fitzgerald et al., 2011; Venugopal, 2011). Seaweed can be categorized into three division of multicellular algae classes which is Class Rhodophyceae (red algae), Class Phaeophyceae (brown algae) and Class Chlorophyceae (green algae) (Pelinggon and Tito, 2009). In Asian countries especially in Japan, Korea, and China, human consumption of brown seaweed (66.5%), red seaweed (33%) and green seaweed (5%) is high. All of this seaweed can be found in coastal area in intertidal zone where is enough sunlight is available. Instead of health, this seaweed also contributes to economic and biodiversity of ecological. Okuda (2008) reviewed that seaweed beds play an important role in ecology of aquatic or marine system. It provides food, shelter and also habitat for shellfish fisheries and finfish. Muraoka (2004) claimed that seaweed can be used as carbon sink as one-fourth of the total amount of carbon dioxide was absorb by the ocean per year. Picture of the three class of algae is shown in Figure 2-5.

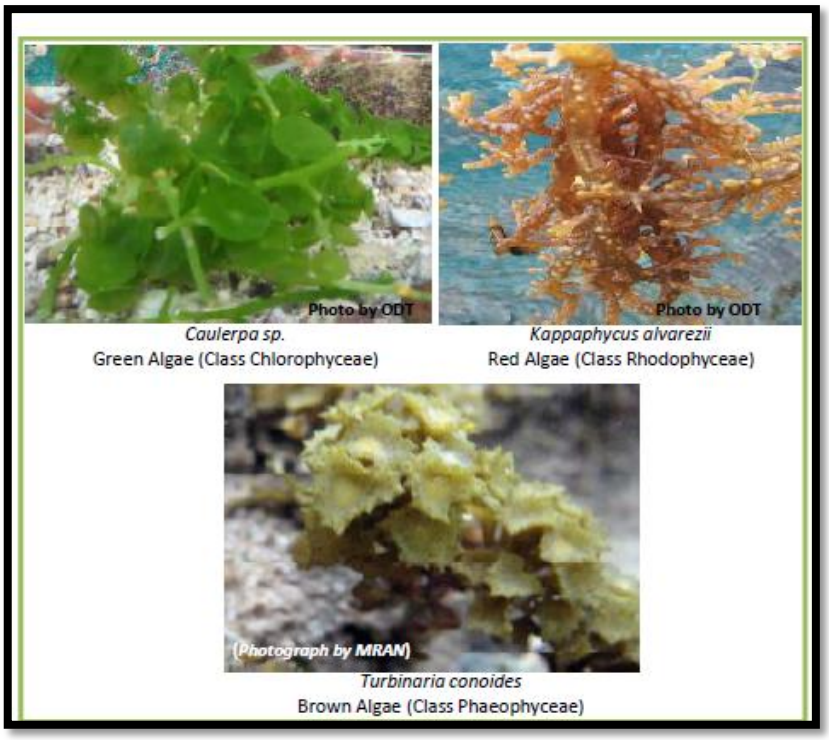

Figure 2-5: Major group of seaweeds

(Pelinggon and Tito, 2009)

In production of capsule, kappa carrageenan is used as it has higher gel strength and forming brittle structure but stable. Extraction of kappa carrageenan can be done by the process of extraction from the red seaweed or algae (*Rhodophyta*). According to Distantina et al. (2011) *Eucheuma cottonii* is good source of kappa carrageenan and it mainly harvested in Indonesia and Philippines. The main component of *Eucheuma cottonii* is kappa carrageenan and consists of less than 10% of iota types (Lee, 2008; De Ruiter & Rudolph, 1997). Table 2-3 shows the red algae species.

Table 2-3: Red algae species

(Jaspars and Folmer, 2013)

Official name	Common name	Major types of nutrients
Chondrus crispus	Irish moss	• rich in carrageenans (kappa and lambda) (50% D.W.) • floridoside (10% D.W.) • taurine (5% D.W.) • beta-carotene • vitamin B complex • rich in carrageenans (esp. iota) • lectins
Eucheuma denticulatum (formerly *Euchema spinosum*)	spinosum	• rich in carrageenans (esp. iota) • lectins
Gigartina sp.	-	• rich in carrageenans (kappa)
Gracilaria sp. (major source of agar)	ogo, ogonori (Japan), sea moss	• rich in agar (25% D.W.) • carrageenans
Kappaphycus alvarezii (formerly *Eucheuma cottonii)*	cottonii	• carrageenan (kappa) (22% D.W.)
Mastocarpus stellatus	carrageen moss	• carrageenans (kappa and lambda)
Palmaria palmata	söl (Iceland), dulse	• rich in floridoside (25% D.W.) • iodine • carotenoids • vitamin B complex

In Malaysia, *Kappaphycus alvarezii* and *Euchema spinosom* can be found in coastal of Sabah(i.e. Lahad Datu, Kunak, Semporna) as it a only main producer in Malaysia (Sade et. al., 2006). Falshaw et al. (2001) showed that more kappa carrageenan can be extract from *C.cripus* than *G.skottsbergii* and *S.crispata*. Yermak et al. (1999) had conducted the extraction and isolation of carrageenan from *Gigartinaceae* and *Tichocarpaceae* types which include *C. armatus, C. pinnulatus, I.cornucopia* and *T. crinitus* .From the research, conclude that kappa has higher composition than iota (i) and mixture of kC and β. Bixler (1996) stated that *Kappaphycus alvarezii* is used for main production of kappa carrageenan.

2.6 Carrageenan

Carrageenan is hydrocolloid that can be extracted from red seaweed by alkaline treatment (Paola, 2010). In India, carrageenan was sold sold at Rs 1 lakh per tone (Vijayalakshmi, 2003) and this industry achieved 3% growth per year. In year 2000, worlwide sales has reached 310 millions US$ (Ruiter and Velde). CP Kelco (USA), Quest International (The Netherlands) and FMC Corporation (USA) are the company that dominates this carrageenan market at the end of 20s century. In this research, carrageenan is used as raw material for production of hard capsule shell as it is derived from plant and it suitable for all type of consumers.

Table 2-4: Comparison of property between gelatin, carrageenan and agar (Martin Lersch, 2010)

Property	Gelatin	Carrageenan	Agar
Thermoreversible	Yes	Yes	Yes
Strength	Soft	Soft	Hard
Elasticity	Elastic	Elastic	Brittle
Shear Thinning	No	Yes	No
Hydration	50° C	70° C	90° C
Melting Temperature	25-40° C	45-80° C*	80-90° C
Viscosity	Low	Medium	Low
Gelling Concentration	0.6-1.7%	1.0-1.5%	0.2%
Syneresis	No	No	Yes

From Table 2-4, carrageenan shows the most potential material to replace the gelatin in production of hard capsule compared to agar because it isothermally reversible has

suitable melting temperature, elastic and higher gelling concentration. Although the melting temperature of carrageenan is minimum at 45° C but it can be modified by the process of cross linking so that the capsule can be melt based on human body temperature. Carrageenan can be found in main cell wall material of red algae (*Rhodophyta*) and it is derivatives of polysaccharides (linear sulfated polysaccharides) that can be extracted from red seaweed like *Eunheuma cottonii*, *Gigartina pistillata* and *Chondrus crispus* (De Ruiter and Rudolph, 1997). It consist of potassium, sodium, magnesium, and calcium sulfate esters of galactose and 3,6-anhydro-galactose copolymers in its structure (Paola, 2010). In food industry, they are used as stabilizer, thickening agent and gelling agent (Distantina et al., 2011). Number and position of sulphate groups, the presence of 3,6-anhydro-D-galactose, and conformation of the pyranose ring are the main factors that determined classes and the rheological behaviour of the carrageenan (Pereira et al., 2012). In red seaweed, commonly kappa (k), iota (i), lambda (λ), mu, nu, xi, θ, and β carrageenan can be found naturally in the form of mixture or hybrid.

Kappa, iota and lambda are three main commercial classes with mu and nu are precursor for the iota and kappa type when they are treats with alkali (Falshaw et al., 2001; Pereira et al., 2013). For kappa carrageenan, its structure consist of 25% of sulfate group and 34% of 3,6-anhydrogalactose while for iota consist of 32% and 30% respectively. In lambda structure have a little or no 3,6-anhydrogalactose and contains 35% ester sulfate. Comparing all type of carrageenan, it is found that kappa that consist one group of sulfate per disaccharides has higher gel strength and than iota and lambda. This sulfate group affects the solubility temperature and gel strength of carrageenan. Higher degree of sulfation lowers the solubility temperature of the carrageenan and lowered the gels strength. The presence of 3,6-anhydrogalactose bridges affect the ability to forming gels as sulfate group is transeliminate in the process of alkaline extraction.