EXTRACTION AND ISOLATION OF KAPPA CARRAGEENAN FROM RED SEAWEEDS

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EXTRACTION AND ISOLATION OF KAPPA CARRAGEENAN FROM RED SEAWEEDS

NUR NABILLAH BT MOHD ARIF

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
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JANUARY 2014

SUPERVISOR'S DECLARATION

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

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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.

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Dedication

To my beloved parents, Mohd Arif and Siti Haslina

ACKNOWLEDGEMENT

In the name of Allah S.W.T the Most Beneficent and the Most Merciful. The deepest sense of gratitude to the Almighty for the strength and ability to complete this undergraduate research project. Infinite thanks we brace upon Him.

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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.

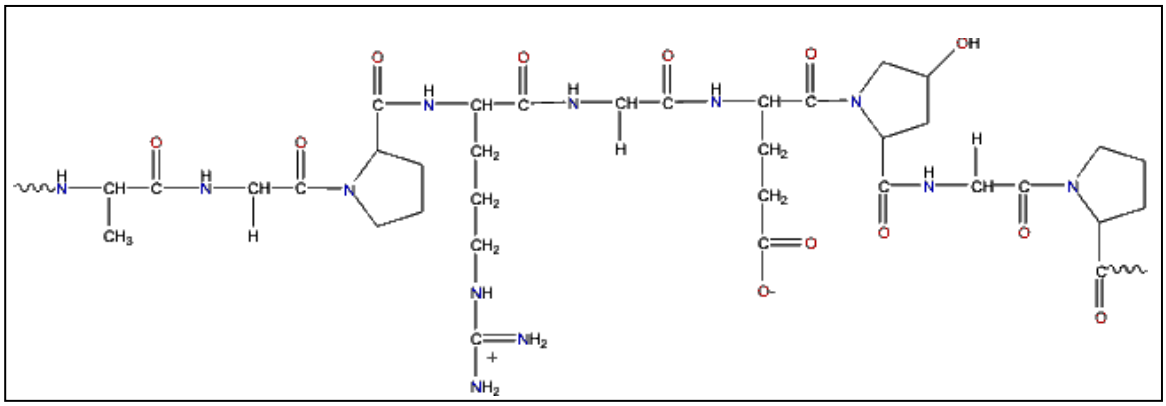


Figure 2-1: Structural unit of gelatin
(Martin Chaplin, 2012)

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.

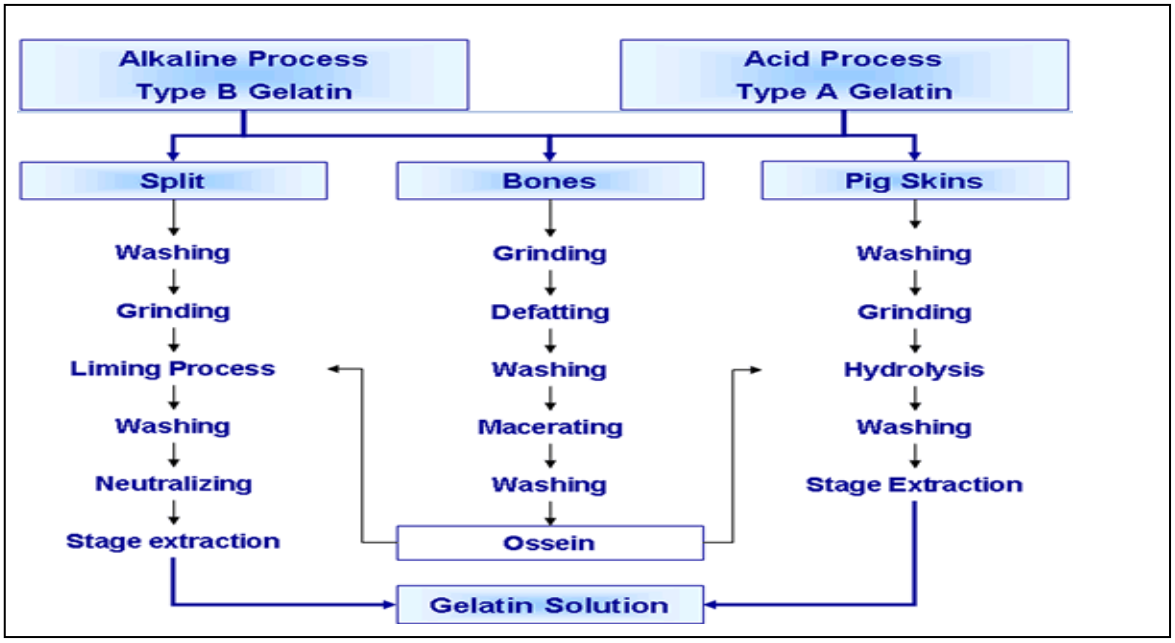


Figure 2-2: Manufacturing process of gelatin

(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.

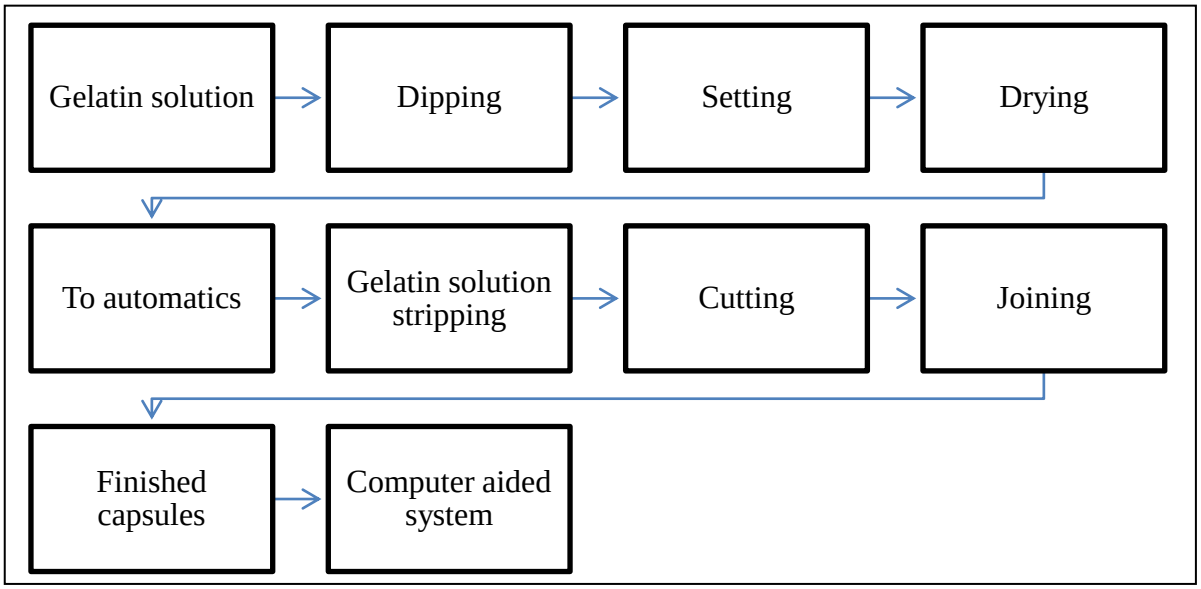


Figure 2-3: Manufacturing of hard gelatin capsule shell
(Mohan et al., 2013)

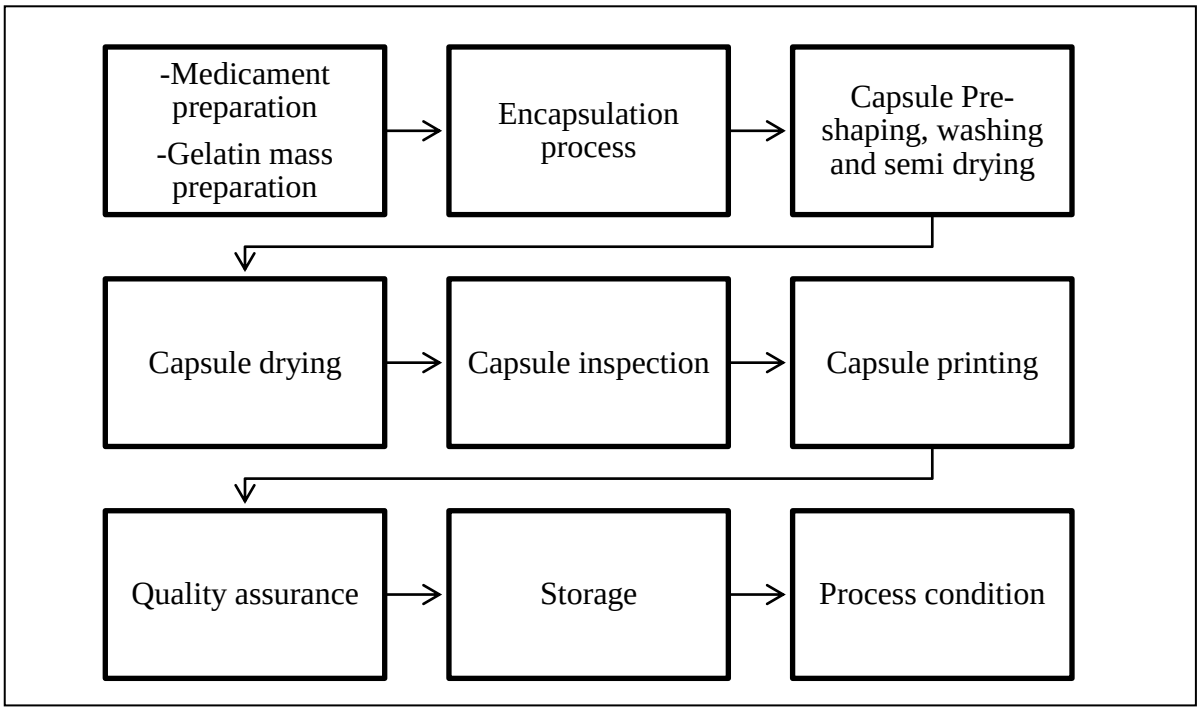


Figure 2-4: Manufacturing of soft gelatin capsule shell

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.

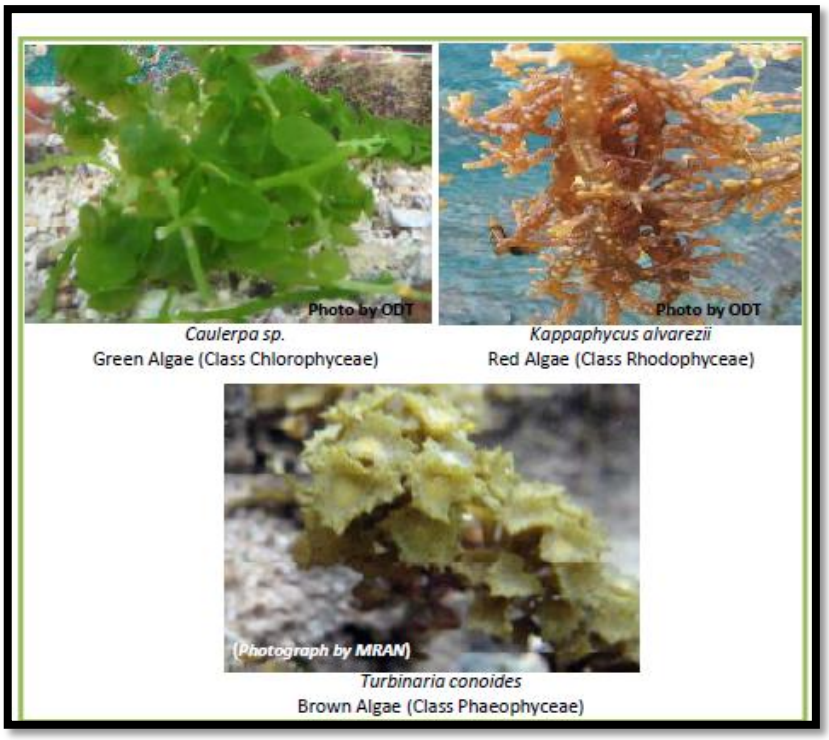


Figure 2-5: Major group of seaweeds

(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.

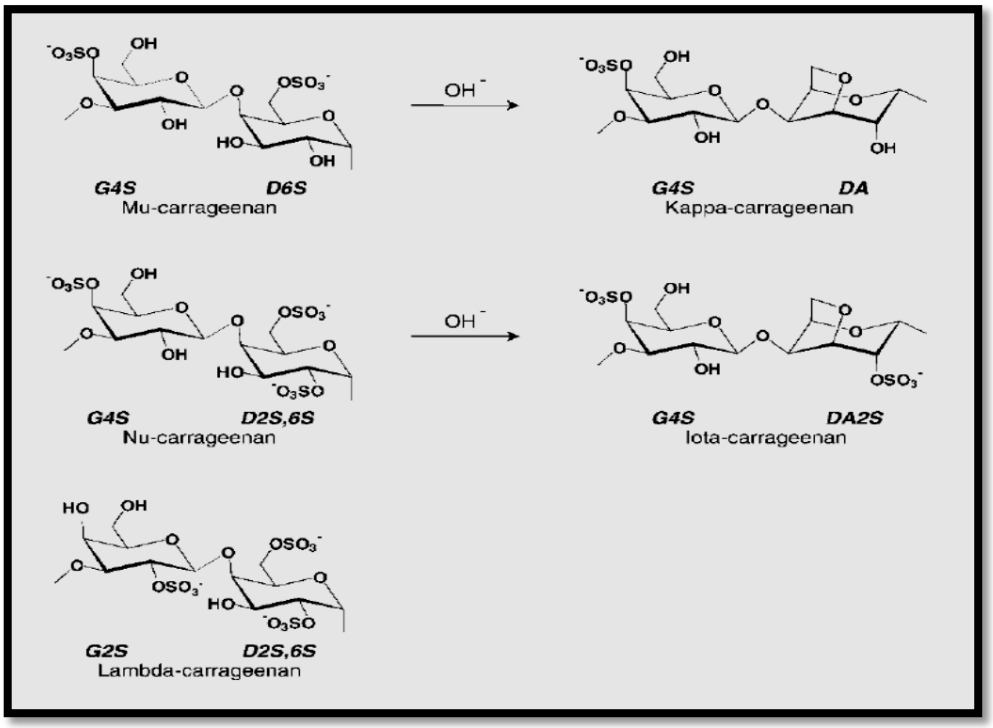


Figure 2-6: Structures of carrageenan

(Knutsen et al., 1994)

2.7 Kappa Carrageenan

Based on the three type of carrageenan, kappa carrageenan is chosen for this research. Kappa carrageenan can be extract from *Kappaphycus alvarezii* and *Euchema spinosom* .In Malaysia, both can be found in coastal of Sabah(i.e. Lahad Datu, Kunak and Semporna) as it a only main producer in Malaysia (Sade et al., 2006). Different type of seaweed consist of different differ composition kappa carrageenan. *Eucheuma cottonii , C.cripus, Gigartina stellata, Hypnea spp and Furcellaria* are stated as predominantly source for kappa carrageenan (Rideout et al., 1998). 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. Distantina et al. (2011) stated that yield, chemical and physical properties influence its commercial value in industry. All of these properties depend on the structure of carrageenans that mainly consist of sulphate ester group and the 3, 6-anhydrogalactose rings (De Ruiter

and Rudolph, 1997). Kappa carrageenan has lower degree of sulfation (one sulphate group) and it has higher melting temperature that iota (Martin Lersch, 2010). Besides that, it forms brittle and firm gel instead of soft gel (De Ruiter and Rudolph, 1997). Compare to iota and lambda, kappa carrageenan has higher gel strength, thermal reversible and it undergo synerisis process (De Ruiter and Rudolph, 1997; Martin Lersch, 2010).

Table 2-5: Comparison of kappa, iota and lambda carrageenan type
(Chan et al., 2011)

	Kappa	Iota	Lambda
Type of gel	Strong and brittle with syneresis	Elastic and cohesive without syneresis.	Non-gelling
Effect of cations	Gels most strongly with potassium ions	Gels most strongly with calcium ions	Non-gelling
At the neutral and alkaline pH	Stable	Stable	Stable
At acid pH	Salt;Hydrolyzed in solution when heated. Stable in gelled form.	Hydrolyzed in solution Stable in gelled form.	

Different extraction process (alkali treatment) affects the yield and quality of carraggeenan (Chandra Mishra et al., 2006).

2.8 Extraction

Extraction is a process of purifying or withdrawing desired material from the solid or liquid that is mixed with partially or not miscible solvent. There are two type of extraction process which is solid–liquid extraction and liquid-liquid extraction. In extraction of kappa carrageenan from the red seaweed, there are different reagent alkali treatment can be use (i.e. KOH, $Ca(OH)_2$, distilled water). According to Mustapha et al., (2011) dried *Kappaphycus alvarezii* must be washed first before the process extraction to reduce salt content. In the research the effect of different alkaline reagent (i.e. KOH and $Ca(OH)_2$) at different concentration (i.e. 0.01 M, 0.1 M and 1.0 M) has been study. Result shows that gel is forming at concentration of KOH more than 0.1 M and this gel solution have higher viscosity. In alkaline reagent of $Ca(OH)_2$, no gel is forming at all concentration and the gel start forming at 60 °C to 80 °C. Distantina et al. (2011) extracted kappa carragenan from *Eunchema Cottonii* by using KOH for alkali treatment.

In the research claimed that KOH influenced the gel strength and viscosity of the carrageenan. Alkali treatments affect the internal arrangement of carrageenan structure by forming helical structure that contributed to firm and brittle gel. Three alkali treatments (aqueous, NaOH and KOH) were done for the extraction of carrageenan from *Hypnea bryoides* (Al-Alawi et al., 2010). It is found that NaOH give the highest yield of carrageenan compared to aqueous (distilled water) and KOH. From the research it can be conclude that different reagent of alkali in extraction process gave different percentage of yield of carrageenan.

2.9 Precipitation

Precipitation is a process where solid is form from a solution due to diffusion or chemical process. For the precipitation of kappa carrageenan Distantina et al. (2011) and Al-Alawi et al. (2010) use ethanol as a medium for precipitation. This is done for isolating kappa from other types of carrageenan likes iota and impurities. Other than ethanol, potassium chloride (KCl) is also used as the medium for the precipitation of kappa carrageenan. In the extraction of kappa carrageenan from *Chondrus crispus* , different alkali metal ion (LiCl, NaCl, KCl, CsCl) is used to study the effect of shielding effect of electrostatic repulsion in rheological and physicochemical properties (Watase and Nishinari, 1982).

2.10 Summary of literature review

There some issue or problem rises as pig and animal was use as source of gelatin in the production of capsule shell. This problem related to the religious and culture reason. Haug et al. (2013) stated that it is religious reason for Muslims, Jews and Hindus to accepting gelatin from pig sources. Besides that, gelatin give some disadvantages in the characteristic of capsule and can cause some allergic to the consumer. Chicken skin, fish, HPMC , and red seaweed (kappa carrageenan) are found to be potential material to replace gelatin (Sabron et al., 2013; Irwandi et al., 2009; Al-Tabakha, 2010; Murano , 1998).In this research red seaweed is used as it can be found in coastal of Sabah (Sade et al., 2006). This seaweed produced kappa carrageenan which is the raw material that is used in the production of capsule shell. This type of carrageenan resulted in production of higher gel strength and forming brittle structure but stable (De Ruiter and Rudolph, 1997; Martin Lersch, 2010). There is some process need to be done to get this raw

material (kappa carrageenan) from red seaweed which included extraction using alkali treatment and precipitation process (Distantina et al., 2011; Al-Alawi et al., 2010). There is some parameter that is needs to be study (i.e. time, temperature and concentration) so that it optimized both process.

3 MATERIALS AND METHODS

3.1 Overview

This paper presents the method of extraction and isolation of kappa carrageenan from the red seaweed. In extraction process, time, temperature and concentration of alkaline reagent are the parameter that needs to be study. In isolation process, alcohol precipitation was used to isolate kappa carrageenan from the extraction solution. After the extraction and isolation process, several analysis such as water holding capacity, viscosity, yield analysis and swelling test was conducted to characterize the kappa carrageenan.

3.2 Chemicals

All chemicals were obtained from Sigma-Aldrich (Potassium hydroxide (98.0%), isopropanol (99.7%), and buffer solution pH1, pH7, pH13). Distilled water was also use as solvent in dilution of potassium hydroxide at different concentration.

3.3 Materials and method

3.3.1 Raw material

Sample of the dried red seaweed algae of the species *Eunheuma cottonii* was obtained from Sabah state, Malaysia. All the seaweed was washed with tap water to remove any visible foreign material and also to eliminate and reduce the salt content. Then, it was dried under the sun until it reaches constant weight.

3.3.2 Extraction

Dilution of KOH (solid form) in distilled water is done according to the desired concentration (0.03M, 0.05M, 0.1M) at different temperature (90°C, 100°C) and at different time (1 hour 30 min, 2 hour 30 min, 3 hour 30 min). KOH solution was heated up on hot plate until the desired temperature reached constant. Then the seaweed was added to the KOH solution at constant ratio (10/500; g/mL). After extraction process, alcohol precipitation was done.

3.3.3 Alcohol precipitation

Filtrate in extraction solution was filter using filter paper and directly poured into 3 volume of isopropanol which lead to the precipitation of carrageenan. This process takes about 30 minutes and the precipitated carrageenan was collected by filtration process and then it was dried in the oven at 50 °C to 60 °C until constant weight was achieved.

3.3.4 Characterization of kappa carrageenan

3.3.4.1 Yield analysis

Constant weight of dried kappa carrageenan was collected and the yield of kappa carrageenan was defined as the ratio of dried kappa carrageenan weight to dried seaweed weight which is 10g.

$$\text{Yield} = \frac{\text{dried carrageenan weight}}{\text{dried seaweed weight}}$$

3.3.4.2 Rheological analysis

0.2g of dried carrageenan was diluted in 30 ml of distilled water was prepared for 6.8 ml samples container. The sample was heat in water bath with temperature of 80°C for 20 to 30 minutes until the dried carrageenan was thoroughly diluted in distilled water. Next the spindle No. 18 and sample container was attached to the viscometer and the speed was set to 50 rpm. The viscometer was start and constant reading of viscosity was taken.

3.3.4.3 Physicochemical analysis

a) Water holding capacity

0.56g of dried kappa carrageenan was diluted in 8mL of distilled water to form a gel. This solution was set inside a 15ml centrifuge tube and the initial weight of the gel sample was recorded. Then it was stored overnight at room temperature 25 °C. At 4000 rpm for 30 minutes at 25°C, the gel was centrifuged. After the centrifugation runs, the

supernatant was removed and the gel was weighed. The water holding capacity (WHC) of the gel is then calculated:

$$\text{Water holding capacity } (\%) = \frac{W_t}{W_o} \times 100 \qquad \text{(eq 1)}$$

where W_o is the initial weight of gel and W_t is the weight of gel at day.

b) ***Swelling studies***

Swelling studies of kappa carrageenan were carried out in buffer solutions of pH1, pH 7, and pH13 at room temperature. Sample of the dried kappa carrageenan was weighed and immersed in Petri dish filled with 30mL of the each buffer solution. At different time intervals, the samples were moved out and were wiped using filter paper to remove excess liquid and weighed. The swelling ratio is then calculated:

$$\text{Swelling ratio, } (\%) = \frac{W_1 - W_o}{W_o} \qquad \text{(eq 2)}$$

where W_1 is weigh of dried kappa carrageenan and W_2 is weigh of samples after excess liquid was removed.

3.4 Summary

This thesis presents experimental study of extraction and isolation of kappa carrageenan from red seaweed. Concentration, temperature and time are the parameter that was study in this work. The kappa carrageenan that was extracted and isolated was compared in the analysis (i.e. yield analysis, rheological and physicochemical analysis).

4 RESULT

Concentration (M)	Temperature, ºC	Time	Sample	Yield ,%	Viscosity, mPa.s	Water holding capacity (%)	Swelling analysis, %		
							pH1	pH7	pH13
0.03	90	1h 30 min	A1	52.2	2247	97.36	3.09	6.34	7.29
		2h 30 min	A2	45.0	836.0	90.69	1.19	25.73	2.39
		3h 30 min	A3	24.7	837.8	100.60	4.05	0.22	6.75
	100	1h 30 min	B1	48.1	336.3	99.29	3.03	12.54	4.24
		2h 30 min	B2	43.9	413.7	100.24	3.94	9.69	4.07
		3h 30 min	B3	33.4	447.5	98.57	4.92	13.10	7.99
0.05	90	1h 30 min	C1	44.3	400.7	97.91	2.53	9.01	10.86
		2h 30 min	C2	33.0	463.7	97.40	4.71	7.91	7.42
		3h 30 min	C3	45.2	470.3	98.01	3.71	7.15	3.96
	100	1h 30 min	D1	52.7	195.4	98.40	2.36	5.46	5.01
		2h 30 min	D2	39.4	458.0	99.88	4.15	7.42	4.60
		3h 30 min	D3	54.6	136.8	98.36	2.18	4.41	3.26
0.1	90	1h 30 min	E1	51.3	338.5	97.78	4.25	5.21	6.47
		2h 30 min	E2	67.2	257.4	90.72	1.96	4.31	3.54
		3h 30 min	E3	57.0	267.9	89.39	1.64	4.32	4.06
	100	1h 30 min	F1	43.4	463.7	97.89	3.57	7.64	5.52
		2h 30 min	F2	79.3	537.7	83.65	2.27	4.17	1.85
		3h 30 min	F3	94.5	245.0	82.38	1.64	3.49	2.07

4.1 Extraction and isolation of kappa carrageenan

In extraction process, sulphates group (SO_4) in carrageenan is removed through desulfation at 6-position of galactose unit of carrageenan as the OH^- part of the reagent penetrates into seaweed. Then potassium sulphate (K_2SO_4) is formed as the K^+ part of reagent combined with the removed sulphates group (SO_4).

For isolation process, carrageenan is insoluble in alcohol and the filtrate turns into a coagulum of carrageenan.

4.2 FTIR Spectra of extracted and isolated kappa carrageenan

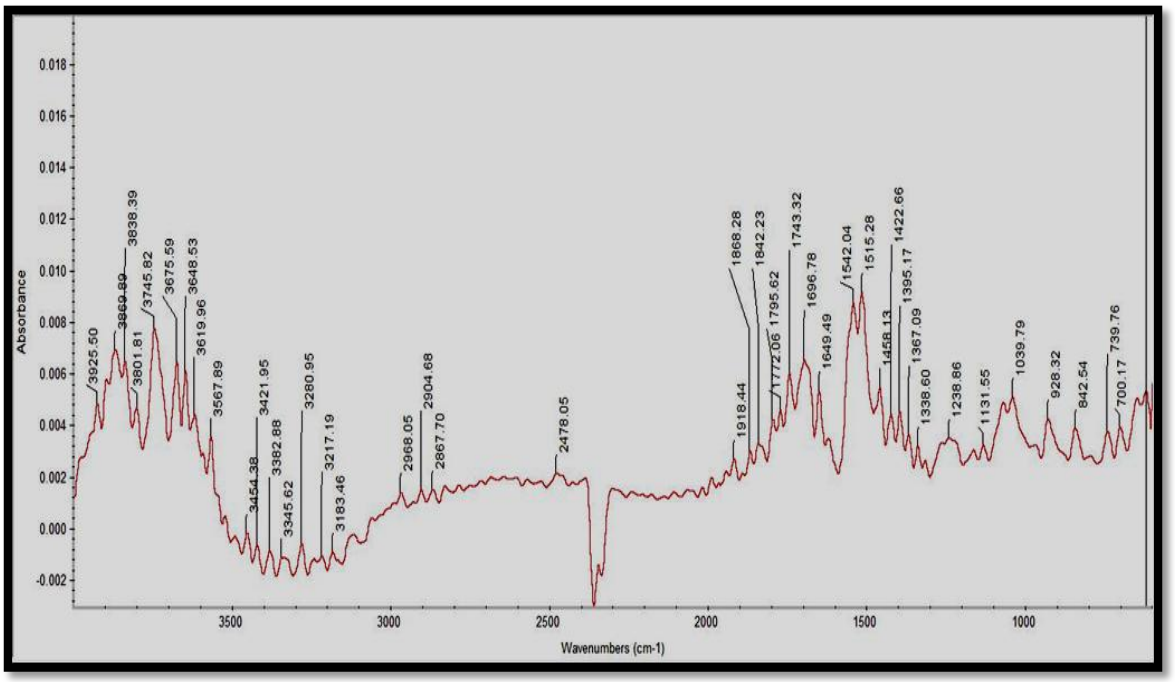


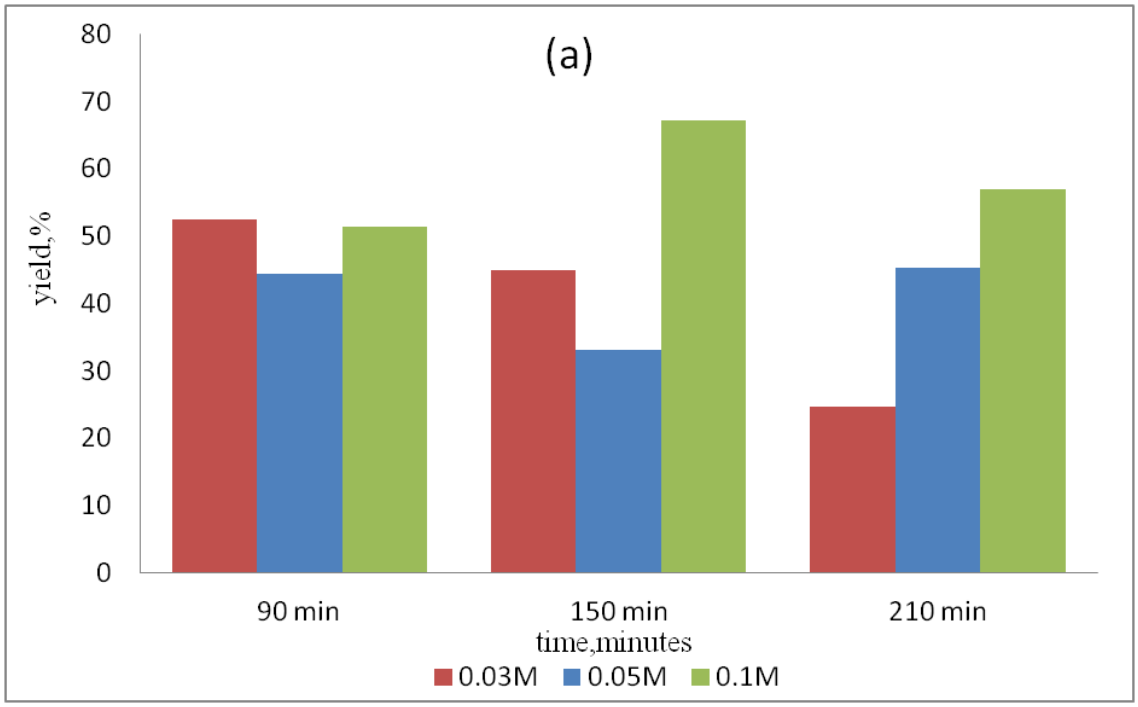
Figure 4-1: FTIR spectra of extracted carrageenan

FTIR Spectra shows the presence of S=O of sulphate ester in 1210-1238 cm^{-1} region and 1039-1080 cm^{-1} (cause to glycosidic linkage) in all carrageenan type. The other chemical groups are characteristics of given carrageenan type, namely 3,6 anhydro-D-galactose at 928-933 cm^{-1}, D-galactose-4-sulfate at 842-850 cm^{-1} and 3,6 anhydro-D-galactose-2-sulfate at 800-805 cm^{-1}. Both kappa and iota carrageenan FTIR spectra show a band at 842-850 cm^{-1}, and the 800-805 cm^{-1} band is distinctive of iota type.

4.3 Characterization of kappa carrageenan

4.3.1 Yield analysis

From Figure 4-2, most of the data shows that an increasing of potassium hydroxide concentration increased percentage of yield. Sample F3 shows the highest yield at 94 % compared to others sample. Low yield of kappa carrageenan is due to the degradation of polysaccharides. This similar result was also found by other researcher (Distantine et al., 2011). In previous work, we have conducted extraction at higher concentration (i.e. 0.3M, 0.5M, and 1M) and it was found that the filtrate turns as number of small particulate and could not be filtered. It shows that a low molecular carrageenan is precipitated. At 90 °C and 100 °C, difference in percentage of yield is not significant. On the other hand extraction time only affect the ratio of k-monomer polysaccharide and it contributing to the low yield of kappa carrageenan. Therefore alkaline extraction at concentration of 0.03 M, 0.05 M and 0.1 M at 90 °C and 100 °C for 90 minutes, 150 minutes and 210 minutes are acceptable for the extraction of kappa carrageenan from red seaweed.



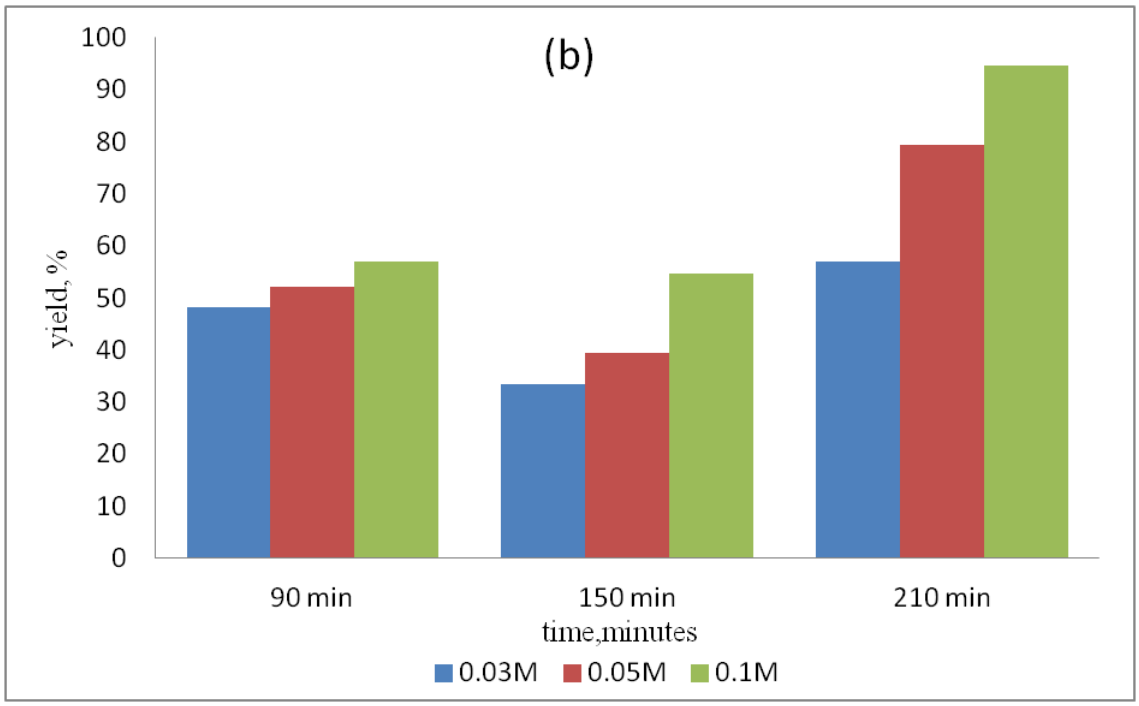
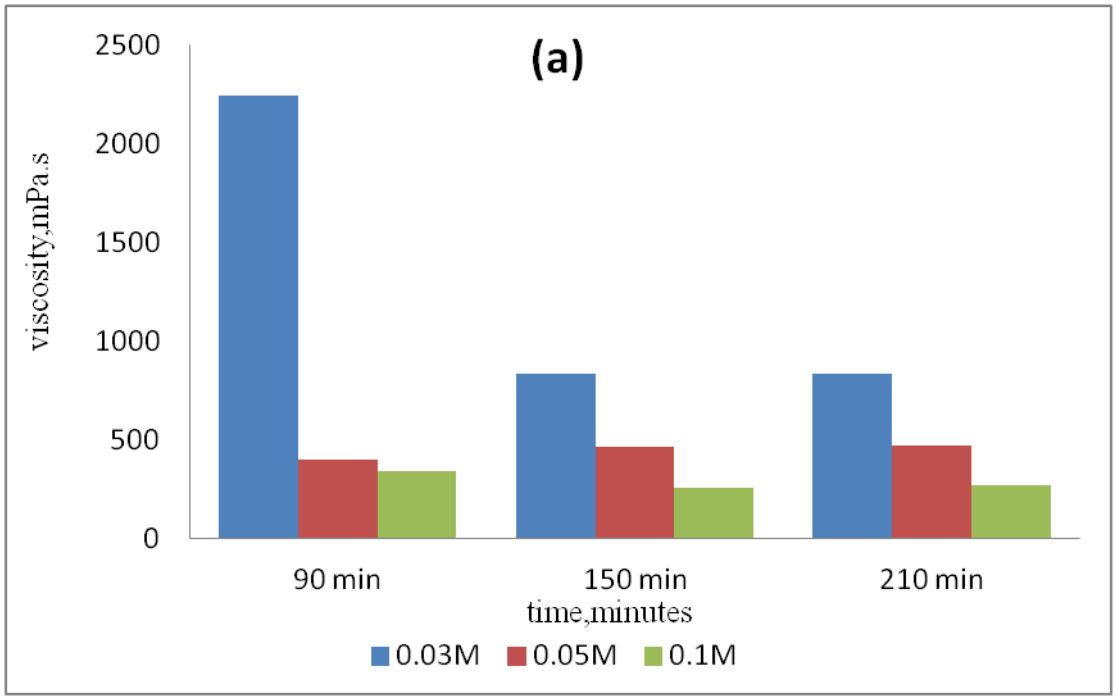


Figure 4-2: Yield analysis of kappa carrageenan with different of KOH with different extraction time at different temperature (a) 90°C (b) 100 °C

4.3.2 Rheological analysis

From Figure 4-3, most of the graph shows a trend where the concentration increases as the viscosity decreased. This viscosity is affected by the degradation of polysaccharide. As the concentration of KOH increased, polysaccharide chain become more compact due to the intramolecular interactions between polymer and the solvent molecules (Wang and W.Cui , 2005).Besides that, more degradation of polysaccharide occurred when the concentration of the alkaline solution increased. Other than alkaline concentration, temperature also affects the viscosity of the solution. Sample A1 have higher viscosity than sample B1 although they have same concentration and extraction time due to difference in temperature. When the temperature increased, it caused disentanglement and conformational change of polysaccharides chains.



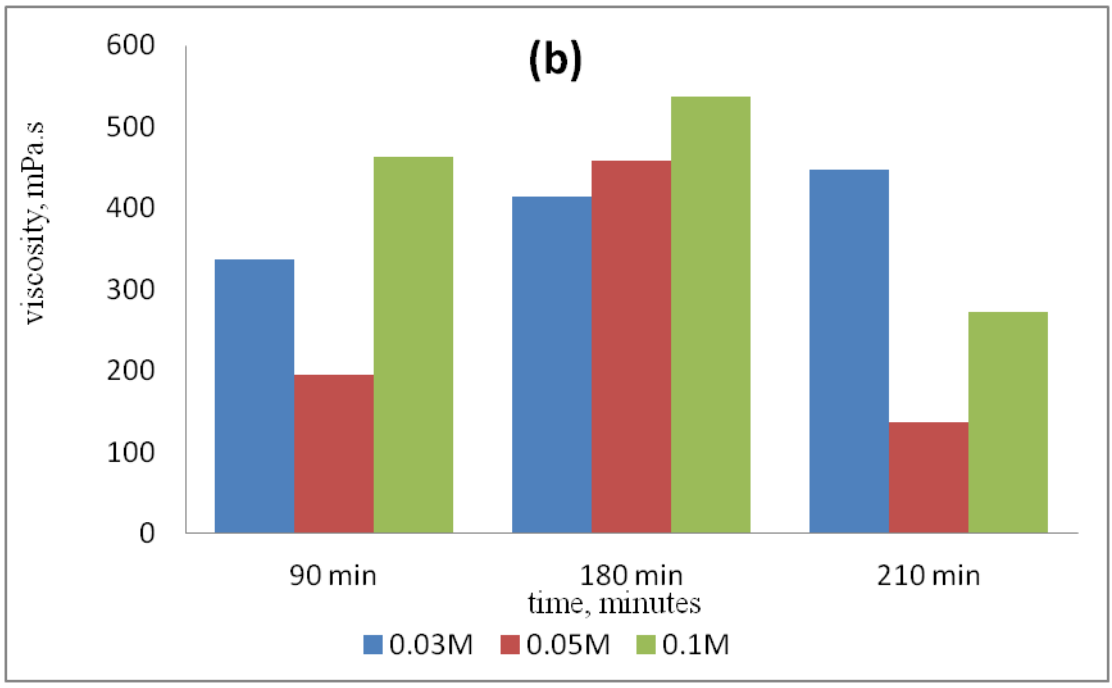
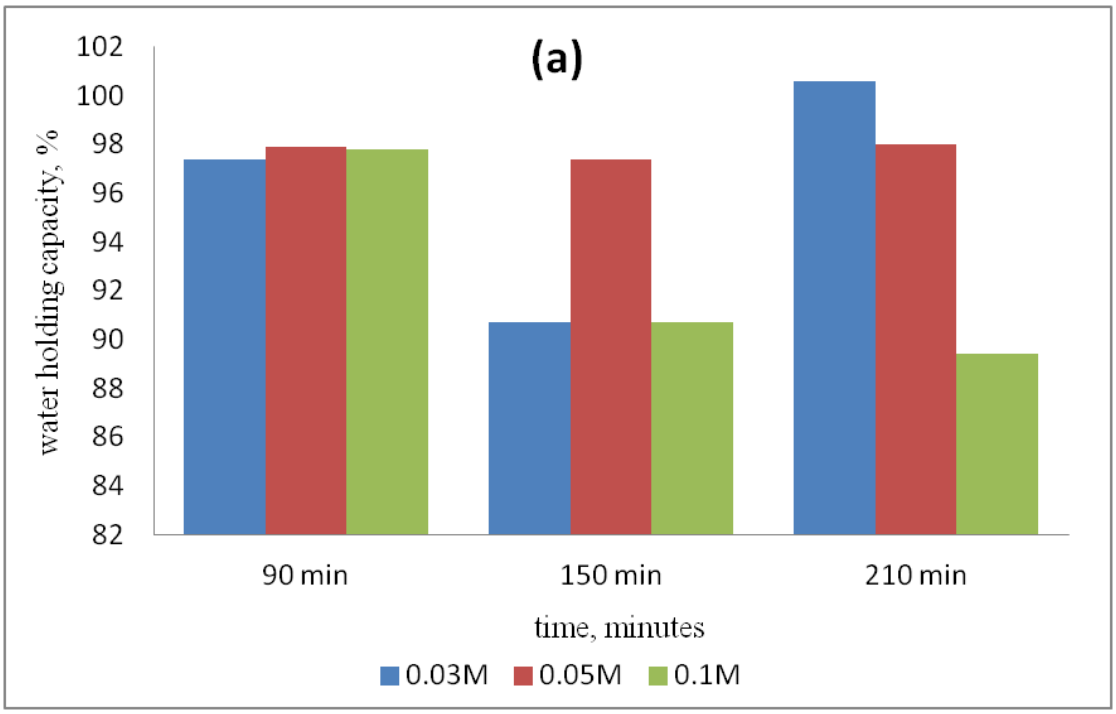


Figure 4-3: Viscosity analysis of kappa carrageenan with different concentration of KOH with different extraction time at different temperature (a) 90°C (b) 100°C

4.3.3 Physicochemical analysis

(a) Water holding capacity

A water holding capacity is the abilities of carrageenan gel to retain its water when external force is applied. This analysis was done at storage temperature of 25°C. From Figure 3, sample A3 (100.60%) has the highest percentage of water holding capacity while sample F3 (82.38%) has the lowest percentage. Most of the sample shows more than 90% of water holding capacity and it shows that the kappa carrageenan has good water holding capacity. This is due to the double helical structure of carrageenan gel holds the water molecules firmer within the interstices of the three dimensional framework. This similar result was also found by Chan et al. (2013).



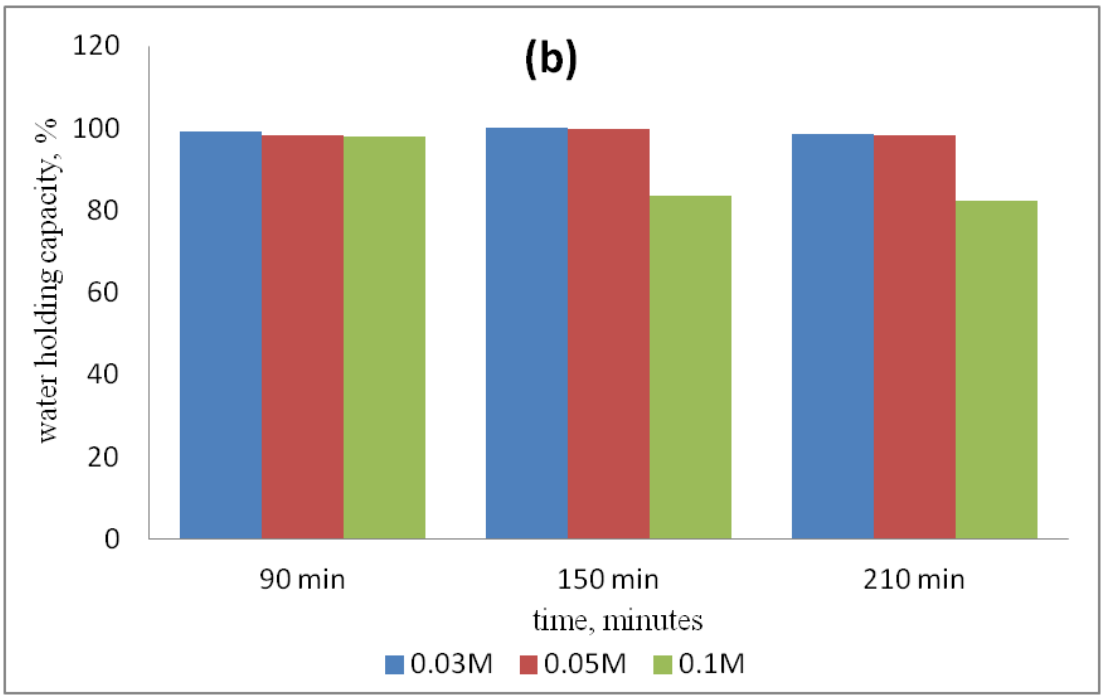
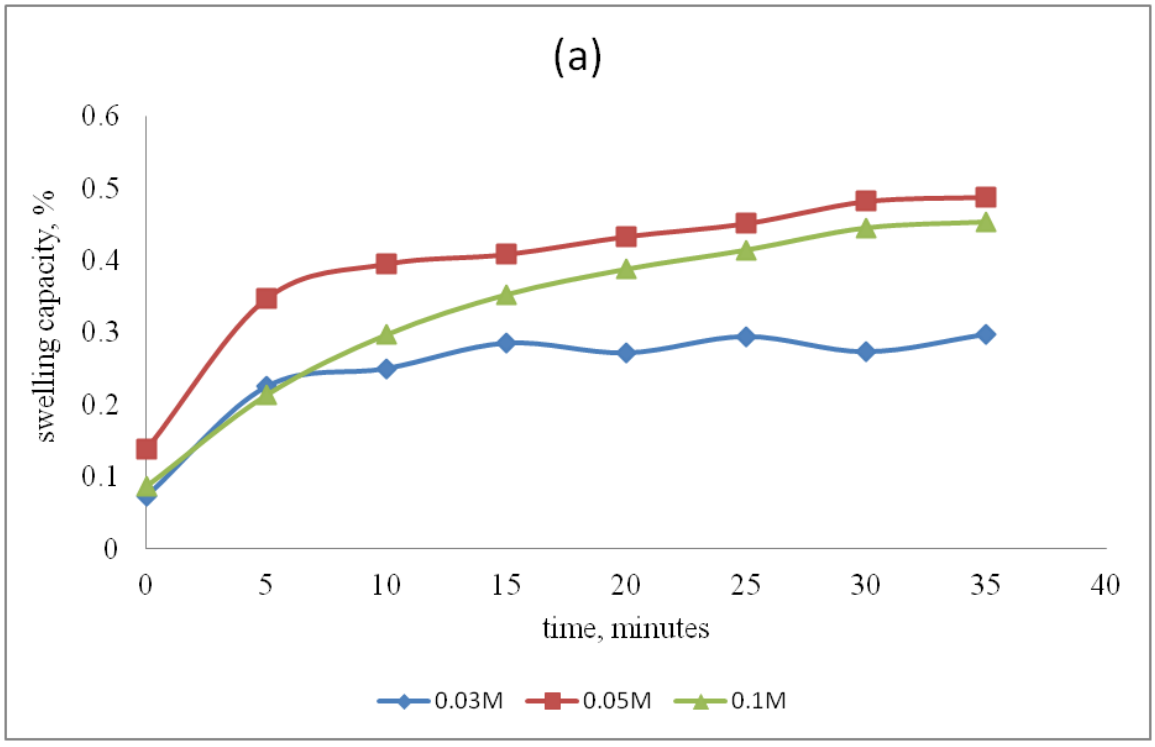
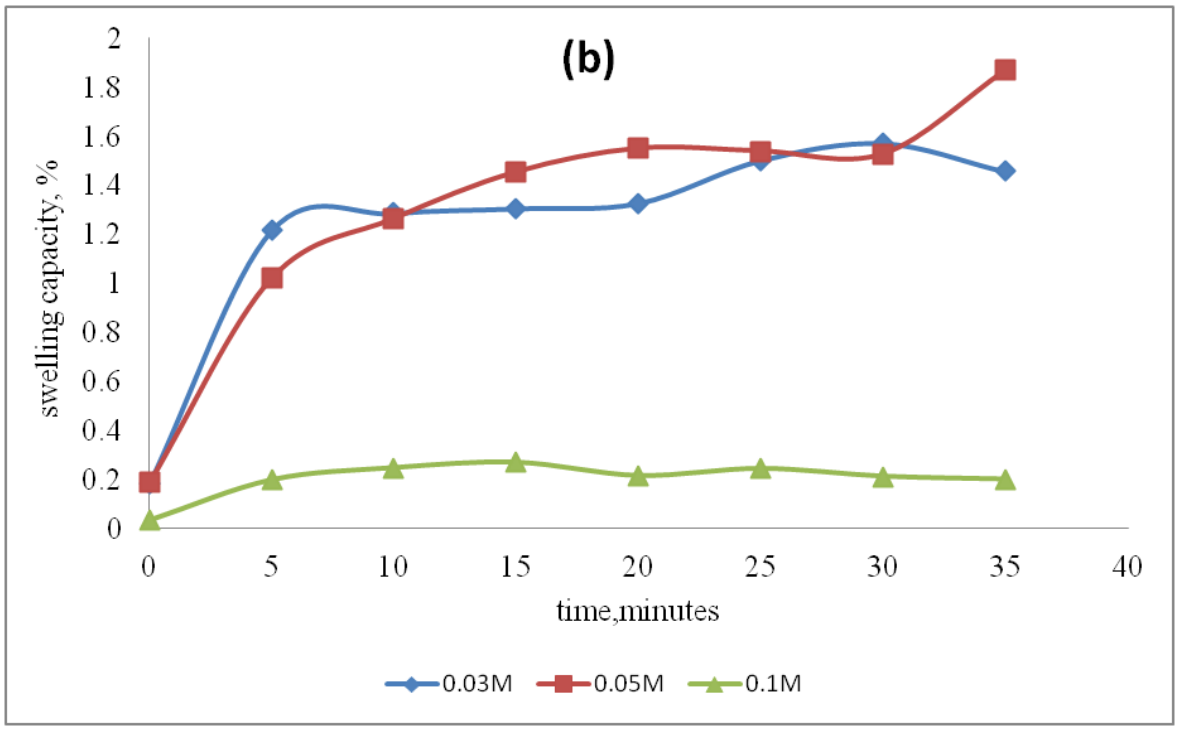


Figure 4-4: Water holding capacity analysis of kappa carrageenan with different concentration of KOH with different extraction time at different temperature (a) 90°C (b) 100°C

(b) Swelling analysis

Swelling rate in all medium initially was high as it is control by ionic chemical potential difference. In acidic medium (pH 1), highest swelling percentage belong to sample E1 that swelled up to 4.25 % and followed by sample A1 that swelled up to 3.09 %. The results shows that protonation of carboxylic group lead to the formation of hydrogen bond that decreases the rate of swelling. In neutral medium (pH 7), sample exhibits larger swelling increment than acidic medium. Sample C1 has largest swelling increment followed by sample A1 that swelled 6.34 %. In alkaline medium (pH 13), all of the sample exhibit more swelling than acidic and neutral medium due to the ionization of carboxylic acid group that breaks the hydrogen bonds. This cause the structure of kappa carrageenan to expand and more water was allowed to diffuse in. This similar finding was also found by Hezaveh and Muhamad (2013). Sample C1 has the highest swelling increment at 10.86% while sample A1 at 7.29 % and sample E1 at 6.47 %. Swelling test for different sample (different time and different temperature) shows the same result as this analysis and the result is shown in appendices.





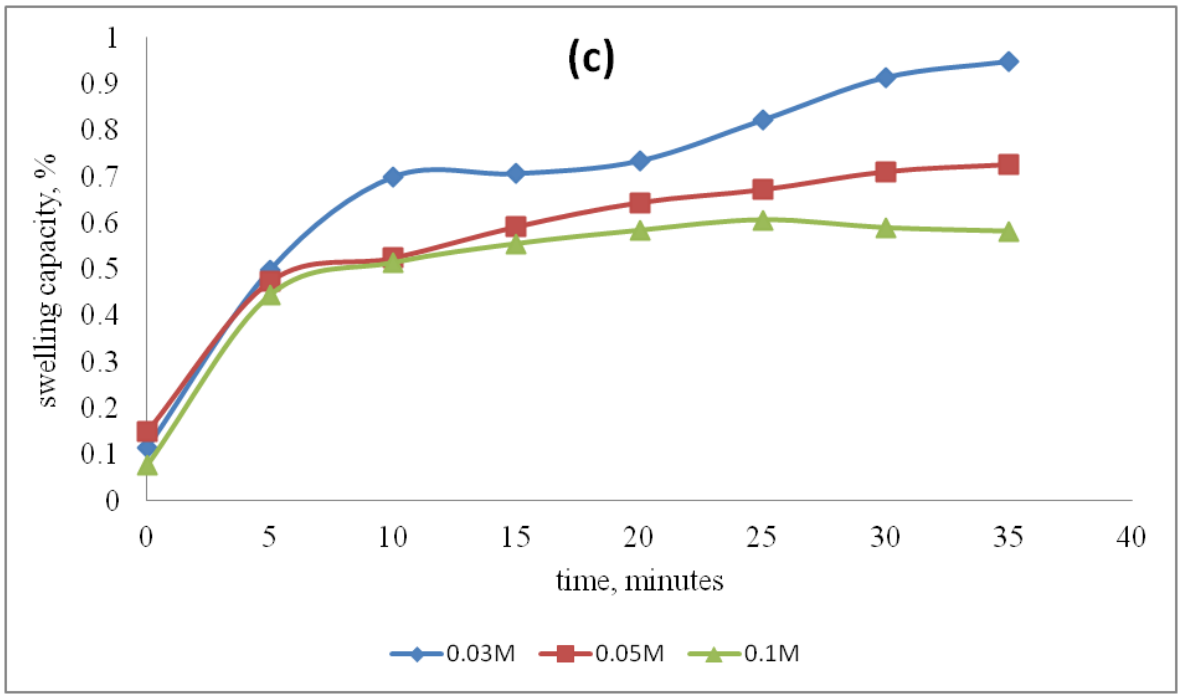


Figure 4-5: Swelling analysis of kappa carrageenan with different concentration of KOH at extraction time of 90 minutes at temperature 90 °C at different (a) pH1, (b) pH7 and (c) pH13

5 CONCLUSION

5.1 Conclusion

Extraction and isolation of kappa carrageenan has been successfully conducted for this experiment. From this experiment, it was found that alkaline extraction caused degradation of polysaccharides chain. Higher concentration of KOH will lead towards the lower yield and lower viscosity of extracted kappa carrageenan. In this research, temperature and time gave insignificant affect in yield, rheological and physicochemical characteristic as there only small difference between the temperature applied and it only affect the ratio of k monomer polysaccharide.

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6 APPENDIX A

Figure 6-1: Raw seaweed

Figure 6-2: Extraction process for concentration 0.03M

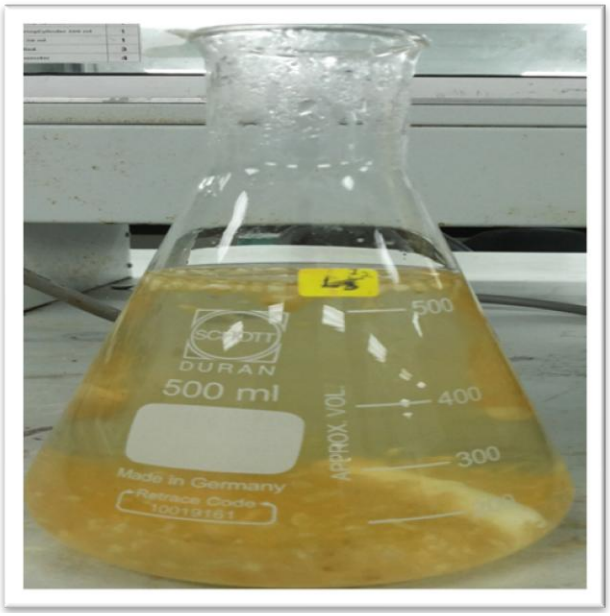

Figure 6-3: Extraction process for 0.1M

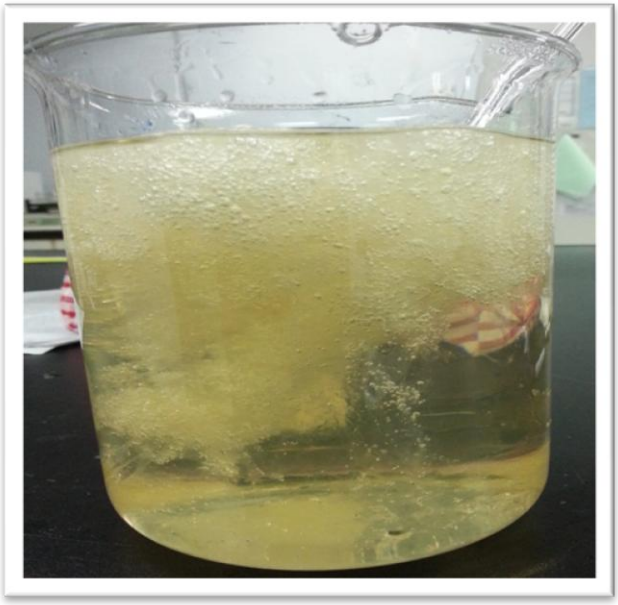

Figure 6-4: Alcohol precipitation for 0.03M

Figure 6-5: Alcohol precipitation for 0.1M

Figure 6-6: Kappa carrageenan that is filtrate from extracted solution

Figure 6-7: Isolated kappa carrageenan

Figure 6-8: Seaweed residue

Figure 6-9: Dry kappa carrageenan after extracted and isolated from the process

Table 6-1: List of samples

Concentration (M)	Temperature, ºC	Time	Labels/Symbol
0.03	90	1h 30 min	A1
		2h 30 min	A2
		3h 30 min	A3
	100	1h 30 min	B1
		2h 30 min	B2
		3h 30 min	B3
0.05	90	1h 30 min	C1
		2h 30 min	C2
		3h 30 min	C3
	100	1h 30 min	D1
		2h 30 min	D2
		3h 30 min	D3
0.1	90	1h 30 min	E1
		2h 30 min	E2
		3h 30 min	E3
	100	1h 30 min	F1
		2h 30 min	F2
		3h 30 min	F3

Table 6-2: Raw data for yield analysis

Sample	Dry weight carrageenan	Yield ,%
A1	5.22	52.2
A2	4.50	45.0
A3	2.47	24.7
B1	4.81	48.1
B2	4.39	43.9
B3	3.34	33.4
C1	4.43	44.3
C2	3.30	33.0
C3	4.52	45.2
D1	5.27	52.7
D2	3.94	39.4
D3	5.46	54.6
E1	5.13	51.3
E2	6.72	67.2
E3	5.70	57.0
F1	4.34	43.4
F2	7.93	79.3
F3	9.45	94.5

Temperature, ºC	Concentration, M	1h 30min	2h 30 min	3h 30 min
90	0.03	52.2	45.0	24.7
	0.05	44.3	33.0	45.2
	0.1	51.3	67.2	57.0
100	0.03	48.1	33.4	33.4
	0.05	52.0	39.4	54.6
	0.1	57.0	79.3	94.5

Table 6-3: Raw data for viscosity analysis

Labels/Symbol	Viscosity, mPa.s
A1	2247
A2	192.0
A3	837.8
B1	336.3
B2	413.7
B3	447.5
C1	400.7
C2	463.7
C3	470.3
D1	195.4
D2	460.1
D3	136.8
E1	338.5
E2	257.4
E3	267.9
F1	463.7
F2	537.7
F3	245.0

Temperature, ºC	Concentration, M	1h 30min	2h 30 min	3h 30 min
90	0.03	2247	192	837.8
	0.05	400.7	463.7	470.3
	0.1	338.5	567.2	267.9
100	0.03	336.3	413.7	447.5
	0.05	390.3	460.1	136.8
	0.1	463.7	537.7	1245.0

Table 6-4: Raw data for water holding capacity analysis

Sample	Initial weight of gel, Wo	Weight of gel after centrifugation, Wt	Water holding capacity (%)
A1	17.05	16.60	97.36
A2	16.23	14.72	90.69
A3	16.55	16.65	100.60
B1	16.67	16.54	99.29
B2	16.82	16.86	100.24
B3	16.83	16.59	98.57
C1	17.22	16.86	97.91
C2	13.82	13.46	97.40
C3	17.09	16.75	98.01
D1	12.21	12.02	98.40
D2	13.52	13.50	99.88
D3	14.84	14.60	98.36
E1	14.86	14.54	97.78
E2	14.98	13.59	90.72
E3	11.78	10.53	89.39
F1	18.02	17.64	97.89
F2	14.43	12.07	83.65
F3	16.52	13.61	82.38

Temperature, ºC	Concentration, M	1h 30min	2h 30 min	3h 30 min
90	0.03	97.36	90.69	100.60
	0.05	97.91	97.40	98.01
	0.1	97.78	90.72	89.39
100	0.03	99.29	100.24	98.57
	0.05	98.40	99.88	98.36
	0.1	97.89	83.65	82.38

Table 6-5: Raw data for swelling analysis

Sample	time, min	pH1	pH7	pH13
A1	0	0.0727	0.1854	0.1143
	5	0.2250	1.2172	0.4992
	10	0.2500	1.2867	0.6991
	15	0.2853	1.3059	0.7059
	20	0.2717	1.3278	0.7336
	25	0.2944	1.5025	0.8217
	30	0.2733	1.5721	0.9136
	35	0.2974	0.2487	0.9481
A2	0	0.1669	0.1166	0.1192
	5	0.3097	1.1326	0.2930
	10	0.3552	3.1366	0.3901
	15	0.3585	3.3271	0.3802
	20	0.3643	3.1825	0.3945
	25	0.3841	3.1410	0.4081
	30	0.3667	3.2776	0.3940
	35	0.3663	3.1170	0.4035
A3	0	0.0477	0.1580	0.0372
	5	0.1693	0.1750	0.1936
	10	0.1948	0.1956	0.2404
	15	0.2056	0.2028	0.2653
	20	0.2203	0.2032	0.2736
	25	0.2340	0.2067	0.2796
	30	0.2400	0.2114	0.2821
	35	0.2408	0.1926	0.2884
B1	0	0.0770	0.0955	0.0724
	5	0.2427	0.7166	0.2248
	10	0.2520	1.0097	0.2451
	15	0.2676	1.1159	0.2614
	20	0.2851	1.1565	0.3242
	25	0.2980	1.2251	0.3881
	30	0.2987	1.2467	0.3559
	35	0.3104	1.2936	0.3797
B2	0	0.0535	0.1323	0.0822
	5	0.1904	0.8311	0.3503
	10	0.2010	1.311	0.3763
	15	0.2239	1.1690	0.3869
	20	0.2341	1.3498	0.4152
	25	0.2500	1.3432	0.4496
	30	0.2579	1.4027	0.4344
	35	0.2644	1.4148	0.4173
B3	0	0.0395	0.1128	0.0432
	5	0.01686	0.9666	0.3930
	10	0.1766	1.1643	0.3987
	15	0.1933	1.2853	0.4132
	20	0.2023	1.3268	0.4224
	25	0.2133	1.5519	04817

	30	0.2242	1.4441	0.4412
	35	0.2340	1.5910	0.3886
C1	0	0.1382	0.1866	0.1491
	5	0.3474	1.0204	0.4732
	10	0.3954	1.2635	0.5235
	15	0.4087	1.4536	0.5912
	20	0.4335	1.5496	0.6433
	25	0.4515	1.5385	0.6712
	30	0.4822	1.5273	0.7101
	35	0.4882	1.8680	0.7254
C2	0	0.0954	0.0796	0.0486
	5	0.3508	0.4270	0.2979
	10	0.4152	0.5032	0.3240
	15	0.4535	0.5810	0.3547
	20	0.4832	0.6388	0.3733
	25	0.5093	0.6958	0.3855
	30	0.5291	0.6816	0.4041
	35	0.5455	0.7092	0.4090
C3	0	0.1458	0.1081	0.1207
	5	0.4552	0.5138	0.4606
	10	0.5496	0.6158	0.5065
	15	0.5826	0.7089	0.5501
	20	0.6069	0.7695	0.5693
	25	0.6369	0.8087	0.5861
	30	0.6691	0.8419	0.5975
	35	0.6867	0.8805	0.5985
D1	0	0.17440	0.1987	0.0709
	5	0.3414	0.5909	0.2470
	10	0.3994	0.7236	0.2875
	15	0.4553	0.9119	0.3282
	20	0.4956	0.9510	0.3593
	25	0.5731	1.0520	0.3815
	30	0.5965	1.2548	0.4161
	35	0.5875	1.2851	0.4261
D2	0	0.0689	0.0667	0.0624
	5	0.1990	0.3052	0.2281
	10	0.2488	0.3925	0.2636
	15	0.2900	0.4531	0.2492
	20	0.3209	0.4887	0.3163
	25	0.3354	0.5220	0.3265
	30	0.3427	0.5414	0.3397
	35	0.3547	0.5613	0.3494
D3	0	0.0672	0.0695	0.0616
	5	0.1239	0.1767	0.1585
	10	0.1530	0.2262	0.1853
	15	0.1679	0.2751	0.2022
	20	0.1798	0.3044	0.2283
	25	0.1950	0.3389	0.2364
	30	0.2032	0.3670	0.2402

	35	0.2139	0.3760	0.2624
E1	0	0.0864	0.0322	0.0779
	5	0.2131	0.1975	0.4450
	10	0.2969	0.2467	0.5144
	15	0.3526	0.2685	0.5551
	20	0.3880	0.2145	0.5839
	25	0.4146	0.2436	0.6065
	30	0.4454	0.2117	0.5894
	35	0.4536	0.2001	0.5819
E2	0	0.0365	0.0688	0.0826
	5	0.0900	0.2585	0.2815
	10	0.0908	0.3062	0.3232
	15	0.0990	0.3135	0.3421
	20	0.1041	0.3349	0.3592
	25	0.1009	0.3539	0.3527
	30	0.1055	0.3592	0.3712
	35	0.1079	0.3653	0.3753
E3	0	0.2028	0.1454	0.1528
	5	0.4166	0.5630	0.5278
	10	0.4483	0.6236	0.6306
	15	0.4775	0.6768	0.6682
	20	0.4968	0.7030	0.7108
	25	0.5108	0.7423	0.7219
	30	0.5253	0.7454	0.7486
	35	0.5345	0.7732	0.7726
F1	0	0.1056	0.0939	0.0540
	5	0.3337	0.4814	0.2802
	10	0.3727	0.5779	0.3084
	15	0.4071	0.6474	0.3191
	20	0.4434	0.6950	0.3260
	25	0.4650	0.7445	0.3367
	30	0.4691	0.7609	0.3485
	35	0.4831	0.8112	0.3520
F2	0	0.1714	0.1706	0.1057
	5	0.3032	0.4963	0.2395
	10	0.3473	0.6164	0.2565
	15	0.3989	0.6690	0.2673
	20	0.4454	0.6640	0.2753
	25	0.4903	0.7961	0.2841
	30	0.5072	0.8212	0.2871
	35	0.5613	0.8823	0.3015
F3	0	0.0866	0.0971	0.0614
	5	0.1546	0.2256	0.1299
	10	0.1752	0.2843	0.1455
	15	0.1865	0.3270	0.1584
	20	0.1974	0.3570	0.1685
	25	0.2159	0.3899	0.1731
	30	0.2213	0.3972	0.1809
	35	0.2289	0.4362	0.1888

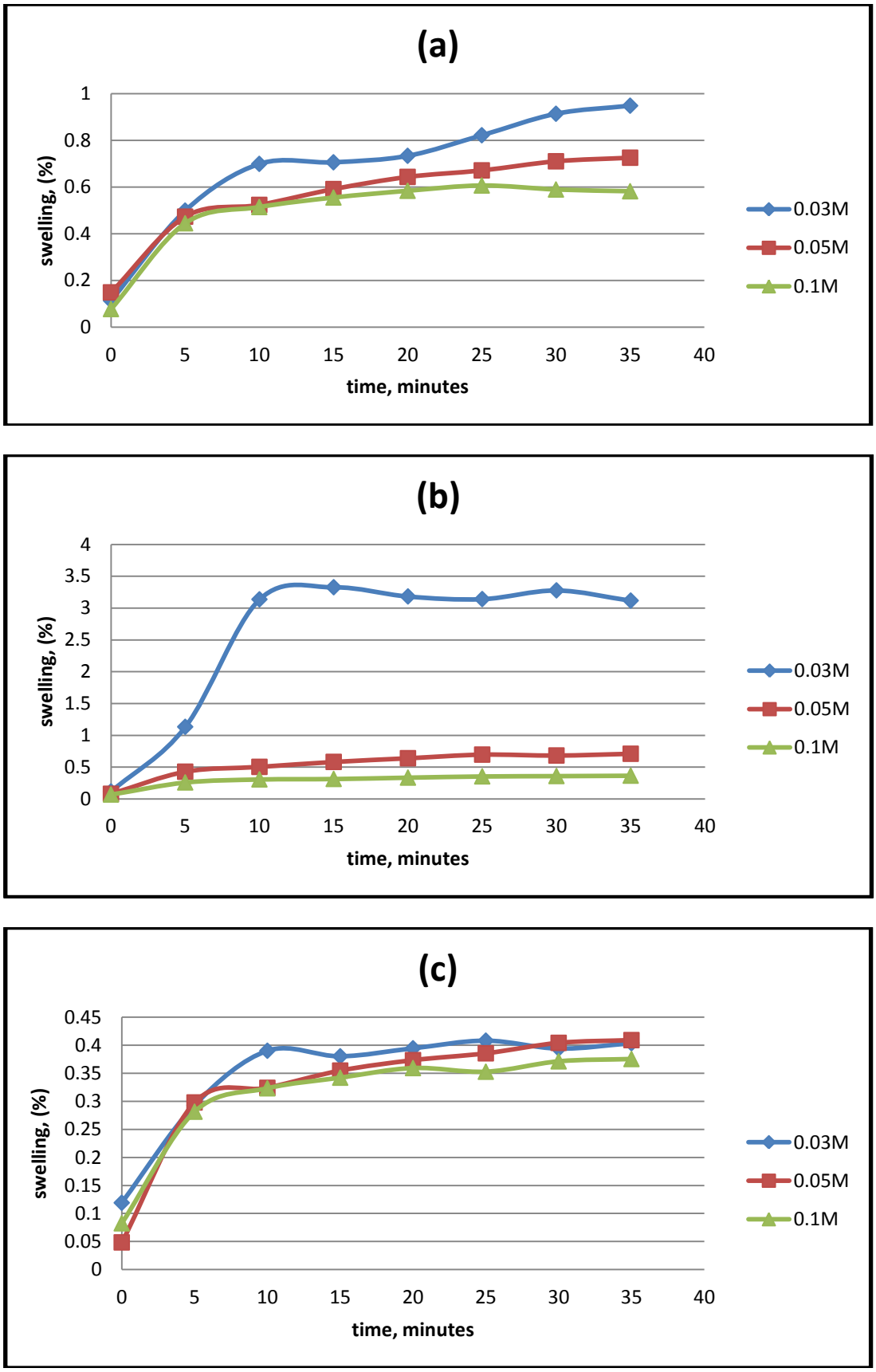

Figure 6-10: Swelling analysis of kappa carrageenan with different concentration of KOH at extraction time of 150 minutes at temperature 90 °C at different (a)pH1, (b) pH7 and (c) pH13

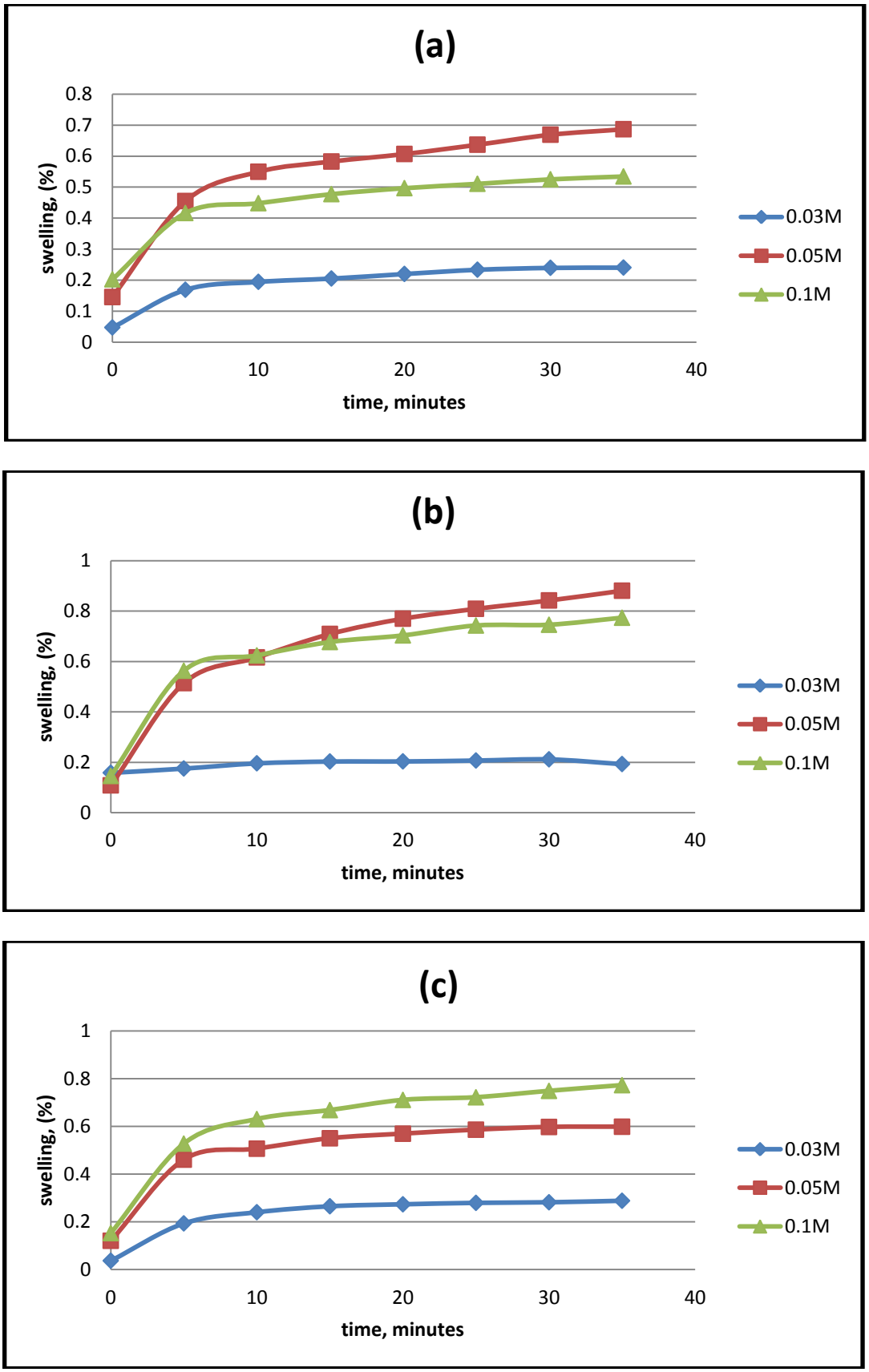

Figure 6-11: Swelling analysis of kappa carrageenan with different concentration of KOH at extraction time of 210 minutes at temperature 90 °C at different (a)pH1, (b) pH7 and (c) pH13

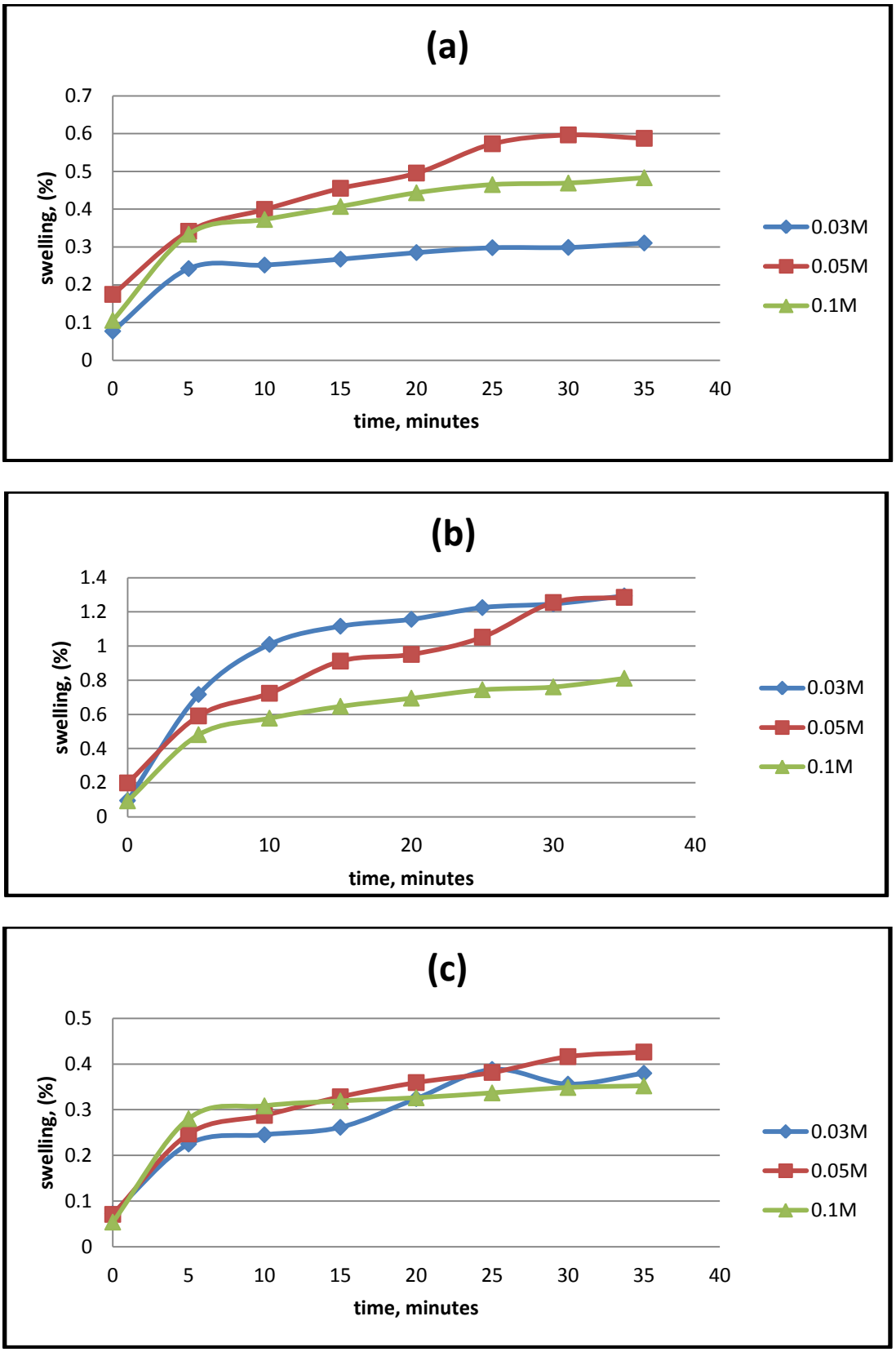

Figure 6-12: Swelling analysis of kappa carrageenan with different concentration of KOH at extraction time of 90 minutes at temperature 100 °C at different (a)pH1, (b) pH7 and (c) pH13

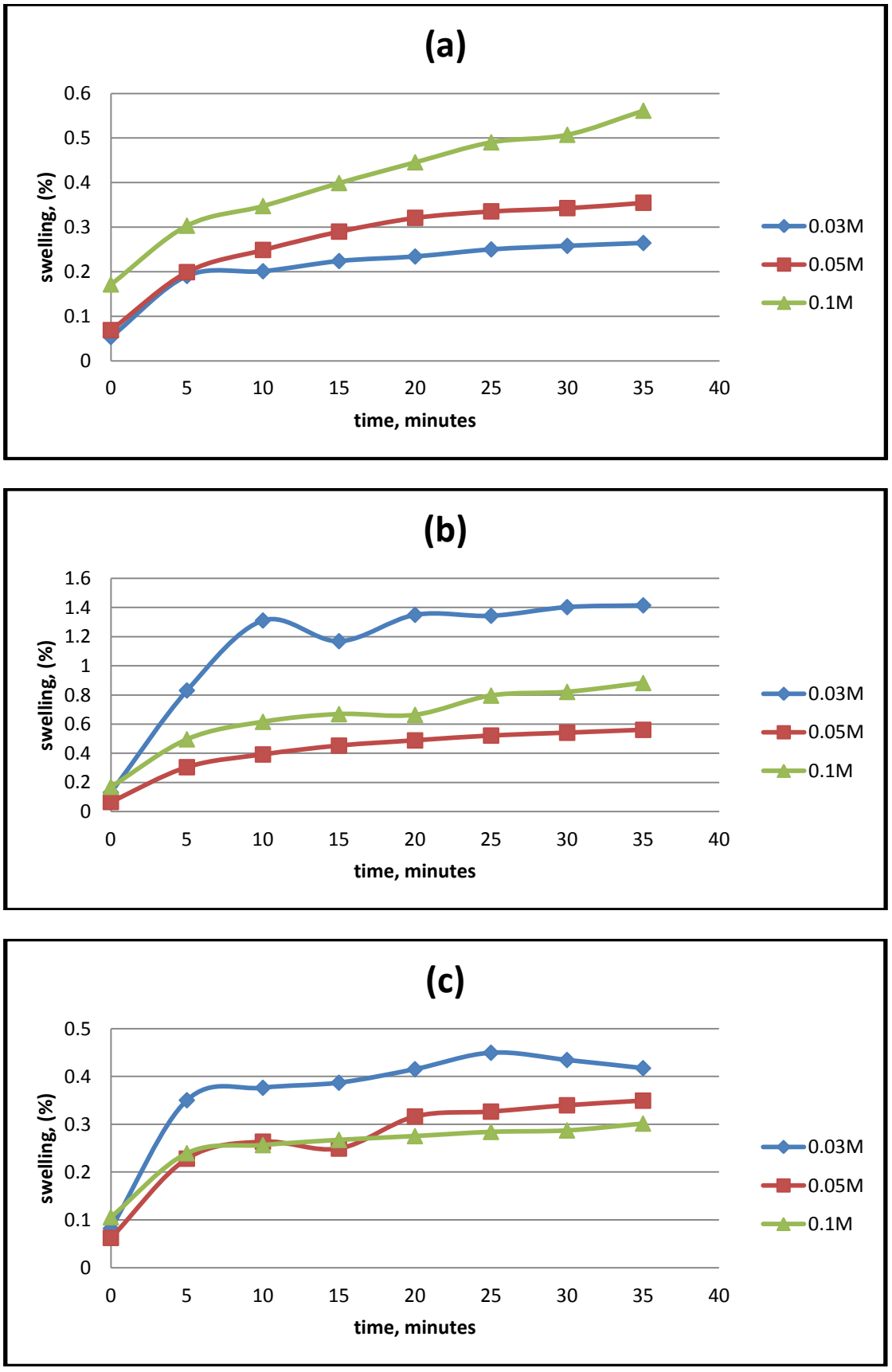

Figure 6-13: Swelling analysis of kappa carrageenan with different concentration of KOH at extraction time of 150 minutes at temperature 100 °C at different (a)pH1, (b) pH7 and (c) pH13

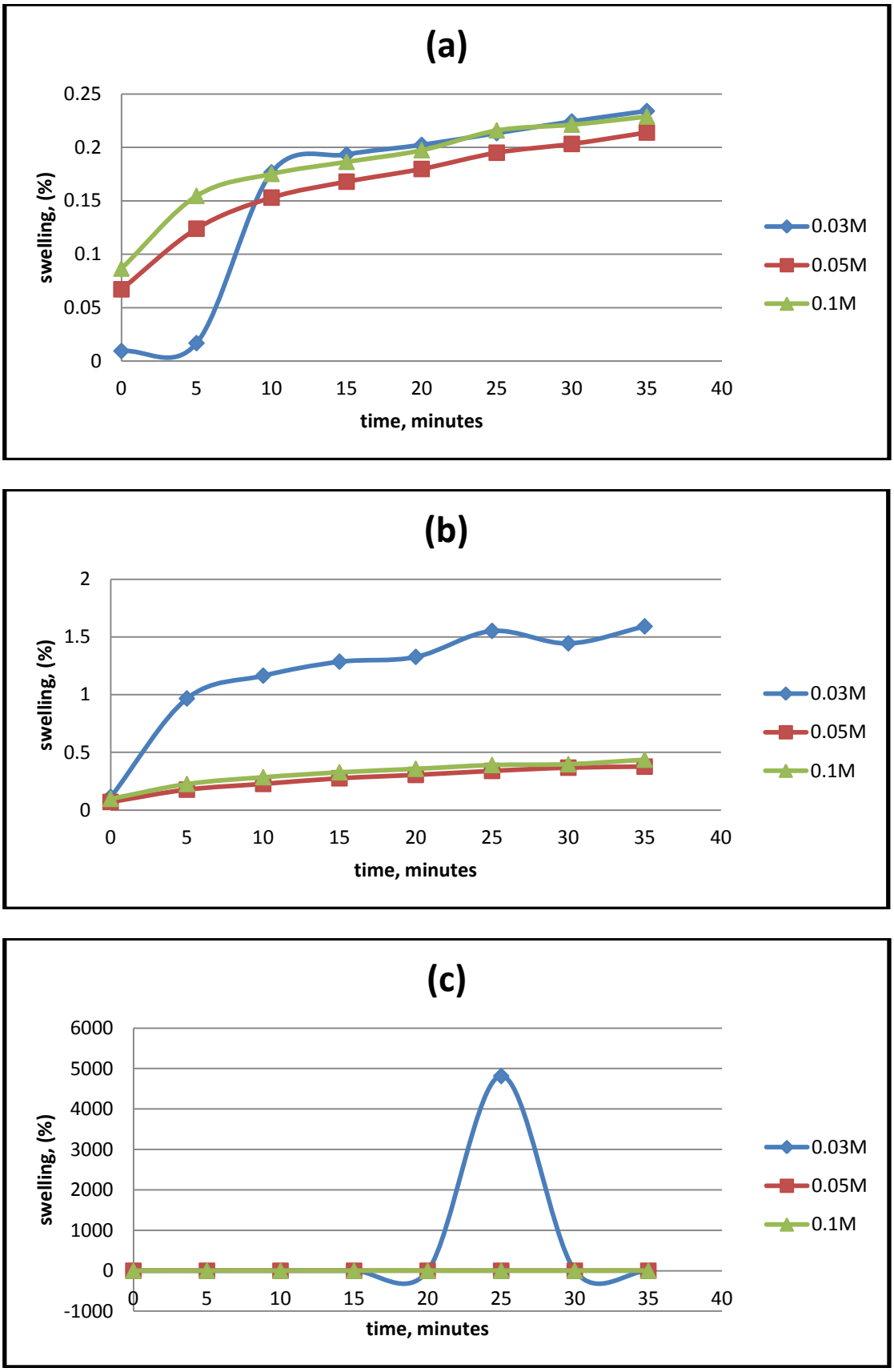

Figure 6-14: Swelling analysis of kappa carrageenan with different concentration of KOH at extraction time of 210 minutes at temperature 100 °C at different (a)pH1, (b) pH7 and (c) pH13