EFFECT OF DIFFERENT DRYING TECHNIQUES ON NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT AND ACETYLCHOLINESTERASE INHIBITION PROPERTIES OF EDIBLE RED SEAWEED, *Kappahycus alvarezii*

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UNIVERSITI MALAYSIA SABAH
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THESIS SUBMITTED IN FULLFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
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2016

DECLARATION

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

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ABSTRACT

Post-harvest process is an important factor that one must take into consideration in order to ensure that good seaweeds are produced and best quality of seaweeds is maintained as to command high value in the market. However, this post-harvest processing affects the quality such as content and activity of bioactive compounds in the seaweeds. The aim of the present study was to investigate the effect of different drying techniques on phytochemical content, antioxidant and acetylcholinesterase inhibition activity as well as nutritional composition of *Kappaphycus alvarezii*; crocodile and tambalang giant morphotypes. The cleaned seaweeds were divided into seven portions and was dried using seven drying conditions; (1) oven-drying at 40 ºC, (2) oven-drying at 80 ºC, (3) sun-drying, (4) hang-drying, (5) sauna-drying, (6) shade-drying and (7) freeze-drying. First, determination of total phenolic, flavonoid, anthocyanin and carotenoid contents were done spectrophotometrically. Oven-drying at 40 ºC extract of crocodile morphotype contained highest value of total phenolic (53.33 mg GAE/100 g), while the oven-drying at 40 ºC extract of tambalang giant morphotype contained highest values of total flavonoid (34.50 mg CE/100 g), total anthocyanin (1.47 mg C-3-GE/ g) and total carotenoid (0.26 mg BC/ g). Sauna-drying extract of tambalang giant morphotype contained lowest values of total phenolic (20.33 mg GAE/100 g) and total carotenoid (0.02 mg BC/ g), while the sauna-drying extract of crocodile morphotype contained lowest values of total flavonoid (9.83 mg CE/100 g) and total anthocyanin (0.53 mg C-3-GE/ g). Evaluation of antioxidant activity was conducted using DPPH free radical scavenging assay, ferric reducing power (FRAP) and radical scavenging ABTS assays. Oven-drying at 80 ºC extract of crocodile morphotype displayed highest scavenging activity (93.29 %), while the oven-drying at 40 ºC extract of crocodile morphotype displayed strongest reducing ability (7.81 mM Fe^{2+}/ g) and antioxidant capacity (0.54 mg AEAC/ g). Acetylcholinesterase is an enzyme known to breakdown the neurotransmitter acetylcholine which leads to Alzheimer's disease. Acetylcholinesterase inhibition assay was done and the result showed that oven-drying at 40 ºC extracts of both morphotypes displayed highest acetylcholinesterase inhibition activity with the values of 45.03 % (crocodile morphotype) and 24.89 % (tambalang giant morphotype), when tested at 100 mg/ml concentration. However, there was no acetylcholinesterase inhibition activity detected in sauna-drying and freeze-drying extracts in both morphotypes. Based on correlation analysis, all dried samples showed positive correlation between phytochemical content and antioxidant, positive correlation between phytochemical content and acetylcholinesterase inhibition effect and positive correlation between antioxidant and acetylcholinesterase inhibition effect. Proximate analysis test showed that freeze-drying samples of both morphotypes contained lowest amount of ash content, while the others show no significant differences. Shade-drying samples of both morphotypes contained lowest amount of moisture content, while the others show no significant differences. Freeze-drying samples (crocodile and tambalang giant morphotypes) contained highest percentage of crude lipid (1.26 % and 1.21 %), crude fiber (8.28 % and 8.42 %) and crude protein (14.10 % and 14.69 %), while the oven-drying 80 ºC samples (crocodile and tambalang giant morphotype) contained highest percentage of carbohydrate (55.14 % and 54.24 %). Lowest percentage of crude lipid, crude fiber and crude protein were found in

sun-drying and sauna-drying samples of both morphotypes, while carbohydrate in the oven-drying at 40 ºC sample of both morphotypes. Analysis for major and trace mineral elements showed that the oven-drying at 40 ºC samples of both morphotypes contained highest value of Na, Ca, Mg and Mn, while the shade-drying samples of both morphotypes contained highest value of K, Fe and Zn. Lower mineral elements was tested in sun-drying and sauna-drying samples of both morphotypes. Based on the drying techniques studied, oven-drying at 40 ºC is the best drying technique used to retain the phytochemical content, antioxidant and acetylcholinesterase inhibition activity and some mineral compounds in seaweeds, while freeze-drying is the best drying technique used to retain the nutritional content in seaweeds. As a conclusion, post-harvest process such as drying could greatly influence the occurrence of phytochemical content, antioxidant and acetylcholinesterase inhibition activity as well as nutrient content in the dried seaweeds.

ABSTRAK

KESAN TEKNIK PENGERINGAN YANG BERBEZA TERHADAP KOMPOSISI PEMAKANAN DAN FITOKIMIA, AKTIVITI ANTIOKSIDA DAN PERENCATAN ENZIM ASETILKOLINESTERAS DALAM RUMPAI LAUT MERAH, Kappaphycus alvarezii

Proses lepas-tuai adalah faktor penting yang perlu diambil kira untuk memastikan rumpai laut terbaik dihasilkan dan kualiti terbaik rumpai laut dikekalkan untuk menguasai nilai yang tinggi di pasaran. Walau bagaimanapun, pemprosesan lepas-tuai ini memberi kesan kepada kualiti seperti kandungan dan aktiviti sebatian bioaktif dalam rumpai laut. Objektif kajian ini dijalankan adalah untuk mengkaji kesan teknik pengeringan yang berbeza pada kandungan fitokimia, antioksida dan aktiviti perencatan enzim asetilkolinesteras dan juga komposisi pemakanan dalam Kappaphycus alvarezii; 'buaya morphotype' dan 'tambalang giant morphotype'. Rumpai laut yang telah dibersihkan dibahagikan kepada tujuh bahagian dan dikeringkan menggunakan tujuh kaedah pengeringan; (1) pengeringan-ketuhar pada 40 ºC, (2) pengeringan-ketuhar pada 80 ºC, (3) pengeringan-matahari, (4) pengeringan-gantung, (5) pengeringan-sauna, (6) pengeringan-teduh dan (7) pengeringan-sejukbeku. Pertama, penentuan kandungan fenolik, flavonoid, antosianin dan karoten dijalankan menggunakan kaedah spektrofotometrik. Ekstrak pengeringan-ketuhar pada 40 ºC untuk 'buaya morphotype' mengandungi jumlah fenolik yang paling tinggi (53.33 mg GAE/100 g), manakala ekstrak pengeringan-ketuhar pada 40 ºC untuk 'tambalang giant morphotype' mengandungi jumlah flavonoid (34.50 mg CE/100 g), jumlah antosianin (1.47 mg C-3-GE/ g) dan jumlah karoten yang paling tinggi (0.26 mg BC/ g). Ekstrak pengeringan-sauna untuk 'tambalang giant morphotype' mengandungi jumlah fenolik (20.33 mg GAE/100 g) dan jumlah karoten (0.02 mg BC/g) yang paling rendah, manakala ekstrak pengeringan-sauna untuk 'buaya morphotype' mengandungi jumlah flavonoid (9.83 CE mg/100 g) dan jumlah antosianin (0.53 mg C-3-GE/ g) yang paling rendah. Penentuan aktiviti antioksida dijalankan menggunakan ujian penghapusan radikal bebas DPPH, ujian penghapusan ABTS dan ujian penurunan ferik kepada ferus FRAP. Ekstrak pengeringan-ketuhar pada 80 ºC untuk 'buaya morphotype' menunjukkan aktiviti penghapusan radikal bebas tertinggi dengan nilai 93.29 %, manakala ekstrak pengeringan-ketuhar pada 40 ºC untuk 'buaya morphotype' menunjukkan keupayaan penurunan yang kuat (7.81 mM Fe^{2+}/ g) dan kapasiti antioksidan yang tinggi (0.54 mg AEAC/ g). Asetilkolinesteras merupakan enzim yang bertindak merencatkan aktiviti neurotransmitter asetilkolin dan menyebabkan penyakit Alzheimer. Ujian perencatan asetilkolinesteras telah dijalankan dan hasilnya menunjukkan ekstrak pengeringan-ketuhar pada 40 ºC untuk kedua-dua 'morphotype' mencatatkan aktiviti perencatan asetikolinesteras yang tinggi dengan nilai 45.03 % ('buaya morphotype') dan 24.89 % ('tambalang giant morphotype'), apabila diuji pada 100 mg/ml kepekatan. Walau bagaimanapun, tiada aktiviti perencatan asetilkolinesteras dikesan dalam ekstrak pengeringan-sauna dan pengeringan-sejukbeku untuk kedua-dua 'morphotype'. Berdasarkan analisa kolerasi, kesemua sampel kering menunjukkan korelasi positif antara kandungan fitokimia dengan antioksida, korelasi positif antara kandungan fitokimia dengan kesan perencatan asetilkolinestras dan korelasi positif antara antioksida dengan

kesan perencatan asetilkolinestras. Ujian analisis proksimat menunjukkan sampel pengeringan-sejukbeku untuk kedua-dua 'morphotype' mengandungi kandungan abu yang rendah, manakala sampel pengeringan lain tidak menunjukkan perbezaan yang signifikan dalam kandungan abu. Sampel pengeringan-teduh untuk kedua-dua 'morphotype' mengandungi kandungan lembapan yang rendah, manakala sampel pengeringan lain tidak menunjukkan perbezaan yang signifikan dalam kandungan kelembapan. Sampel pengeringan-sejukbeku ('buaya morphotype' dan 'tambalang giant morphotype') menunjukkan peratusan tertinggi bagi lemak (1.26 % dan 1.21 %), serat kasar (8.28 % dan 8.42 %) dan protein mentah (14.10 % dan 14.69 %), manakala sampel pengeringan-ketuhar pada 80 ºC ('buaya morphotype' dan 'tambalang giant morphotype') menunjukkan peratusan tertinggi bagi karbohidrat (55.14 % dan 54.24 %). Peratusan terendah lemak, serat kasar dan protein mentah ditemui dalam sampel pengeringan-matahari dan pengeringan-sauna untuk kedua-dua 'morphotype', manakala karbohidrat dalam sampel pengeringan-ketuhar pada 40 ºC untuk kedua-dua 'morphotype'. Analisis untuk unsur-unsur mineral utama dan surih menunjukkan sampel pengeringan-ketuhar pada 40 ºC untuk kedua-dua 'morphotype' mengandungi Na, Ca, Mg dan Mn yang paling tinggi, manakala sampel pengeringan-teduh untuk kedua-dua 'morphotype' mengandungi K, Fe dan Zn yang paling tinggi. Unsur-unsur mineral yang rendah terkandung dalam sampel pengeringan-matahari dan pengeringan-sauna untuk kedua-dua 'morphotype'. Berdasarkan teknik-teknik pengeringan yang dikaji, pengeringan-ketuhar pada 40 ºC adalah teknik pengeringan yang terbaik digunakan untuk mengekalkan kandungan fitokimia, antioksida dan aktiviti perencatan asetikolinesteras dan beberapa kandungan mineral di dalam rumpai laut, manakala pengeringan-sejukbeku adalah teknik pengeringan yang terbaik digunakan untuk mengekalkan kandungan pemakanan di dalam rumpai laut. Kesimpulannya, proses lepas-tuai seperti pengeringan mempengaruhi kandungan fitokimia, aktiviti antioksida dan aktiviti perencatan enzim asetilkolinesteras dan juga kandungan pemakanan dalam rumpai laut kering.

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LIST OF ABBREVIATIONS

%	**-**	Percent
ºC	**-**	Degree Celsius
<	**-**	Less than
>	**-**	More than
±	-	Plus-minus
α	-	Alpha
β	-	Beta
h	-	Hour(s)
min	-	Minute(s)
µl	-	Microliter
ml	-	Mililiter
mM	-	Milimolar
M	-	Molar
cm	-	Centimeter
L	-	Liter
µg	-	Microliter
mg	-	Miligram
g	-	Gram
U	-	Unit(s)
nm	-	Nanometer
rpm	-	Revolution per minute
ABTS	-	2'2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
AD	**-**	Alzheimer's disease
BHA	-	Butylated hydroxyanisole
BHT	-	Butylated hydroxytoluene
ChE	-	Cholinesterase
ChEIs	-	Cholinesterase inhibitors
CNS	-	Centre Nervous System

DPPH	-	2,2-diphenyl-1-picrylhydrazyl
FRAP	-	Ferric reducing ability of plasma
GSH	-	Glutathione
PG	-	Propylgallate
ROS	-	Reactive oxygen species
TBHQ	-	Butylated hydroxyquinone
UVA-UVB	**-**	Ultraviolet A – Ultraviolet B

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Seaweeds are marine organisms that have been widely cultivated to provide raw materials for food, cosmetic and biomedical product (Vairappan *et al.,* 2014). Fresh and dry seaweeds are extensively consumed by people especially living in the coastal area. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods (Robeldo and Pelegrin, 1997). Certain edible seaweeds contain significant quantities of lipids, protein, vitamins and minerals (Norziah and Ching, 2000; Wong and Cheung, 2001), although nutrient contents vary with species, geographical location, season and temperature (Dawes *et al.,* 1993; Kaehler and Kennish, 1996).

Seaweeds are considered to be a rich source of antioxidant. Recently, they have received significant attention for their potential as a source of natural antioxidants. A number of active antioxidant compounds have been isolated and identified in seaweeds such as phlorotannins, sulphated polysaccharides and carotenoid pigments (Sabeena and Jacobsen, 2013). These compounds have potential to be used as neuroprotective agents due to their effectiveness in inhibiting acetylcholinesterase enzyme, which catalyzes the breakdown of acetylcholine and could be useful for treatment of Alzheimer's disease (Pangestuti and Kim, 2011). According to Ranganayaki *et al.,* (2014), seaweed is a promising candidate for drug production because it is relatively easy to obtain adequate, reliable and most importantly renewable supplies for aquaculture.

Kappaphycus alvarezii (Doty ex P. C. Silva, 1996) is a red algae species that has been introduced throughout the tropics for mariculture (Sellers *et al.,* 2015). *K. alvarezii* is a native to the Philippines and easternmost Sabah, Malaysia and this species occurs in shallow reef areas on sandy coral to rocky substrate (Trono,

1992). Over past decades, cultivated varieties of *K. alvarezii* have been introduced to shallow tropical marine environment around the world, including sites in Africa, South East Asia and South America (Sellers *et al.,* 2015). This species have a rapid growing rate and can double its biomass in 15 - 30 days when cultivated in appropriate sites (Trono, 1992).

K. alvarezii has been widely cultivated in Southeast Asian countries for food and for production of *kappa* carrageenan (Kumar *et al.,* 2008). It is a main source of *kappa* carrageenan which is used as an additive in food, pharmaceutical and cosmetic products (Prud'homme van Reine and Toron, 2001). It also has been used in herbal medicine for its anti-inflammatory, diuretic, choleretic and hemostatic properties and has been approved for food use in Japan, Korea and Taiwan (Ranganayaki *et al.,* 2014). In India, *K. alvarezii* powder has been used as a spice ingredient to enhance the nutritional quality because of the ash, protein and crude fiber content, the high amount of Vitamin E and the small amounts of niacin and Vitamin B2 (Ranganayaki *et al.,* 2014).

It has been estimated that the total production of this species in Malaysia is about 1800 tan per year (dry weight) (Prud'homme van Reine and Toron, 2001). Most of the culture farms are located in Semporna, east coast of Malaysia and handling by individual farmer or fishermen. The farmers are usually handling the preliminary processing such as harvesting the crops, sun dried and packed, before sold to processors. According to Vairappan *et al.,* (2014), moisture content in the processed seaweed biomass plays an important role in its market acceptance and determines the quality of the end product. The preferable moisture content is about 35 % which give more stability for longer storage (Rolando and Oliver, 2009).

Dried foods are processed with the goal of maintaining their quality such as flavor, texture, convenience, and functionality, increasing their nutritional content and reducing anti-nutritional factors or toxins (Bonazzi and Dumoulin, 2011). Severe drying processing generally results in higher nutritional loss, resulting in poor quality products. Hence, post-harvest handling should take into consideration of these aspects especially in the present economic context when buyer and

consumer demand for supply chain, availability, habits, nutritional value and so on in the dried food products (Bonazzi and Dumoulin, 2011). Thus, seven drying techniques were used in this study in order to learn the effects of drying process on the content and activity of bioactive compounds in *K. alvarezii*.

1.2 Problem of Statement

Drying process is an important step in post-harvest handling, which involves the removal of moisture. Drying helps decrease the water activity (a_w) which eventually retards microbial growth, conserve the desirable qualities and reduces the storage volume (Gupta *et al.,* 2011a). Water activity is a parameter used to predict the growth of bacterial, yeast and mold. According to Sandulachi (2012), controlling the level of water activity can helps to increase the product stability. However, enzymatic and non-enzymatic processes that might occur during the drying process of fresh plant tissue may lead to significant changes in composition of phytochemicals (Gupta *et al.,* 2011a). Drying process also might affect antioxidant capacity, nutritional and physical quality of herbs (Joshi and Metha, 2010). According to Vairappan *et al.,* (2014), the biological and chemical changes occur during the drying process of seaweeds has caused an inevitably damages in cell morphology, pigments and primary metabolites of seaweeds. Therefore, the present study was conducted to determine the effect of different drying techniques on the nutritional and phytochemical composition, antioxidant and acetylcholinesterase inhibition activity of *K. alvarezii.* It is expected that the finding of the present study can provide useful knowledge of the optimum post-harvest drying treatment for *K. alvarezii.*

1.3 Objectives

a. To determine the effect of different drying techniques on the phytochemical content of *Kappaphycus alvarezii.*
b. To determine the effect of different drying techniques on the antioxidant activity of *Kappaphycus alvarezii.*
c. To determine the effect of different drying techniques on the acetylcholinesterase inhibition activity of *Kappaphycus alvarezii.*

d. To determine the effect of different drying techniques on the nutritional composition and minerals of *Kappaphycus alvarezii.*

1.4 Hypothesis of Study

Ho: Drying techniques do not have effect on the nutritional and phytochemical constituents, antioxidant and acetylcholinesterase inhibition activity in seaweeds.

Ha: Drying techniques does have effect on the nutritional and phytochemical constituents, antioxidant and acetylcholinesterase inhibition activity in seaweeds.