NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND HYPOTENSIVE EFFECT OF BROWN SEAWEED (*Sargassum polycystum*) ENRICHED BREAD

NORAIDAH BINTI HAINI

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Date: 26 May 2017

Noraidah binti Haini
MN1311001T

CERTIFICATION

NAME : **NORAIDAH BINTI HAINI**

MATRIC NO. : **MN1311001T**

TITLE : **NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND HYPOTENSIVE EFFECT OF BROWN SEAWEED (*Sargassum polycystum*) ENRICHED BREAD**

DEGREE : **MASTER OF SCIENCE (NUTRITION)**

VIVA DATE : **14 MARCH 2017**

CERTIFIED BY;

1. Supervisor Signature

Assoc. Prof. Dr. Patricia Matanjun

2. Co-Supervisor

Dr. Yasmin Ooi Beng Houi

3. Co-Supervisor

Assoc. Prof. Dr. Tan Tek Song

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Noraidah binti Haini
MN1311001T

ABSTRACT

Hypertension is a major risk factor of cardiovascular diseases globally. Studies have shown that there is a positive association between seaweed intake and lower blood pressure as indicated in animal studies as well as limited human trials. Hence, this study was designed to investigate the hypotensive effect of *Sargassum polycystum* (SP) enriched bread on blood pressure among pre-hypertensive subjects. In this open label crossover trial, 12 subjects were randomly divided into two groups, who later received seaweed bread and control bread for 8 weeks with two months washout period. *S. polycystum* powder (5 – 25%) was incorporated with wheat flour in producing yeast breads. These formulated breads were examined for their nutritional composition, antioxidant activities and sensory acceptance. The study showed that nutritional values of seaweed breads were significantly different from the control bread ($p<0.05$); wherein SP enriched breads contained ash (1.16 – 4.74%), moisture (34.65 – 37.57%), crude fat (6.45 – 6.98%), crude protein (8.74 – 8.92%), dietary fibre (2.26 – 9.71%) on dry weight basis, besides having significant composition of macro minerals (Na, K, Ca and Mg) content. Ethanolic extracts of *S. polycystum* enriched breads possessed significantly higher total phenolic content (5.34 – 13.85 mg PGE/g dry extract), ferric reducing antioxidant power (FRAP) (122.89 – 162.06 µM FE/g dry extract) and DPPH radical scavenging activity (2.09 – 9.07 mg/ml) than water extracts ($p<0.05$). Sensory evaluation of breads using hedonic test has shown that bread enriched with 10% SP powder was selected as the best formulation among all seaweed breads ($p<0.05$), whereby its shelf life test showed that it is safe for 7-day consumption. *In vitro* test of anti-hypertensive assay showed that SP powder (0.41 mg/ml) and 10% seaweed bread (3.13 mg/ml) possessed higher ACE-inhibitor capacities than the control bread (25.01 mg/ml) ($p<0.05$). Intervention trial showed that subjects consuming seaweed bread resulted in significantly lower systolic blood pressure, diastolic blood pressure, pulse and total cholesterol ($p<0.05$). On contrary, subjects consuming control bread showed that there was no significant change in diastolic blood pressure but their systolic blood pressure, pulse and total cholesterol were significantly higher than the baseline ($p<0.05$). On the whole, the new therapeutic discovery of hypotensive effect in SP powder would contribute to health-promoting properties of seaweed as well as providing useful information to local industries for potential development of food and health products.

ABSTRAK

KOMPOSISI PEMAKANAN, AKTIVITI ANTIOKSIDAN DAN KESAN HIPOTENSI ROTI DIPERKAYA RUMPAI LAUT PERANG (*Sargassum polycystum*)

Hipertensi merupakan antara risiko utama penyakit kardiovaskular sedunia. Kajian telah menunjukkan bahawa terdapat hubungan yang positif di antara pengambilan rumpai laut terhadap paras tekanan darah seperti yang dinyatakan dalam kajian haiwan dan ujikaji klinikal manusia. Justeru, kajian ini direka untuk mengenal pasti kesan hipotensif roti diperkaya rumpai laut perang, Sargassum polycystum (SP) terhadap tekanan darah dalam kalangan subjek pra-hipertensif. Dalam ujikaji pangkah silang terbuka ini, seramai 12 subjek telah dibahagikan secara rawak kepada dua kumpulan, untuk menerima roti diperkaya rumpai laut dan roti kawalan sepanjang 8 minggu yang diselangi 2 bulan tempoh proses "pencucian". Serbuk S. polycystum (5 – 25%) telah dicampur dengan tepung gandum dalam menghasilkan roti yis. Komposisi pemakanan, aktiviti antioksidan dan penerimaan sensori formulasi roti telah dikenal pasti. Kajian ini menunjukkan komposisi pemakanan roti diperkaya SP mempunyai perbezaan yang signifikan dengan roti kawalan ($p<0.05$); dimana roti diperkaya SP mengandungi abu (1.16 – 4.74%), lembapan (34.65 – 37.57%), lemak (6.45 – 6.98%), protein (8.74– 8.92%), serat diet (2.26– 9.71%) untuk asas berat kering, disamping mempunyai komposisi makro mineral (Na, K, Ca and Mg) yang signifikan. Ekstrak etanol bagi roti diperkaya S. polycystum mengandungi fenolik (5.34 – 13.85 mg PGE/g ekstrak kering), kuasa penurunan Ferik (FRAP) (122.89 – 162.06 μM FE/g eksrak kering) dan aktiviti penghalang radikal DPPH (2.09 – 9.07 mg/ml) yang lebih tinggi berbanding ekstrak air ($p<0.05$). Penilaian sensori roti melalui kaedah hedonik telah menunjukkan roti diperkaya SP (10%) telah dipilih sebagai formulasi terbaik berbanding formulasi yang lain ($p<0.05$), dimana ujian jangka hayat menunjukkan roti tersebut selamat untuk dimakan selama 7 hari. Ujian in vitro anti-hipertensif menunjukkan serbuk SP (0.41 mg/ml) dan roti diperkaya 10% serbuk SP (3.13 mg/ml) mempunyai keupayaan untuk merencat aktiviti ACE yang lebih tinggi berbanding roti kawalan (25.01 mg/ml) ($p<0.05$). Ujikaji intervensi pula menunjukkan subjek yang menerima roti diperkaya SP mempunyai paras tekanan sistolik darah, tekanan diastolik darah, nadi dan kolesterol yang lebih rendah ($p<0.05$). Sebaliknya, subjek yang menerima roti kawalan menunjukkan tiada perubahan tekanan diastolik yang signifikan tetapi mempunyai paras tekanan sistolik, nadi dan kolesterol yang lebih tinggi ($p<0.05$). Keseluruhannya, penemuan terapeutik baru tentang kesan hipertensif serbuk SP boleh menyumbang kepada ciri-ciri kesihatan rumpai laut dan menyediakan maklumat yang berguna kepada industri tempatan untuk potensi pembangunan makanan dan produk kesihatan.

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A1	-	Group A when receives seaweed bread in Stage One
A2	-	Group A when receives control bread in Stage Two
ALA	-	α-linolenic acid
Al	-	Aluminium
ALT	-	alanine aminotransferase
ANOVA	-	Analysis of Variance
ARP	-	Anti-Radical Power
B1	-	Group B when receives seaweed bread in Stage Two
B2	-	Group B when receives control bread in Stage One
BMI	-	Body Mass Index
BP	-	Blood pressure
CVD	-	Cardiovascular Disease
DASH	-	Dietary Approaches to Stop Hypertension
DBP	-	Diastolic blood pressure
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
DW	-	Dry Weight
FBG	-	Fasting blood glucose
FTC	-	Fasting total cholesterol
FRAP	-	Ferric Reducing Antiradical Power
HAT	-	Hydrogen atom transfer
KA	-	*Kappaphycus alvarezii*
LDL-C	-	Low Density Lipoprotein Cholesterol
NHMS	-	National Health and Morbidity Survey
PUFA	-	Polyunsaturated Fatty Acids
PVD	-	Peripheral Vascular Disease
RE	-	Retinol
ROS	-	Reactive Oxygen Species
SBP	-	Systolic blood pressure
S.D	-	Standard deviation
SEM	-	Standard Error Mean

SET	-	Single Electron Transfer
SP	-	*Sargassum polycystum*
TC	-	Total Cholesterol
UV	-	Ultra Violet
WHO	-	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Background

Blood pressure is defined as the force exerted by the blood flow against the tunnel of blood vessels (Stamler, Stamler and Neaton, 1993). Also known as brachial arterial pressure, it is measured on the upper arm where blood circulates away from the heart. This normal arterial pressure is produced as the heart pumps the blood throughout systemic circulation of human body. However, interference from physiology changes might affect the systemic circulation (Levitan, Wolk and Mittleman, 2009), resulting in a lower or a higher blood pressure reading (Lifton, Gharavi and Geller, 2001). Lower blood pressure is also known as hypotension whereby a raised blood pressure is called hypertension (Appel, 2010).

Hypertension is further explained as a continuous elevated systolic blood pressure (SBP) of 140 mmHg or greater and/or diastolic blood pressure (DBP) of 90 mmHg or greater (Ministry of Health Malaysia, 2013). It is an evident major risk factor for heart, cerebrovascular and peripheral diseases globally (Ueshima, Sekikawa, Miura, Turin, Takashima, Kita, Watanabe, Kadota, Okuda and Kadowaki, 2008). Being listed as one of the leading burden of non-communicable diseases, hypertension has caused at least 7.5 million deaths per year worldwide (WHO, 2014). The latest National Health and Morbidity Survey (2015) report shown that the prevalence of hypertension among Malaysians aged 18 and 30 years above has increased to 32.7% and 50.4% respectively, which demonstrated that approximately one third to half of Malaysian adults population are now suffering from hypertension (Ministry of Health Malaysia, 2015). The prevalence has mainly increased with rising awareness on blood pressure control in the hypertensive population (Chobanian, Bakris, Black, Cushman, Green, Izzo, Jones, Materson, Oparil, Wright and Roccella, 2003).

It has also been reported that 37% of Malaysian are belonged to pre-hypertension category, which demonstrated that approximately 11.1 million of Malaysia adult populations are suffering from pre-hypertension, leading to hypertension if left untreated (Ministry of Health Malaysia, 2013). Furthermore, the increased cardiovascular events such as stroke, diabetes and kidney failure are seen closely associated with the incidence of pre-hypertension. There is approximately a third of blood pressure-related deaths from coronary heart disease have been estimated to occur in individuals with pre-hypertension (Greenlund, Croft and Mensah, 2004). Thus, it is vital to raise public and clinical health level awareness on the prevention of pre-hypertension as the pre-hypertensives are at greater risk for progression to hypertension (Ministry of Health Malaysia, 2013; Chobanian *et al.*, 2003). Early possible detection of pre-hypertension and prevention of hypertension among individuals should be assessed through 6 – 12 monthly follow-up and decision regarding pharmacological treatment should be based on the overall of individual cardiovascular risks (Liszka, Mainous, King, Everett and Egan, 2005).

Hence, controlling blood pressure within its normal range is very important (Conlin, 2008). It should be measured frequently (Chia, Keevil and Ching, 2013) because most of hypertension cases in Malaysia have remained undiagnosed (Ministry of Health Malaysia, 2013). The changes in blood pressure can be controlled by self-monitoring at home and workplace (Pickering, 1996). Effective blood pressure control and diagnosis of initial hypertension may help to reduce the burden of non-communicable diseases (Rampal, Rampal, Syed Azhar and Rahman, 2008). Upon diagnosis, the practice on therapeutic lifestyle changes as an alternative way to control blood pressure will be recommended to all individuals with hypertension and pre-hypertension (Ministry of Health Malaysia, 2013).

Therapeutic lifestyle changes such as routine exercise (Montero, Roche and Martinez-Rodriguez, 2014), smoking cessation (Ezzati, Henley, Thun and Lopez, 2005), weight loss management (Costa, 2002), restriction of alcohol intake (Rakic, Puddey, Burke, Dimmitt and Beilin, 1998) and cutting down sodium intake (Graudal, Hubeck-Graudal and Jürgens, 2012) have been discovered to help reduce

blood pressure level (Brook, Appel, Rubenfire, Ogedegbe, Bisognano, Elliott, Fuchs, Hughes, Lackland, Staffileno, Townsend and Rajagopalan, 2013). People are also highly encouraged to practice Dietary Approaches to Stop Hypertension (DASH) Eating Plan as another way of lifestyle changes to control their blood pressure (Boden-Albala and Sacco, 2000). DASH is a combination of diet with plenty of fruits and vegetables which are rich in potassium and calcium content; with a reduced cholesterol content as well as total saturated fat (Houston, 2005). DASH diet has been shown to be safe and effective in lowering blood pressure among general population (Appel, 2010). Furthermore, it has been reported that DASH eating plan is more effective than taking treatment drugs in helping to lower blood pressure in some individuals (Chobanian *et al.*, 2003).

Over the years, studies have reported that non-drug treatment for hypertensive are effective by consuming natural foods containing antioxidants (Galley, Thornton, Howdle, Walker and Webster, 1997), sour milk (Hata, Yamamoto, Ohni, Nakajima, Nakamura and Takano, 1996), immune milk (Sharpe, Gamble and Sharpe, 1994), garlic extracts (Steiner, Khan, Holbert and Lin, 1996) and lactobacillus component (Nakajima, Hata, Osono, Hamura, Kobayashi and Watanuki, 1995). These findings prompted the idea on developing a functional food product which can be useful in delivering health benefit properties (Fleurence, Morancais, Dumay, Decottignies, Turpin, Munier, Garcia-Bueno and Jaouen, 2012). Functional food is a term practically referred to foods or food constituents provided with certain health benefits beyond basic nutrition (Diplock, Aggett, Ashwell, Bornet, Fern and Roberfroid, 1999). Hence, with regards to dissatisfaction of high costs and potentially hazardous side effect of antihypertensive treatment drugs (Gu, Burt, Dillon and Yoon, 2012), the potential of using functional food products for preventive measures is currently on-going.

Seaweed, on the other hand, has been long consumed particularly in Asian countries like China, Japan and Korea (Ismail and Tan, 2002). Seaweed is a type of nutritive edible food which exerts various health benefit properties that reduce the risk of chronic diseases as well as improving the management of chronic diseases (Mohamed, Hashim and Rahman, 2012). Studies have reported that edible

seaweeds are rich in polysaccharides, minerals and vitamins (Holdt and Kraan, 2011). They also contain bioactive compounds with antioxidative, anticancer and antimicrobial properties (Kumar, Ganesan, Suresh and Bhaskar, 2008a). There are three main edible seaweed species available in Malaysia, namely as *Kappaphycus alvarezii* (Rhodophyta), *Caulerpa lentillifera* (Chlorophyta) and *Sargassum polycystum* (Phaeophyta) (Matanjun, Mohammed, Mustapha, Muhammad and Cheng, 2008). Among the three seaweed species mentioned above, the use of *S. polycystum* in bread making application will be the main focus in this study.

S. polycystum is a local brown seaweed species collected from the coastal area of Semporna, Sabah, Malaysia. It has been reported that *S. polycystum* contains high total dietary fiber (39.67%) and ash (42.40%) (Matanjun, Mohammed, Mustapha and Muhammad, 2009). In addition, it has been shown to have *in vitro* (Matanjun *et al.*, 2008) and *in vivo* antioxidant activities (Matanjun, Mohamed, Muhammad and Mustapha, 2010). Protein extracts of *S. polycystum* have also portrayed sequences of ACE inhibitor peptides indicating that it possesses natural anti-hypertensive property (Ahmad, Sulaiman, Awang, Chye and Matanjun, 2014). The above properties of *S. polycystum* are appropriate to be used as a functional ingredient in developing bread product for preventing or treating hypertension.

To date, limited studies have been conducted on the use of seaweed as a food ingredient in bread making. According to Hall, Fairclough, Mahadevana and Paxmana (2012), *Ascophyllum nodosum* enriched bread has reduced subsequent energy intake in healthy, overweight males with no effect on their postprandial glucose concentration. Mamat, Matanjun, Ibrahim, Md. Amin, Hamid and Rameli (2013) have incorporated *Kappaphycus alvarezii* powder in bread making but the study only focused on the textural properties, and none of its nutritional content. Additionally, Fitzgerald, Gallagher, Doran, Auty, Prietod and Hayes (2014b) have shown that the incorporation of protein hydrolysate from red seaweed, *Palmaria palmata* into bread is well accepted by consumers, however, the antihypertensive effect of the bread product on blood pressure using human clinical trial has not been investigated.