MICROPROPAGATION, ACCLIMATIZATION AND CHEMICAL COMPOSITION OF FARMED SEAWEED, *KAPPAPHYCUS ALVAREZII*, IN SABAH

YONG YOONG SOON

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH

2014

MICROPROPAGATION, ACCLIMATIZATION AND CHEMICAL COMPOSITION OF FARMED SEAWEED, *KAPPAPHYCUS ALVAREZII*, IN SABAH

YONG YOONG SOON

THESIS SUBMITTED IN FULLFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH

2014

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL : ____
____
____

IJAZAH : ____
____

SAYA : ___________________________________ **SESI PENGAJIAN** : _______________________________
(HURUF BESAR)

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

☐ SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

☐ TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☐ TIDAK TERHAD

Disahkan oleh:

___________________ ___________________
(TANDATANGAN PENULIS) (TANDATANGAN PUSTAKAWAN)

Alamat Tetap: ________________
____
____
____

____
(NAMA PENYELIA)

TARIKH: _____________ TARIKH: _______________

Catatan:
*Potong yang tidak berkenaan.
*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that the material in this thesis in my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

16 July 2014

Yong Yoong Soon

PB20118061

CERTIFICATION

NAME : **YONG YOONG SOON**

MATRIC NO. : **PB20118061**

TITLE : **MICROPROPAGATION, ACCLIMATIZATION AND CHEMICAL COMPOSITION OF FARMED SEAWEED, *KAPPAPHYCUS ALVAREZII*, IN SABAH**

DEGREE : **MASTER OF SCIENCES (BIOTECHNOLOGY)**

VIVA DATE : **8th JULY 2014**

DECLARED BY

1. MAIN SUPERVISOR
Mr. Wilson Yong Thau Lym Signature

____

2. CO-SUPERVISOR
Prof. Datin Seri Panglima Dr. Ann Anton

____

ACKNOWLEDGEMENT

First of all, I would like to dedicate my appreciation and thanks to both of my supervisor, Mr. Wilson Yong Thau Lym and my co-supervisor, Prof. Datin Seri Panglima Dr. Ann Anton for their guidance, supervision, dedicated efforts and patience throughout this project.

Appreciation is also dedicated to the staffs of the Plant In Vitro Laboratory, Biotechnology Research Institute, where have assisted me towards the completion of this research.

I am also deeply grateful to the postgraduate students of Biotechnology Research Institute for their support and help throughout the past few years.

Last but not least, I wish to give my greatest appreciation to my beloved family for their love and constant moral support.

ABSTRACT

Under the Economic Transformation Programme (ETP), seaweed production in Malaysia is expected to increase to 150,000 metric tonnes annually by 2020. To achieve this goal, micropropagation and subsequent acclimatization of the micropropagated *Kappaphycus alvarezii* prior transplantation to the sea farm is an option to solve the shortage of seedlings. In a series of micropropagation experiments, the highest growth rate was achieved when the *K. alvarezii* micropropagagules were exposed to a salinity of 30 ± 5 ppt, pH of 8.5 ± 1, and 30 mg/L of Natural Seaweed Extract (NSE). In order to achieve the highest survival rate during transplantation, a study was carried out to determine the optimum conditions for acclimatization of micropropagated *K. alvarezii* seedlings prior to transfer to the sea farm. In a two-week acclimatization study, *K. alvarezii* showed the highest growth rate when cultured in seawater enriched with NSE in outdoor nursery tank with complete replacement of culture media daily and 0.40 g/L of culture density. Two batches of acclimatized *K. alvarezii* were transferred to the sea farm for four weeks and their carrageenan quality, physiochemical profile, minerals and trace elements, and fatty acid compositions were chemically analyzed upon harvesting. As compared to the farm propagated *K. alvarezii*, micropropagated *K. alvarezii* showed a significantly higher yield and viscosity of native carrageenan with similar sulphate content profile. Physiochemical profile revealed that both *K. alvarezii* consists of high ash content, low total protein, and low total lipids. The micropropagated *K. alvarezii* showed higher compositions of minerals and trace elements, and lower heavy metal contaminants, as compared to farm propagated *K. alvarezii*. These studies justify the rational and importance of micropropagation and acclimatization in the production of *K. alvarezii* seedlings.

ABSTRAK

Dalam Program Transformasi Ekonomi (ETP), pengeluaran rumpai laut di Malaysia dijangka akan meningkat kepada 150,000 tan metric setahun menjelang tahun 2020. Untuk mencapai matlamat ini, kaedah mikropropagasi diikuti dengan aklimatisasi benih *Kappaphycus alvarezii* sebelum dipindahkan ke ladang adalah salah satu penyelesaian masalah kekurangan benih. Dalam satu siri kajian pengoptimuman mikropropagasi, benih *K. alvarezii* didapati tumbuh dengan subur pada kemasinan 30 ± 5 ppt, pH 8.5 ± 1, dan 30 mg/L NSE (Natural Seaweed Extract). Dalam usaha untuk mencapai kadar hidup tertinggi semasa pemindahan, satu kajian telah dijalankan untuk menentukan keadaan optimum untuk peringkat aklimatisasi sebelum memindahkan benih *K. alvarezii* ke ladang. Dalam dua minggu kajian peringkat penyesuaian, *K. alvarezii* menunjukkan kadar pertumbuhan tertinggi apabila ditanam menggunakan air laut yang telah diperkaya dengan NSE dalam tangki nurseri terbuka dengan 100% pertukaran media harian dan kepadatan 0.40 g/L. Dua kumpulan benih-benih *K. alvarezii* yang telah menjalani proses aklimatisasi telah dipindahkan ke ladang untuk penanaman dan analisis pascatuai dijalankan untuk kualiti karagenan, ciri-ciri fisiokimia, kandungan mineral serta unsur surih, dan komposisi asid lemak. Berbanding dengan *K. alvarezii* biasa, hasil mikropropagasi menunjukkan komposisi karagenan asli yang lebih tinggi dengan ciri-ciri kelikatan walaupun kandungan sulfat adalah serupa. Hasil analisis fisiokimia juga menunjukkan bahawa kedua-dua jenis *K. alvarezii* mempunyai kandungan abu yang tinggi, jumlah protein serta lemak yang rendah. *K. alvarezii* yang menjalani mikropropagasi menunjukkan komposisi mineral dan unsur surih yang lebih tinggi, serta kandungan pencemaran logam berat yang lebih rendah, berbanding dengan *K. alvarezii* yang tidak melalui proses mikropropagasi. Kajian ini membuktikan kepentingan pendekatan kaedah mikropropagasi dan aklimatisasi dalam meningkatkan penghasilan benih-benih *K. alvarezii*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
=	equal
µmol	micromole
α	alpha
cp	Centipoise
g	gram
ha	hectare
kg	kilogram
L	Liter
M	Molarity
mg	milligram
min	minute
mL	mililiter
mt	Metric tonnes
N	Normality
nm	nanometer
°C	Degree Celcius
AP	Acardian Marine Plant Extract Powder
GF	Gofar600
NSE	Natural Seaweed Extract
PES	Provasoli Enriched Seawater
ppm	Part per million
ppt	Part per thousand
pss	Practical salinity scale
psu	Practical salinity unit
rETR	Relative electron transport rate
sp.	species
ß	beta
t	tonnes
UV	Ultraviolet
UVR	Ultraviolet radiation

vol	Volume
ω	omega
H^+	Hydrogen ion
CO_3^{2-}	Carbonate ion
$CaCO_3$	Calcium carbonate
HCO_3^-	Bicarbonate ion
H_2CO_3	Carbonic acid
CO_2	Carbon dioxide
Ca^{2+}	Calcium ion
H_2O	Water

CHAPTER 1

INTRODUCTION

1.1 Seaweed Industry in Malaysia

The seaweed farming industry continues to expand since it was first introduced by the Aquatic Resources Limited in 1978 in Malaysia, and contributed 207,892.40 metric tonnes (mt) of fresh seaweeds in 2010 (Table 1.1), which was valued at RM 83 million (Sade *et* al., 2006). Starting from the east-coast of Sabah in 1978, this industry is growing fast and now recruiting in the Peninsular Malaysia, where the first seaweed farming site was reported in Perak water and contributed to the seaweed production since 2010 (DOF, 2010). With the increasing market demand for seaweeds and their products, especially carrageenan, the seaweed industry has been identified as the third Entry Point Project (EPP), under the National Key Economic Area (NKEA), since the Malaysia government introduced the Economic Transformation Programme (ETP).

Through its transition from agriculture to agribusiness, the Malaysian government aims to increase the annual production of dry seaweeds from 13,500 mt in 2009 to 150,000 mt in 2020. In order to achieve the target and fulfill the worldwide demand of 7.5-8.0 million mt of biomass from the seaweed processing industry, research and development in the field of biotechnology is necessary. This study focuses on the optimization of abiotic factors during micropropagation of *Kappaphycus alvarezii*, acclimatization through nursery set-up, and chemical profilling of the seaweed components, especially the carrageenan profile.

Table 1.1: Annual fresh seaweed productions

Year	***2006***	***2007***	***2008***	***2009***	***2010***
Area (HA)	5,949.37	6,684.19	7,730.57	7,538.46	7,940.50
Culturists	520	738	950	943	1156
Production (MT)	43,200.00	90,268.50	111,298.20	138,855.90	207,892.40
Value (RM ,000)	6,912.00	18,053.70	22,259.60	27,771.18	83,159.06

Source: Department of Fisheries, Ministry of Agriculture & Agro-Based Industry Malaysia (DOF, 2006-2010)

1.2 Seaweed Micropropagation

Application of *in vitro* technique to micropropagate seaweeds has been introduced in *Chondrus crispus* to produce healthy and rapidly growing seaweed seedlings since 1970s (Chen & Taylor, 1978). However, seaweed micropropagation and seaweed tissue culture are still underdeveloped as compared to plant tissue culture, which has been developed and continued for almost 80 years (Baweja *et al.*, 2009). Research on callus induction and micropropagation have revealed the optimum media, especially the concentration of plant growth regulators and the use of seaweed extracts as fertilizer.

Unlike terrestrial plants, the plant growth regulators for growth improvement which are available in the market are different from seaweed natural produced hormones, such as spermine, spermidine, and polyamines (Baweja *et al.*, 2009). In order to overcome this issue, the application of seaweed extracts as fertilizer will provide most of the essential components for seaweed cultures.

1.3 Significance of study

The seaweed industry worldwide uses 7.5–8.0 million tonnes of wet seaweeds annually with a majority of them derived from cultivated farms, as the demand for seaweed based-products exceeds the supply of seaweed raw material from natural stocks. Most of the seaweeds are cultivated using seedlings produced by segmenting ready-to-sell fresh seaweeds. Through this process, parasites or pathogens from the ready-to-sell fresh seaweeds may be re-introduced, thus reducing the productivity of the farm. In order to increase the productivity, this study proposes to produce high quality and healthy seaweed seedlings using micropropagation techniques.

However, the rationale of applying biotechnology technique for seaweed seedlings production is still unrevealed. The quality of the carrageenan and chemical compositions of the micropropagated seaweeds still remain unknown. By applying micropropagation technique for seaweed production, the uses of plant growth hormones and fertilizers, which are known to be toxic to human health, may remain in the seaweed cultures, thus the seaweed products may become harmful as well. Besides, the carrageenan quality of the micropropagated seaweeds may not achieve market standard. Thus, the study on acclimatization and evaluation of chemical compositions of micropropagated seaweeds is necessary.

1.4 Objectives of Study

This study is divided into three parts. I: optimization of the abiotic factors of micropropagation of *Kappaphycus alvarezii*, viz. the effect of fertilizers, salinity, and pH of the culture medium; II: acclimatization of the micropropagated *K. alvarezii* to the seaweed farm in Semporna, Sabah, through an outdoor nursery; III: comparison of the chemical and physical properties of the extracts of the micropropagated seaweeds with those of the commonly farm-propagated seaweeds.

The specific objectives of this study are:-

a) To optimize the pH, salinity, and addition of fertilizer in culture medium for micropropagation of *K. alvarezii*.
b) To compare different fertilizers, frequencies of media replacement, and densities of culture during acclimatization of the micropropagated *K. alvarezii*.
c) To compare the chemical properties of micropropagated *K. alvarezii* with those of the farm propagated *K. alvarezii*.
d) To determine the added value of micropropagated *K. alvarezii* compared to farm propagated *K. alvarezii* based on the contents of fatty acids and mineral content.

CHAPTER 2

LITERATURE REVIEW

2.1 *Kappaphycus alvarezii*

Kappaphycus alvarezii (Figure 2.1) or formally recognized as *Eucheuma cottonii*, and commercially called as "cottonii", is the most common carrageenophyte cultivated in Malaysia, especially in the east coast of Sabah (McHugh, 2003; Phang, 2006; Sade *et al*., 2006). Classified under the Phylum Rhodophyta; Class Florideophyceae; Order Gigartinales; and Family Solieriaceae, *K. alvarezii* is well known for its high content of kappa-carrageenan, which is in high demand for food production industry (McHugh, 2003; Guiry & Guiry, 2012).

Figure 2.1: *Kappaphycus alvarezii* varieties which are widely cultivated in Semporna, Sabah.

Morphologically similar to other *Kappaphycus* species, *K. alvarezii* exhibits a wide range of gross morphology, colour and general appearance. Their morphology often changes due to their environmental factors (Neish, 2008). Thus, it is hard to distinguish species with reference to their morphological characteristic. *K. alvarezii* is tough, fleshy and firm, and able to grow up to 2 m. The thalli of *K. alvarezii* are course, with axes and branches with 1-2 cm in diameter, with major axes relatively straight, and lacking secondary branches near apices (Patterson-Edward & Bhatt, 2012).

Naturally, *K. alvarezii* is found mostly in the Southeast Asia waters, China, and some in Northern America (Figure 2.2) (Ask & Azazan, 2002; Guiry & Guiry, 2012). However, potential farming sites for *K. alvarezii* cover a much larger area, which lies within ± 10º latitude, notably Southeast Asian countries extending to Brazil and Hawaii (Figure 2.3). Currently, most of the *K. alvarezii* farming sites are found in the Southeast Asian region, primarily Philippines, Indonesia, Malaysia, and Brunei, which make up about 60% of the total farming sites globally (Hayashi *et al*., 2010).

The life cycle of *K. alvarezii* is triphasic (Figure 2.4), which consists of gametophyte (N), tetrasporophyte (2N) and carposporophyte (2N) phases (Azanza-Corrales, 1990; Azanza-Corrales *et al*., 1992; Neish, 2008). Vegetative regeneration of plants will repeat the same reproductive phases. Thus, the ratio of reproductive phase may reflect the original plants introduced in the area (Azanza-Corrales *et al*., 1992). However, the mechanisms and factors favoring spore release are not determined.

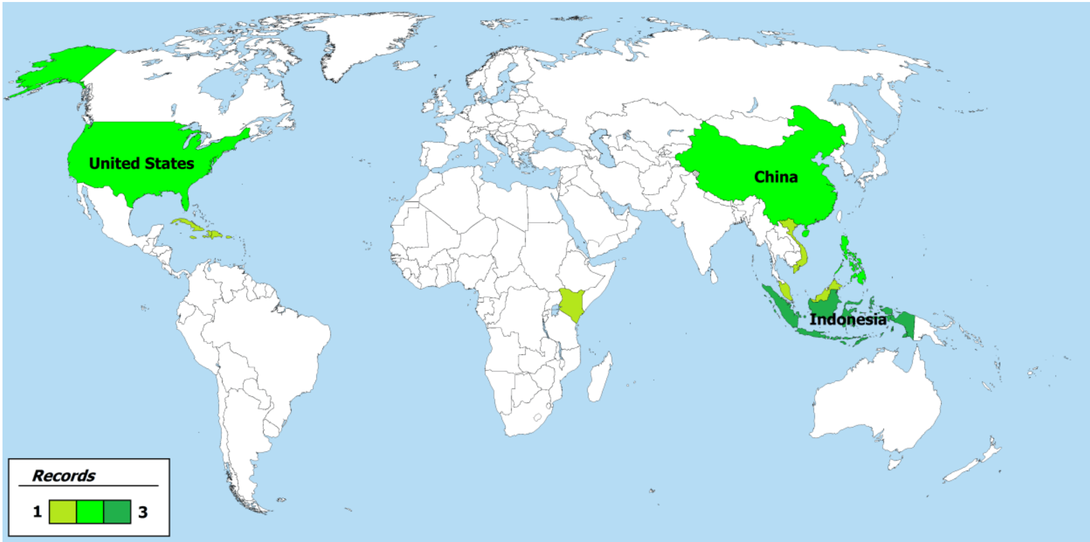

Figure 2.2: Natural distribution of *Kappaphycus alvarezii*, which cover Southest Asia, East Africa, and some of the countries in Northern America.

Source: Guiry and Guiry (2012)

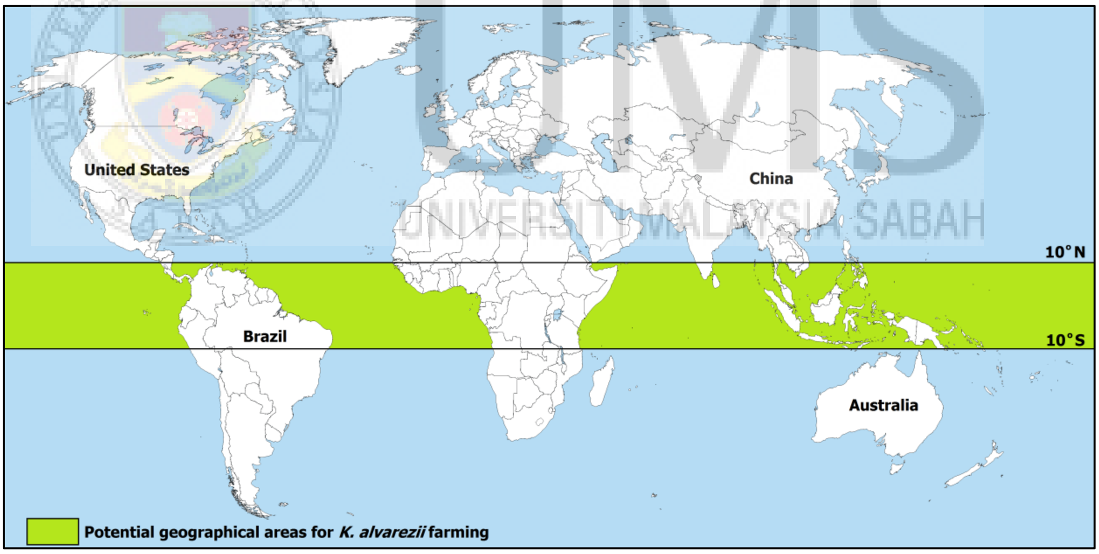

Figure 2.3: Potential global geographical areas for the *Kappaphycus alvarezii* farming.

Source: Hayashi *et al*. (2010)

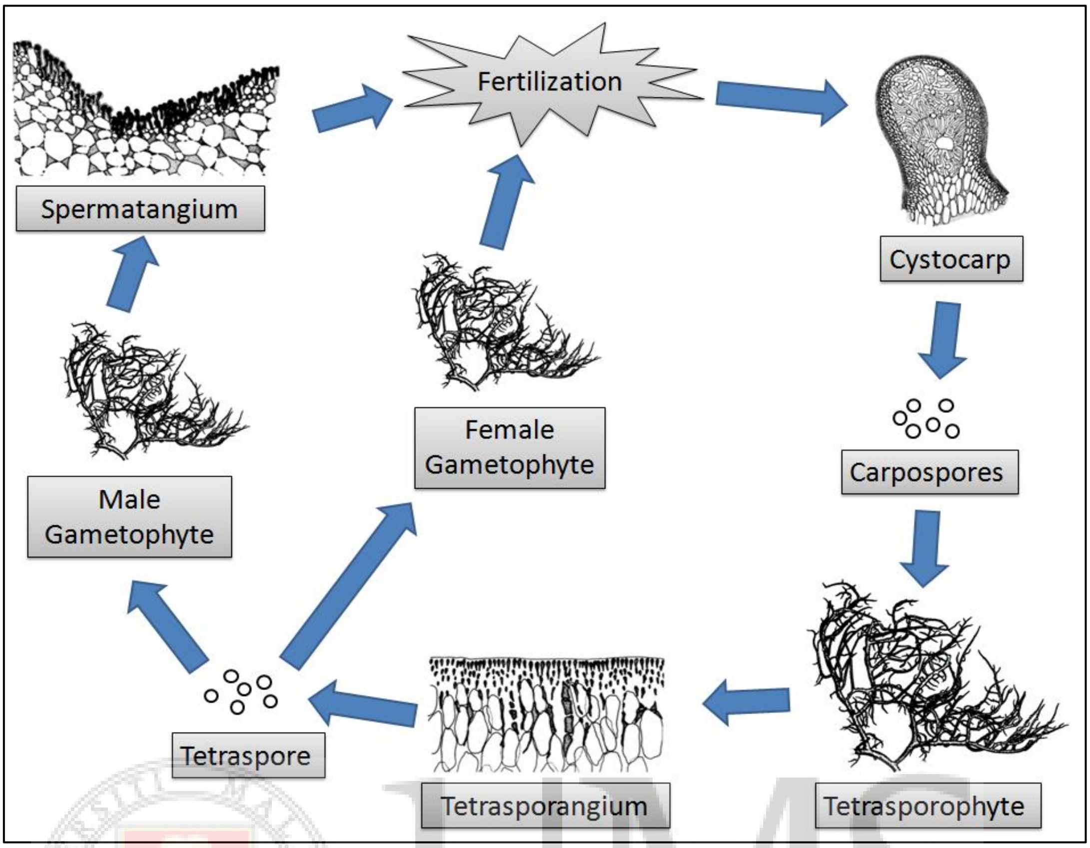

Figure 2.4: Triphasic life cycle of *K. alvarezii*, consisting of gametophyte (N), tetrasporophyte (2N) and carposporophyte (2N) phases.

Source: Neish (2008)

2.2 Carrageenan

The application of carrageenan was first described and applied in Ireland around 1810, when *Chondrus crispus* was boiled with milk to make pudding. The gelling properties of *C. crispus* were utilized for centuries in herbal and pharmaceutical preparation. Since 1862, the term "carrageenin" was given to the extract of *C. crispus*, and was later changed to "carrageenan" by the American Chemical Society to reflect the use of –an as an affix denoting its structure as polysaccharide (Watson, 2007).

In red seaweeds, such as *Kappaphycus, Eucheuma,* and *Chondrus*, carrageenan is part of cell wall component associated with cellulose and xylan (Mabeau & Fleurence, 1993). Carrageenan is a high molecular weight linear polysaccharide made up of repeating galactose units, where 3,6-anhydrogalactose (3,6-AG), both sulphated and nonsulphated, joined by alternating α1-3, β1-4 glycosidic linkages (Flick, 1991). The number and position of the ester sulphate groups on the repeating galactose units determine the properties of the carrageenan; whereby carrageenan containing more sulphate groups has lower gel strength (He *et al.,* 2001; Flick, 1991). Three carrageenans of main commercially interest are iota-, kappa-, and lambda-carrageenan, which can be obtained naturally or through alkaline treatments as illustrated in Figure 2.5 (McHugh, 2003).

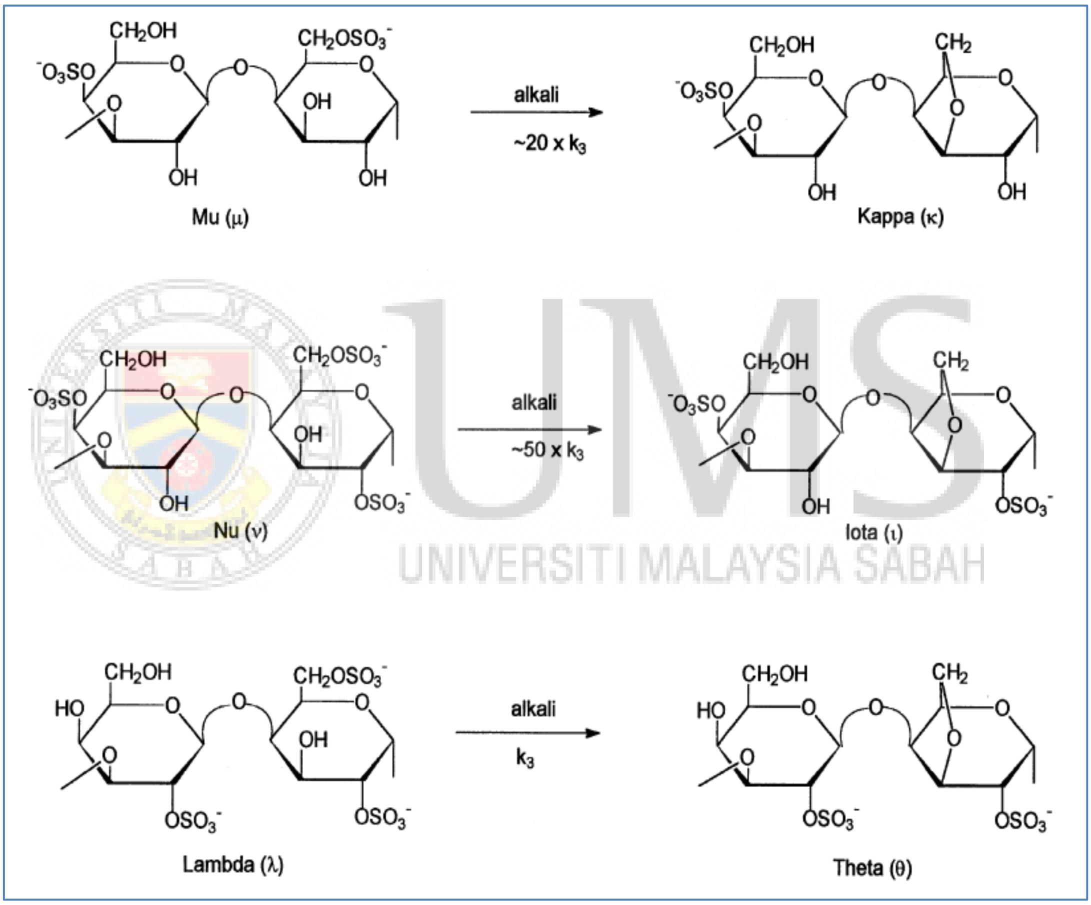

Figure 2.5: Principal carrageenan repeating unit structures showing conversions that occur on alkaline treatment, which is useful in improving the gel qualities.

Source: Falshaw *et al.* (2001)