EFFECT OF STEAMING ON ANTIOXIDANT ACTIVITY IN EXTRACTS OF *Ipomoea batatas* ROOTS

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Product Development Technology) with Honours

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled "**Effect of steaming on antioxidant activity in extract of *Ipomoea batatas* roots**" by **NUR HAZIRAH BINTI ABDUL RAZAK**, matric number **F15A0148** has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agriculture Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Effect of Steaming on Antioxidant Activity in Extracts of *Ipomoea batatas* Roots

ABSTRACT

Ipomoea batatas are one of the important plant sources in term of their nutritional content which known to have high antioxidant. A heat stable antioxidant is important to ensure their effectiveness for neutralize the free radical in human body. The purpose of this study is to determine the effect of steaming on antioxidant activity in *Ipomoea batatas* root extracts. Fresh root was steamed at 0, 10, 20, 30 and 40 minutes respectively. The steamed roots were extracted using ethanol. The antioxidant activity, total phenolic and flavonoid content of the extracts were determined by 2,2-diphenyl-1-picrylhydrazyl, Folin-ciocalteu and Aluminium chloride assay, respectively. All result show significant difference ($p \leq 0.05$) except for DPPH assay. Antioxidant activity shows highest value at 30 min 17.561 ± 0.211 %, total phenolic shows highest value at 20 min 16.802 ± 0.676 mg BHTE/g raw material and flavonoid shows highest value at 10 min 27.861 ± 0.621 mg QE/g raw material. It can be conclude that steaming increase the antioxidant activity however further increase of steaming time may destroyed antioxidant in the samples extracts.

Keywords**:** *Ipomoea batatas*, antioxidant, DPPH, total phenolic, total flavonoid

Kesan Pengukusan Terhadap Aktiviti Antioksidan di Dalam Ekstrak Akar *Ipomoea batatas*

ABSTRAK

Ipomoea batatas adalah salah satu sumber tumbuhan penting dalam kandungan nutrisi yang diketahui mempunyai antioksidan yang tinggi. Antioksidan stabil haba adalah penting untuk memastikan keberkesanannya bagi meneutralkan radikal bebas dalam tubuh manusia. Tujuan kajian ini adalah untuk menentukan kesan pengukusan pada aktiviti antioksidan di dalam ekstrak akar *Ipomoea batatas*. Akar segar dikukus pada 0, 10, 20, 30 dan 40 minit masing-masing. Akar yang dikukus diekstrak menggunakan etanol. Aktiviti antioksidan, jumlah kandungan fenol dan flavonoid ekstrak telah ditentukan oleh ujian 2,2-diphenyl-1-picrylhydrazyl, Folin-ciocalteu dan Aluminium klorida. Semua keputusan menunjukkan perbezaan yang signifikan ($p \leq 0.05$) kecuali ujian DPPH. Aktiviti antioksidan menunjukkan nilai tertinggi pada 30 min 17.561 ± 0.211, nilai fenolik tertinggi dilihat pada 20 min 16.802 ± 0.676 dan flavonoid menunjukkan nilai tertinggi pada 10 min 27.861 ± 0.621. Kesimpulan boleh dibuat bahawa pengukusan meningkatkan aktiviti antioksidan tetapi penambahan masa pengukusan boleh memusnahkan antioksidan dalam ekstrak sampel.

Kata kunci: *Ipomoea batatas*, antioksidan, DPPH, jumlah fenolik, jumlah flavonoid

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LIST OF ABBREVIATIONS

g	gram
min	minute
nm	nanometre
µg	microgram
µl	microlitre
ml	millilitre
M	molar
rpm	revolutions per minute
mg/ml	microgram per mililitre
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toulene
DPPH	2,2-diphenyl-1-picryhydrazyl
GAE	Gallic acid equivalent
HAT	Hydrogen-atom transfer
QE	Quercetin equivalent
R^2	Correlation coefficient
RM	Raw material
SET	Single electron transfer
SD	Standard deviation
Sig	Significant
TFC	Total flavonoid content
TPC	Total phenolic content
UV-VIS	Ultraviolet visible

LIST OF SYMBOLS

ºC	Degree Celcius
%	Percent
≤	Less than or equal
±	Plus-minus
:	Ratio
µ	micro

CHAPTER 1

INTRODUCTION

1.1 Research background

Ipomoea batatas or sweet potatoes are one of the important plant sources in term of their nutritional content and health benefits for human consumption. In several studies, orange-fleshed of sweet potatoes have been proven to be a better source of bioavailable beta carotene than green leafy vegetables. In some countries such as Africa, Caribbean and India, sweet potatoes have been observed as a highly effective way to provide sufficient amounts of daily Vitamin A to school age children (Mateljan, 2018). Purple-fleshed sweet potato contain important antioxidants compound such as anthocyanins, peonidins and cyanidins.

Recent studies showed, steaming is the best cooking method to preserve anthocyanins in sweet potatoes. The effect of steaming on antioxidant properties was interesting since only two minutes of steaming can deactivate peroxidase enzyme that might broke down anthocyanins compound in the sweet potato. The deactivation of peroxidase make natural anthocyanin extracts from sweet potato that used for food

colouring become more stable than synthetic food colourings (Mateljan, 2018). Colour related pigments including anthocyanins in sweet potatoes were equally valuable for their antioxidant and anti-inflammatory properties of this tuber

1.2 Problem statement

Sweet potatoes contain numerous phytochemicals which contribute to their health benefits hence consumption of sweet potatoes have increased significantly in recent years. Although there are many research on antioxidant activity of this particular plant were conducted, however there is no information on effect of heat treatment such as steaming on antioxidant activities in sweet potatoes. Longer steaming time of sweet potatoes may cause loss of nutrient in the sweet potatoes as well as destroyed the antioxidant activities. Therefore, this study will be an attempt to evaluate antioxidant properties of sweet potatoes and to study the effect of steaming time on its antioxidant properties. In addition, this plant has many varieties and further research is needed to identify the potential antioxidant compound present in sweet potatoes.

1.3 Hypothesis

H_0: Steaming do not have significant effect on antioxidant activity in *Ipomoea batatas*

H_1: Steaming has significant effect on antioxidant activity in *Ipomoea batatas*

1.4 Objectives

The objectives of this study are:

1. To extract *Ipomea batatas* root at different steaming time using ethanol
2. To determine the effect of steaming on antioxidant activity in extract of *Ipomoea batatas* roots

1.5 Scope of study

This study was focused on the effect of steaming time on antioxidant activity in root extracts of *Ipomoea batatas*. The sample of *Ipomoea batatas* used in this study was orange-flesh and was bought from Jeli market in Kelantan on 11th November 2018. Ethanol solvent has been used to extract the sample because it has been known as a good solvent for extract polyphenol and also non-toxic for human. The antioxidant activity in *Ipomoea batatas* extracts were determine by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, Total Phenolic Content (TPC) assay and Total Flavonoid Content (TFC) assay. The experiment was conducted in Biology Laboratory of University Malaysia Kelantan.

1.6 Significance of study

This study was able to provide information regarding the use of *Ipomoea batatas* on their antioxidant properties. This study also added information on how steaming affect the antioxidant activity and verify the claim made by the indigenous people. The finding on antioxidant properties of this plant can lead to the exploitation of the plant and increase knowledge of the tuber for medicinal purpose. *Ipomoea batatas* can be an alternative for synthetic antioxidant such as BHT and BHA in food preservation.

CHAPTER 2

LITERATURE REVIEW

2.1 *Ipomoea batatas*

Ipomea batatas or its common name sweet potato, is a root crop that is known to have high starch content and can be found widely in most part of the world. This crop rich in nutritional content and has short growing season which make it an ideal crop for development. This crop is easy to grown because its' require only a little or no fertilizer and is widely grown by smallholder farmer in many parts of the world. Sweet potato root can be described as long and tapered while the colour of the flesh ranges between cream, white (highest starch) to orange (high in carotene) to purple (Michael, 2013). Figure 2.1 shows different colour range of sweet potato flesh.

Figure 2.1 Colour of *Ipomoea batatas* flesh (Hendon, 2018)

Ipomoea batatas is belong to the family of convolvulaceae. The plant is categorized as herbaceous perennial vine, has palmately-lobed or heart shaped leaves and has medium-sized sympatelous (Mohanraj & Sivasankar, 2014). *Ipomoea batatas* is an annual plant crop and can be planted by vegetative propagation using either stem cuttings or storage roots. The stem is in shape of cylindrical and the length is influenced by the water presence in the soil and also the growth habit of the cultivar. This plant crop is originated from either Central or South America (Morales, 2009). The crop were introduced to India and Africa by European explorers in early 1500s, China by 1594, while Japan and Taiwan by 1597 (Horton, 1998). With an annual production of 115 million metric tons, sweet potato has been ranked seventh among food crop worldwide. Sweet potato has many varieties and were classified according to their flesh colour and shape of the tuber. Table 2.1 shows several types of sweet potatoes which are Hannah, Japanese, Purple, Jewel and Garnet yams/sweet potatoes.

Table 2.1: Type of sweet potatoes (Hendon, 2018)

Types	Description
Hannah yams/sweet potato	Skin: cream coloured and smooth Flesh: cream/ whitish coloured that becomes yellow when baked Taste: sweet, firm and dry when cooked
Japanese yams/sweet potato	Skin: purple and fairly smooth Flesh: whitish flesh that turns golden when cooked Taste: very sweet and fairly firm inside
Purple yams/sweet potato	Skin: deep purple Flesh: deep purple Taste: not very sweet and dry inside
Jewel yams/sweet potatoes	Skin: orange/copper Flesh: deep orange Taste: mildly sweet and fairly firm inside
Garnet yams/sweet potatoes	Skin: Reddish/ dark orange Flesh: orange Taste: Mildly moist and pretty moist inside

2.1.1 Traditional uses

According to Hartwell, (1971), the old folk used the leaf as decoction for remedies to cure tumour of the mouth and throat. Sweet potato also has been used as folk remedy for various disease such as fever, asthma, diarrhea, stomach stress, nausea, ciguatera, burns, bug bites, and also tumour (Duke & Wain, 1981). Besides, the sweet potato tubers were eaten as vegetable with various cooking method such as boiled, baked fried or dried and ground into flour to make bread, biscuits and other pastries. In Malaysia, the leafy tops is eaten as vegetable and sold in markets.

2.1.2 Phytochemical and pharmacological studies

Sweet potatoes contain various type of phytochemical compound. Sweet potato tuber contain source of flavonoids and phenolic compound such as beta carotene and vitamin A. One of the compound that has long be known is β-carotene that can prevented night blindness and other symptoms cause by lack of vitamin A (Berg et al, 2000). Every molecule of beta carotene will produced two molecules of vitamin A in our liver (Chichili, Nohr, Scaffer, Lintig & Biesalski, 2005).

Vitamin A is necessary to help the body fight against infections and stay resistant to any further infections. In addition, carotenoids in sweet potatoes also have bioactive compound such as vitamin C and anthocyanin (Ghasemzadeh et al., 2016). The presence of anthocyanins compound in purple sweet potatoes provide free radical scavenging activity, gives protection to the liver and memory enhancing effect. Flavonoids provide

protection from oral cavity and lung cancer. Besides, the sweet potato tubers also have various essential vitamin such as thiamine (vitamin B1), panthothenic acid (vitamin B5), pyridoxine (vitamin B6), riboflavin and niacin (Mohanraj & Sivasankar, 2014). These vitamins are needed during metabolism which act as co-factors for most enzymes. The tubers also have plenty of minerals such as, magnesium, iron, manganese, calcium and potassium that are needed for protein, enzyme and carbohydrate metabolism (Woolfe, 1992).

The leaves of sweet potatoes also have plenty of phytochemical compound such as phenolic acid, alkaloid, flavonoid, triterpenes/steroids, tannins, coumarins as well as saponins. Phenolic compounds, alkaloid and glycolipids have demonstrated pharmacological characteristic such as anti-bacterial, hepatoprotectant, antihistamine and various biological effects (Meira et al., 2012). In addition, caffeoulquinic acid derivatives from the leaf of sweet potato has properties of antimutagenicity as well as prevent proliferation of human cancer cells that arise from stomach cancer, promyelocytic leukemia and colon cancer (Basnet et al., 1996).

Sweet potato also has been used to treat several disease in many country of the world. Some of the disease are diabetes, hypertension, kidney ailments, fatigue, dysentery, meningitis, inflammation, constipation, arthritis and hydrocephaly (Mohanraj & Sivasankar, 2014). Besides, they also have demonstrated antimicrobial, anticoagulant, anticancer activities, analgesic and hypotensive (Miera et al., 2012). In addition, the antioxidant capacity of sweet potato is 42.94% as compared to ascorbic acid and the total antioxidant present in purple fleshed sweet potato is higher than the cream fleshed (Teow, 2007). Total phenolic content can be used as an indicator for antioxidant of sweet potatoes and has been found highest in leaves and stem end of the roots.

2.2 Steaming

Steaming is a cooking technique that use steam to cook the food. Steaming can be done by using a food steamer or any kitchen appliance that is made specifically to cook food with steam. Steaming also can be credited as one of the healthiest cooking method and the process is easier and quicker compared to other cooking technique (Borah, 2018). Steaming involves the process of boiling water continuously, making the water molecule to vaporize into steam then brought heat to the nearby food hence cooking the food. Steaming usually done by using circular food steamer that is made from metal, bamboo or wood. The food and the boiling water are kept separately known as compartment steaming but steaming has a direct contact with the steam.

This will give a moist texture to the food. Steaming allows the food to preserve their natural colour, shape, flavour and nutritious value better than boiled or simmered in water (USDA, 2018). Steaming can retain up to 50 percent nutrients in the food compared to other cooking method and also does not require any fats when cooking (Alfaro, 2018). Examples of food that can be cooked using steaming technique are vegetables, poultry, fish, meat, pastry, breads and rice. According to Sikia and Mahanta (2013), among various cooking method, steaming were identified as the most suitable method in most of the cases based on phytochemical retention and antioxidant activities.

2.3 Antioxidant

Antioxidant is a compound that is man-made or natural substance that can inhibits the oxidation process. Oxidation is known as a chemical reaction that produce free radicals that can damage the cells of organism. Free radical is a compound that has unpaired electron, electrically charged molecule, unstable and is highly reactive (Anarson, 2017). It can attach and damage normal cells such as DNA. Free radicals are produce naturally by human body and often resulted in cell damage that leads to cancer development (Marturana, 2017). Besides, it also can come from outside source for example, smoking or toxin however, most of free radicals come from normal metabolism in human body.

Figure 2.2 shows the mechanism of antioxidant. The mechanism of antioxidants occur when a molecule loses an electron and changed into a free radical, then the molecule of antioxidant will act by donating an electron to the free radicals and neutralizing it (Anarson, 2017). Hence, this mechanism will prevent the free radical from causing damage to cells in human body. Antioxidant can be said as a healthy compound and can be found naturally and synthetically. Most consumers prefer natural antioxidant and it it easier to gain legislative approval than synthetic additives do (Maslarova & Heinonen, 2001).

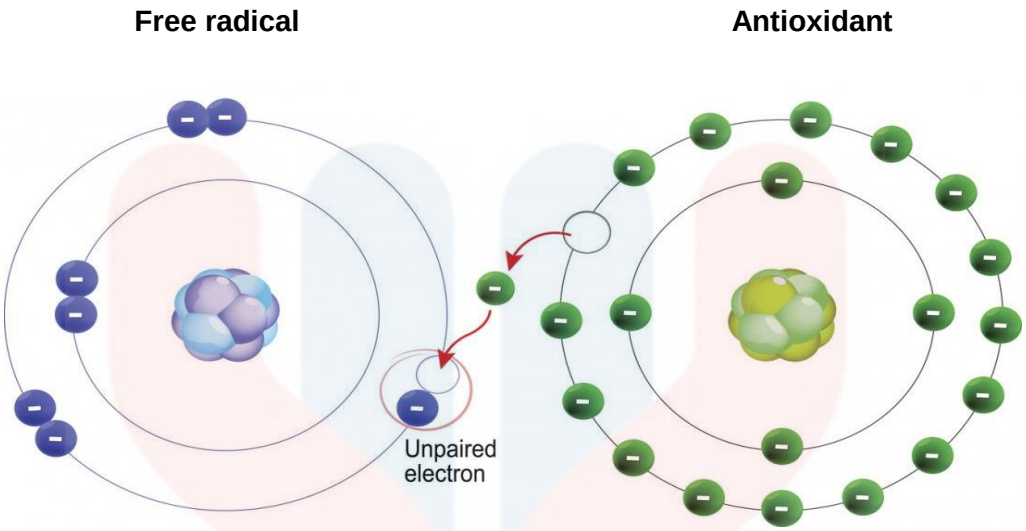

Figure 2.2: Mechanism of antioxidant (Anarson, 2017)

There are many source of natural antioxidant for example in fruits, vegetables, whole grain, herbs, spices, coffee, black tea, edible bean and many more (Akbarirad, Ardabili, Kazemeini, & Khaneghah, 2016). Table 2.2 showed the examples of the most common source of natural antioxidant. The antioxidant in food helps to prevent damage caused by oxidative stress. For example, vitamin E act as a significant role to prevent cardio vascular disease while flavonoids, beta carotene and vitamin C delay of chronic degenerative and ageing (Winter, 2013). In addition, food containing antioxidants not only prevent chronic diseases but also can help to prevent lipid oxidation of food occurring.

Table 2.2: Most common natural antioxidant and their typical sources (Carr et al., 2000; Urquiaga & Leighton, 2000; McGhie & Walton, 2007)

Compound name	Natural source
Ascorbic acid	Most fruits (particularly citrus fruits), some vegetables, tomatoes
Tochopherols	Cereal grains, broccoli, brussels sprouts, cauliflower, cooking oils, almonds, hazelnuts.
Beta-carotene	Vegetables such as red paprika, spinach, parsley, tomatoes, carrots, sweet potatoes, apricots and papaya
Flavonoids	Potatoes, tomatoes, lettuce, onions, wheat, concord grapes, black tea
Anthocyanins	High content in red wines
Various polyphenols	Teas, as well as many red/purple hued fruits or vegetables, such as concord grapes, red cabbage, blueberries, blackberries and berries
Lycopene	Tomatoes, papaya, watermelon, pink grapefruit, guava, the skin of red grapes
CoQ10	Wheat bran

2.3.1 Roles of antioxidant in food and human health

Antioxidant such as vitamins C, E, or natural antioxidants like phenolics, flavonoids, terpenoids, coumarins and tannins that are present abundance in diet plant food have been discovered since past few decades can prevented oxidative stress and specific human diseases (Perumalla & Hettiarachchy, 2011). Hence, there is an increase interest in exploring the range of antioxidant that may be used as food ingredient to prevent oxidation of food. In addition, phenolic extracts made from various plant extracts such as grape seed were known to have antimicrobial properties against foodborne pathogens (Almajano, Carbo, Jimenez, & Gordon, 2008; Perumalla & Hettiarachchy, 2011). Antioxidant from natural plant extracts and synthetic antioxidant such as BHA and BHT have been widely used as preservatives, additives or supplement in many food industries (Zulueta, Esteve, Frasquet, & Frígola, 2007). Increasing the consumption of dietery antioxidant can help maintaining the antioxidant status and also contribute to normal physiological function of human bodies. However, there is no specific daily "total antioxidant" intake recommended because of complexity and diversity of antioxidant (Kaliora, Dedoussis, & Schmidt, 2006).

There are various chronic diseases such as diabetes, heart disease, cancer and macular degeneration were influenced by cellular oxidative damage. According to Ames et al. (1993), antioxidants can help to enhance blood flow to the heart and brain, lower the risks from cardiovascular and Alzheimer's diseases, prevent cancer that cause by DNA damage and also help to prevent blood vessel injuries. Human body contain natural endogenous antioxidant system where it function was to overcome the production of free radicals. Polyphenols, lutein, and lycopene are example of phytochemical antioxidants

and are able to give protection to human body from oxidation damage (Moon & Shibamoto).

2.4 Assay for determination of antioxidant activity

According to Huang and Prior (2005), antioxidant assays were classified into two categories based on their chemical reaction which are Hydrogen Atom Transfer (HAT) based assay and Single Electron Transfer (SET) based assay. HAT-based method can be described as the ability of an antioxidant to quench free radicals by hydrogen donation to form stable compound while HAT-based method used to detect a potential antioxidant to transfer one electron to reduce any compound which may include carbonyls, metals as well as radicals. Both reactions have the same end result however, the kinetics and potential side reactions were not similar (Skowyra, 2014).

2.4.1 DPPH assay

The most popular assay used in natural product of antioxidant studies is known as a standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. This assay is preferred among researcher because the method used is very simple and sensitive. DPPH is in a form of dark-coloured crystalline powder consist of stable free radical molecule. It has a few crystalline forms where its' vary by the melting point (m.p.) and lattice symmetry. The commercial powder has a mixture of phases that melts at ≈ 130 ºC. DPPH-I is orthorhombic (m.p. 106 ºC), DPPH-II is amorphous (m.p. 137 ºC) while DPPH-III is

triclinic (m.p. 128 ºC) (Keirs et al., 1976). The principle of DPPH assay relies on the theory that a hydrogen donor is an antioxidant.

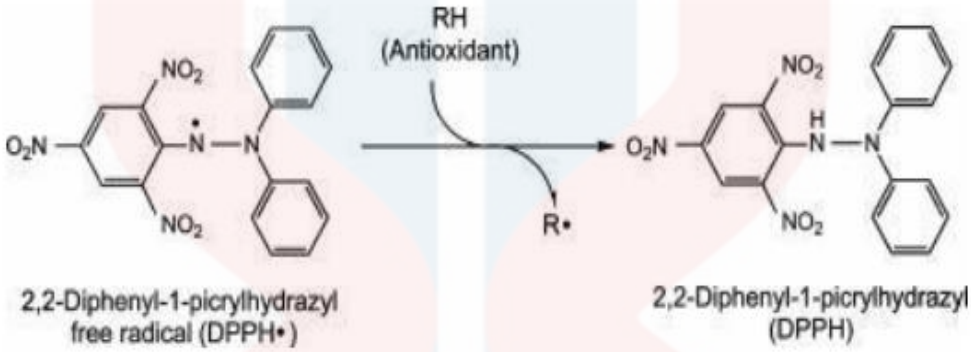

Figure 2.3: DPPH• free radical conversion to DPPH by anti-oxidant compound (Lewis, 2012)

The Figure 2.3 explained that DPPH• accept hydrogen from an antioxidant and the antioxidants effect is proportional to the disappearance of DPPH• in the test samples (Lewis, 2012). The DPPH• is commonly monitored using UV spectrophotometer because of its accuracy and simplicity. When the free radicals have been scavenged, the colour will changed from purple to yellow and followed by formation of DPPH when hydrogen is absorb from an antioxidant (Moon & Shibamoto, 2009). This reaction is stoichiometric with respect to the number of hydrogen atom absorbed and DPPH• showed a strong absorption maximum at 517 nm (purple). Disadvantages of using this assay are because several antioxidants such as carotenoids have spectra that overlap with DPPH at 515 nm and interfere with the results. Besides, DPPH radical can only be dissolved hence it become a limitation when interpreting the role of hydrophilic antioxidants (Arnao, 2000; Karadag et al., 2009).

2.4.2 Total phenolic content (TPC) assay

Common method for phenolic quantification is based on Folin-Ciocalteu method. The Folin-cioucalteu reagent consist of phosphomolybdic/ phosphotungstic acid complexes (Singleton & Rossi, 1965). Total phenolic content assay techniques is based on oxidation/ reduction reaction where it involves transfer of single electron (SET) from phenolic compound to form blue chromophore constituted by a phosphotungstic/ phosphomolybdic complex in alkaline solution and can be observed spectrophotometrically in range 750-765 nm (Prior, Wu & Schaich, 2005). The maximum absorption relies on the concentration of phenolic compound. Advantages using this assay due to convenient, precise, simple and reproducible. This assay also shows a good linear correlation with various assay such as DPPH, FRAP, TEAC as well as ORAC (Gallego et al., 2013; Karadag et al., 2009). However, there are some disadvantages using this assay which are suffers from interference from sugar, aromatic amines, sulphur acids and ferrous ion (Fe^{2+}). Some of inorganic substance can give false value to this assay. Besides, if this assay was carried out in aqueous phase it become not applicable for lipophilic antioxidants.

2.4.3 Total flavonoid content (TFC) assay

The most common method used in this assay was aluminium chloride method. In this assay, complexation reaction is carried out in the presence of sodium nitrite in alkaline medium. The assay is based on the nitration of any aromatic ring bearing a

catechol group with its three or four positions unsubstituted or not sterically blocked (Rocchetti, 2016). After addition of aluminium a yellow solution of complex was formed which then turned red after addition of sodium hydroxide, and the value of absorbance can be measured at 510 nm. In this assay catechin is preferred as a standard compound.

2.5 Main classes of polyphenolic compounds

Phenolic compound consist a wide variety of molecules that include polyphenol structure such as some hydroxyl groups on aromatic ring. Phenolic compound also consist of molecules with one phenol ring like phenolic acids and phenolic alcohols. Polyphenol were classified into a few classes depending to the number of phenols rings and also structural elements that bind the rings to one another (Skowyra, 2014). Polyphenol compounds are known as plant secondary metabolite and showed significant function in growth and reproduction in plant. Phenolic acids, flavonoids, tannins (hydrolysable and condensed), stilbenes and lignans are the main group of polyphenol (Khan & Dangles, 2014).

2.5.1 Phenolic acid

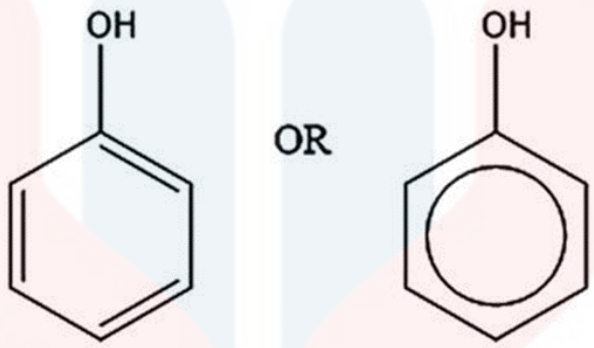

Figure 2.4: Basic structure of phenolic acid (Miguel-Chávez, 2017)

Figure 2.4 depicted the basic structure of phenolic acid consist of aromatic ring attach with one or more hydroxyl (OH) substituent. One-third of dietary phenols consist of phenolic acids and divided into two subgroups which are hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids have in common the C6-C1 structure while hydroxycinnamic acids are aromatic compound with three carbon side chain (Bravo, 1998). The source of phenolic acids can be found abundance in food of plant origin such as vegetables and fruits (Hertog, Hollman, Katan, & Kromhout, 1993). In addition, agro industrial by product have been explored as sources of natural antioxidant due to good sources of phenolic acids. Phenolic can present in the free form and also in conjugated form. Phenolic which commonly stored in cell vacuoles is a free phenolic which extracted with various aqueous alcohol-solvent mixture (Xiong et al., 2014).

For centuries, phenolic compound has been identified its benefit in human health. Phenolic compounds provide advantage effects due to attribute to their antioxidant activity. Phenolic compounds has numerous advantages which can exhibit a wide range

of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Balasundram, Sundram, & Samman, 2006). Determinant of antioxidant potentials of foods could be determined by phenolic compound present, and hence can be a natural source of antioxidant (Parr & Bolwell, 2000). However, phenolic compounds may be exhibit possible roles in carcinogenicity, genotoxicity, thyroid toxicity, interaction with pharmaceuticals, and estrogenic activity (for isoflavones) when consume at high level of concentration (Mennen, Walker, Bennetau-Pelissero, & Scalbert, 2005).

2.5.2 Flavonoid

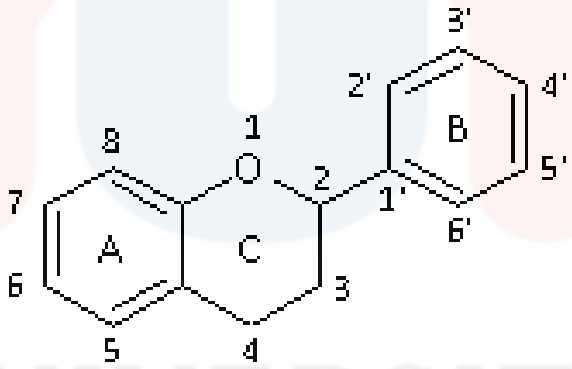

Figure 2.5: skeleton of diphenylpropane

The basic structure of flavonoid is made up of diphenylpropane skeleton. The diphenylpropane skeleton consist of two benzene rings named ring A and B as shown in figure 2.5 and they are linked by three carbon chains which forms a closed pyran ring (C ring, heterocyclic ring containing oxygen) with benzenic A ring (Tazzini, 2014). Thus, their structure is also known as C6-C3-C6. Flavonoids are known as secondary

metabolites of plant. It is functions as a physiological survival where it protect the plant from UV radiation and fungal infection (Kshatriyaa & Nazeruddin, 2013).

Besides, flavonoids are also identified as a plant pigment that can be found abundance in flowers and fruits. Red, yellow, blue and purple are the common colour for the pigments. The pigments are located in the plastids and cytoplasm of flowering plants. Other pigments which include carotenoids, chlorophylls betalains and certain flavonoids have important role in fruit ripening and capturing variants of light within the UV spectrum (Anderson, 2017). There are some important groups of flavonoids which include flavanols, flavones, anthocyanidins, flavanones and isoflavones.

Different classes of flavonoid have different pharmacological activities. Flavones has antioxidant benefit which can delay drug metabolizing activity. Anthocyanidin, which can be found in red and purple fruits such as pomegranates, are associate with heart health. Flavonones and flavanols can help in cardiovascular health and inflammatory activity while isoflavones can help in reducing the risk of hormonal cancer. (Szalay, 2015).

CHAPTER 3

MATERIAL AND METHOD

3.1 Materials

3.1.1 Chemicals and reagent

Chemicals and reagent that has been used in this study are ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), gallic acid, sodium carbonate, folin-ciocalteu reagent, dimethyl sulfoxide, methanol, aluminium chloride hexahydrate, sodium nitrite, sodium hydroxide and quercetin. All of these chemical were purchased from Sigma U.S.A.

3.1.2 Machine and equipment

UV-vis spectrophotometer, centrifuge, vortex, analytical balance, steamer, gas stove, micropipette, mortar and pestle, test tube 10 mL, test tube rack, centrifuge tube 2

ml and 25 mL, cuvette, blue cap bottle 500 mL, beaker, aluminium foil, spatula and dropper.

3.2 Methods

3.2.1 Plant material

Ipomoea batatas or sweet potato (orange flesh) were bought from Jeli market in Kelantan. The sample were washed and the skin were peeled off. Then the sample were cut in dice and put aside. Steamer were used to steam the sample. The lower layer was filled with 1 L water and allowed to boil. After the water was boiled, the sample were put in the second layer of steamer and cover with lid for 10 minutes. The step were repeated for 20, 30 and 40 minutes. The steamed sample is then crushed using mortar and pestle and kept in an air tight container.

3.2.2 Preparation of plant extract

Plant extract was prepared using ethanol solvent. Steamed extract of 10 g were soaked in 25 mL ethanol for 24 hours in room temperature. After 24 hours, 1.5 mL extract was transferred into 2 mL micro centrifuge tube and were spin at 10,000 rpm for 15 minutes using centrifuge machine. The extract were kept at room temperature until used for assay.

3.2.3 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

DPPH radical scavenging activity was determined by previous method explained by Akter et al., 2010. DPPH solution was prepared by adding 100 mL of ethanol into 0.004 g DPPH. The working solution was prepared by adding 2 mL sample with 2 mL DPPH solution. The mixtures were vortex and incubate in dark at room temperature for 30 minutes. At the same moment, a control containing 2 mL ethanol and 2 mL DPPH were prepared. After 30 minutes the absorbance was measured at 517 nm. BHT was used to make the standard calibration curve. The ability to scavenge DPPH radical was calculated as follows: DPPH radical scavenging activity (%) = [($Abs_{control}$ – Abs_{sample}) / $Abs_{control}$ x 100].

3.2.4 Total phenolic content (TPC)

Total phenolic content of *Ipomoea batatas* were determined quantitatively using Folin Ciocalteu method with minor modifications (Singleton and Rossi, 1965). In brief, 1 mL extract were diluted with 1 mL dimethyl sulfoxide (DMSO), then 0.5 mL were transferred into a test tube. The extract were added with 1.5 mL of 10% Folin-ciocalteu reagent and vortex thoroughly. The extract were incubate for 5 minutes. After 5 minutes, the mixture were added with 2 mL of sodium carbonate (75 g/L). Control were prepared by adding 1.5 mL folin-ciocalteu reagent with 0.5 mL DMSO and after 5 minutes, sodium carbonate were added. The working solution and the control were incubated at room temperature for 2 hours. Then the absorbance were measured at 765 nm using a UV-vis

spectrophotometer. The absorbance values were compared with Gallic Acid standard curve. The total phenolic content of samples were expressed as mg gallic acid equivalent (GAE) per gram raw material of extract (mg GAE/g RM). All samples were analyse in three replicates.

3.2.5 Total flavonoid content (TFC)

Total flavonoid content were determine by using aluminium chloride method with minor modification (Marina, Ribarova & Atanassova, 2005). For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock solution was prepared by dissolving 0.01 g in 10 mL methanol. A volume of 0.3 mL sample is added with 150 µl of 0.3 M $AlCl_3$ hexahydrate. The mixture was allowed to stand for 5 minutes, followed by addition of 1 mL of 1M NaOH . After incubate for 15 minutes, the absorbance was measured at 506 nm. The total flavonoid contain was expressed as Quercetin equivalent per weight of raw material (mg QE/ g RM).

3.2.6 Statistical analysis

All measurement were carried out in three replicates and the data were reported as mean ± standard deviations. Significant differences at $p \leq 0.05$ among the means from triplicate were determined by t-test using Microsoft Excel 2013.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preparation of extract

This study was conducted to investigate the effect of steaming on antioxidant activity, total phenolic content and total flavonoid content in the orange-fleshed root extracts of *Ipomoea batatas*. Steaming method were used in this study among various cooking method due to previous finding by Sikia and Mahanta (2013), steaming were identified as the most suitable method in most of the cases based on phytochemical retention and antioxidant activities. The steaming time used in this study range from 0 min to 40 min. The maximum steaming time chosen was 40 minutes because *I. batatas* texture was completely soft and suitable for consumption at 40 minutes of cooking. Steamed *I. batatas* were extract using ethanol for 24 hours before it were filtered. The colour observed from the *I. batatas* was yellow colour as depicted in Table 4.1. The yellow coloured observed from the *I. batatas* due to the presence of beta-carotene. According to Mateljan (2018), the orange-fleshed *I. batatas* was known to have exceedingly rich in beta-carotene and the intensity of the yellow or orange flesh was directly correlated to its beta-carotene (Mateljan, 2018).

Table 4.1: Colour extract observed from different steaming time of *Ipomoea batatas*.

Steaming time	Colour observed
0 min	Pale yellow
10 min	Intense yellow
20 min	Intense yellow
30 min	Intense yellow
40 min	Intense yellow

4.2 DPPH assay

The evaluation of antioxidant activity of extract by DPPH assay was depicted in Figure 4.1 and 4.2. Figure 4.1 showed BHT (butylated hydroxytoluene) standard calibration curve prepared by plotting percentage of DPPH free radical scavenging versus the concentration with R^2 value of 0.86 and the equation y= 0.369x + 25.888. The concentration used are 6.25, 12.5, 25, 50, 100 and 200 mg/mL. BHT standard was chosen over ascorbic acid because BHT gives large range for the absorbance value thus all the absorbance value for the samples extract falls in range of the standard curve.

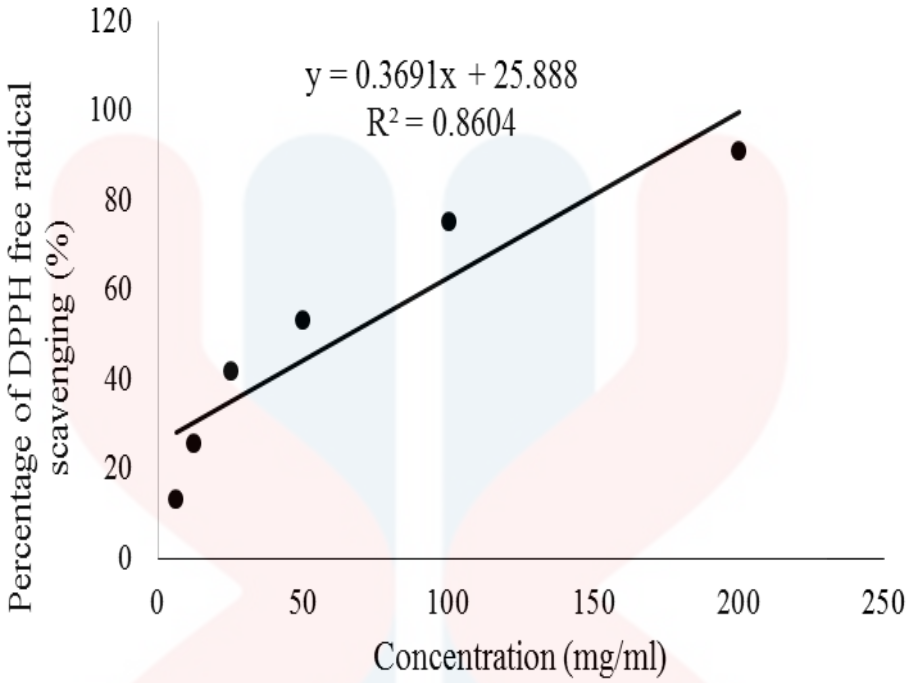

Figure 4.1: Graph of BHT standard calibration curve

Figure 4.2 showed the percentage of DPPH free radical scavenging at different steaming time which are 0 min (non-steamed), 10 min, 20 min, 30 min, 40 min and the value obtained are 16.220±0.484 %, 14.695±0.460 %, 17.561±0.211 %, 14.451±0.588 % and 15.61±0.642 %, respectively. Based on the results obtained, antioxidant in 20 min extracts were scavenged free radical more effectively followed by 0 min extract and the lowest antioxidant activity was in extract of 30 min steaming time. The data was analyse using t-test to compare the mean among the samples. The results obtained has significant when $p \leq 0.05$. Based on the t-test data, all the samples showed significant different ($p \leq 0.05$), except for paired samples of 10 min with 30 min, 0 min with 40 min and 30 min with 40 min which have $p \geq 0.05$ hence the samples can be said as having the same efficiency in scavenged the free radical.

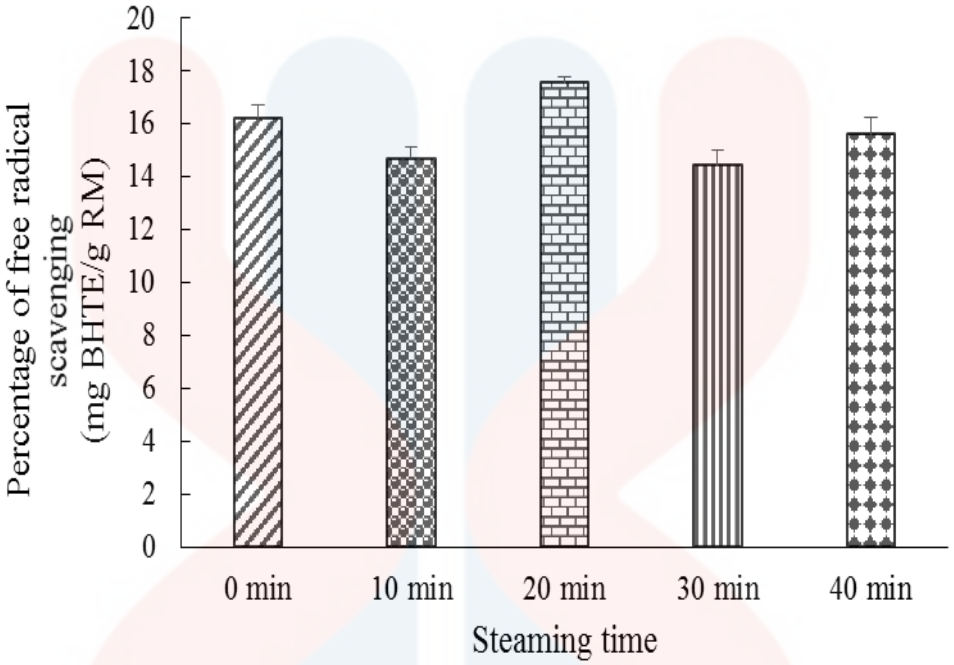

Figure 4.2: Antioxidant activity of the steamed *Ipomoea batatas* root extract by DPPH assay

This data clearly showed that steaming increased the antioxidant activity of the samples however with further increase of steaming time, the antioxidant in the *I. batatas* might be destroyed. This result was in agreement with several researchers that studies on effect of heat towards antioxidant activity in several plants extract such as carrot puree (Damian & Oroian, 2013), chilli pepper (Shaimaa et al., 2016), eggplant (Arkoub-Djermoune et al., 2016), citrus peel (Jeong et al., 2004), chokeberry (Cristea, 2016) and tropical leafy vegetables (Nwozo, Oso & Oyinloye, 2016). The high antioxidant activity in the sample extracts might be due to the presence of phenolic compounds. Previous study also found that heating does not causes drastic loss in antioxidant values, thus heating promotes antioxidant activity in fruits and vegetables because of the enhancement

of the antioxidant properties of naturally occurring compounds or the formation of novel compound such as Maillard reaction products that have antioxidant activity (Gorinstein et al., 2008; Manzocco et al., 2001).

Another reason for the increase of antioxidant activity due to the food matrix interaction in the orange-fleshed *I. batatas*. Food matrix can be defined as the nutrient and non-nutrients components of food and their molecular relationships such as chemical bond to each other. The orange-fleshed *I. batatas* were known to have exceedingly rich in beta-carotene and the intensity of the yellow or orange flesh was directly correlated to its beta-carotene (Mateljan, 2018). However there is also report suggested that antioxidant activity of most foods is reduced after heating at 65 ºC or 100 ºC (Yin & Cheng, 1998).

4.3 Determination of total phenolic content

Total phenolic content was determine by using the Folin-ciocalteu method as metion by Singleton and Rossi, 1965. Total phenolic content assay techniques is based on oxidation/ reduction reaction where it involves transfer of single electron (SET) from phenolic compound to form blue chromophore constituted by a phosphotungstic/ phosphomolybdic complex in alkaline solution and can be observed spectrophotometrically in range 750-765 nm (Prior, Wu & Schaich, 2005). The results for total phenolic content assay were depicted in Figure 4.3 and 4.4. Figure 4.3 shows a standard calibration curve of gallic acid with R^2 value of 0.929 with the equation y = 0.008x + 0.297.

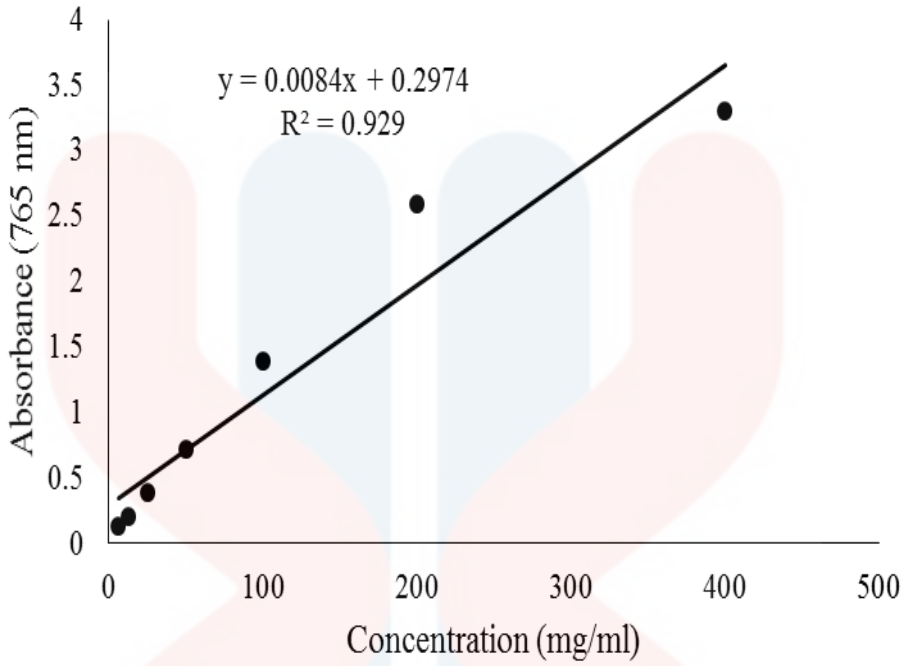

Figure 4.3: Gallic Acid standard calibration curve

Figure 4.4 shows the total phenolic content of *Ipomoea batatas* at different steaming time which are 0 min, 10 min, 20 min, 30 min, 40 min and the value obtained are 7.299±0.076, 12.291±0.008, 16.802±0.676, 8.724±0.063 and 10.482±0.039 mg GAE/ g raw material respectively. Based on Figure 4.4, the highest total phenolic content was sample with 20 min steaming time with value of 16.802 mg GAE/g RM followed by second highest was 10 min sample 12.291 mg GAE/g RM. The lowest total phenolic content was 0 min which is non-steamed sample with value 7.299 mg GAE/g RM. The data was analyse using t-test to compare the significant different among the sample. Based on the t-test data, all the samples showed significant different where $p \leq 0.05$.

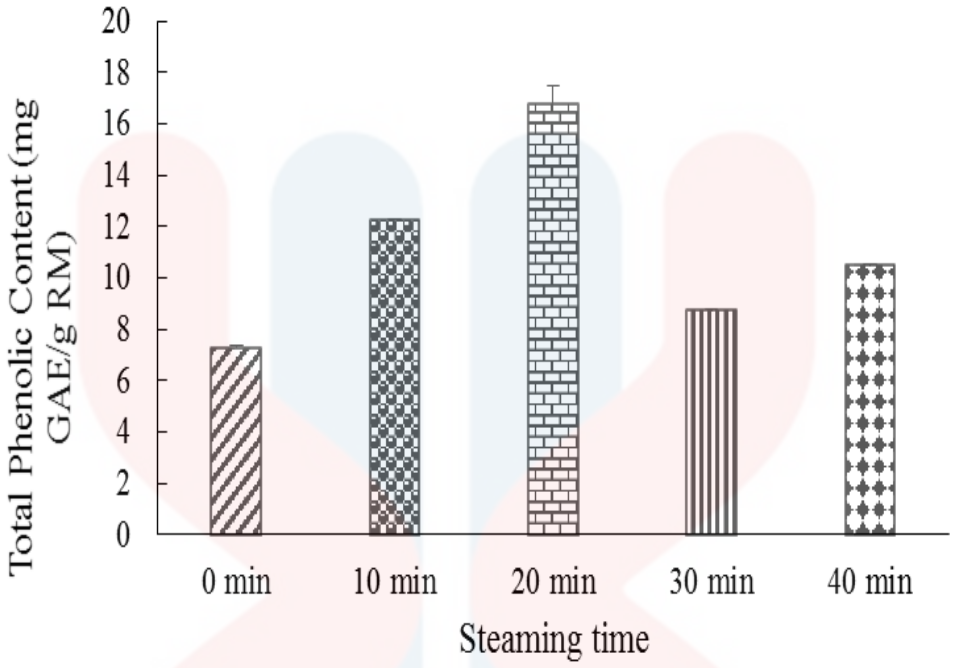

Figure 4.4: Total Phenolic Content (TPC) in steamed *Ipomoea batatas* root extracts

This results are in agreement with previous study by Sharma et al., (2015), where the total phenolic content of six onion varieties was significantly increased after heated at 80 ºC, 100 ºC and 120 ºC for 30 minutes each. The results also in line with Hortova, Suhaj and Simko, (2007) found that thermal treatment on black pepper induced an increase in the content of phenolic substances. The reason for increasing total phenolic content after heating was either due to cleaving of the esterified and glycosylated bond or by Maillard reaction products that are responsible for the increase in total phenolic content after heating (Berset, 1996). In addition, a formation of Maillard products which resulted from degradation of phenolic compounds during thermal treatment have been observed in several experiments where these products showed the significantly higher antioxidant activity than the initial phenolic compounds (Tamanna and Mahmood, 2015; Buchner et al., 2006; Murakmi et al., 2004).

Previous study also found that increase in antioxidant activity of some vegetables after thermal treatment include the liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub-cellular compartments, suppression of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes and production of new non-nutrient antioxidants Maillard reaction products (Odukoya et al., 2007; Morales et al., 2002). Maillard reaction process can prevent enzymatic browning reaction caused by polyphenol oxidase (Billaud et al., 2005). In addition, Ferracane et al., (2008), found that thermal treatments increased the bioavailability of polyphenols most likely as a result of a weakening of the plant biomass allowing for greater bioavailability of polyphenols contained inside the cell walls.

Plant derived products such as fruit and vegetables produce many endogenous phenolic compounds during postharvest handling and processing. The compounds are oxidized by oxidoreductase enzymes like polyphenoloxidase and tyrosinases. This reaction generates highly reactive quinonic compounds that are condensed and polymerized to produce brown pigments thus reduces the quality of food product (Tamanna and Mahmood). Maillard reaction process can prevent this enzymatic process thereby help to maintain the product quality. High temperature used in various cooking methods speed up the maillard reactions and accelerate the evaporation of water. According to Natella et al., (2002) it has been reported that some Maillard reaction process particularly melanoidins have beneficial effects on health such as antioxidant and antibiotic effect.

4.4 Determination of flavonoid content

Evaluation of total flavonoid content in orange-fleshed *Ipomoea batatas* were carried out using aluminium chloride (Marina, Ribarova & Atanassova, 2005). The results for total flavonoid content in the samples extract were depicted in Figure 4.5 and 4.6. Figure 4.5 shows standard calibration curve of Quercetin with R^2 value equal to 0.0994 with the equation y = 0.0003x + 0.0029.

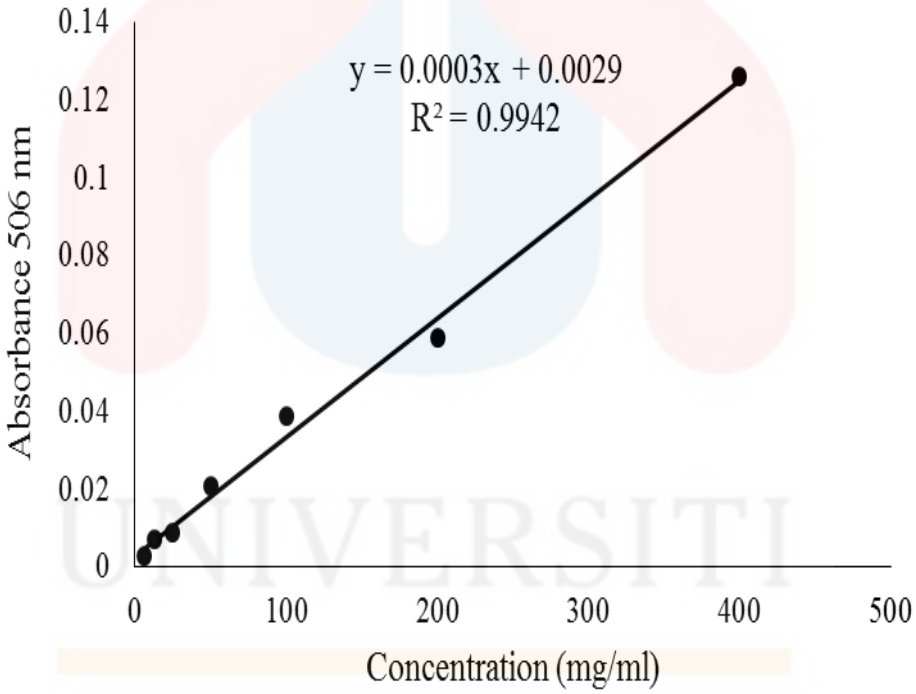

Figure 4.5 Quercetin standard calibration curve

Figure 4.6 shows the total flavonoid content of different steaming time of *I. batatas* which are 0 min, 10 min, 20 min, 30 min, 40 min and the value are 6.296±0.160, 27.861±0.621, 20.498±0.862, 13.770±1.136 and 8.148±0.2778 mg QE/ g raw material respectively. Based on Figure 4.6 graph, the highest total flavonoid content was 10 min sample with the value of 27.861 mg QE/g RM followed by 20 min sample as the second highest total flavonoid content with value of 20.498 mg QE/g RM. The lowest total flavonoid content was 0 min which non-steamed sample with value 6.296 mg QE/g RM. The data was analyse using t-test to compare the significant among the samples. Based on the t-test data, all the samples showed significant different where $p \leq 0.05$.

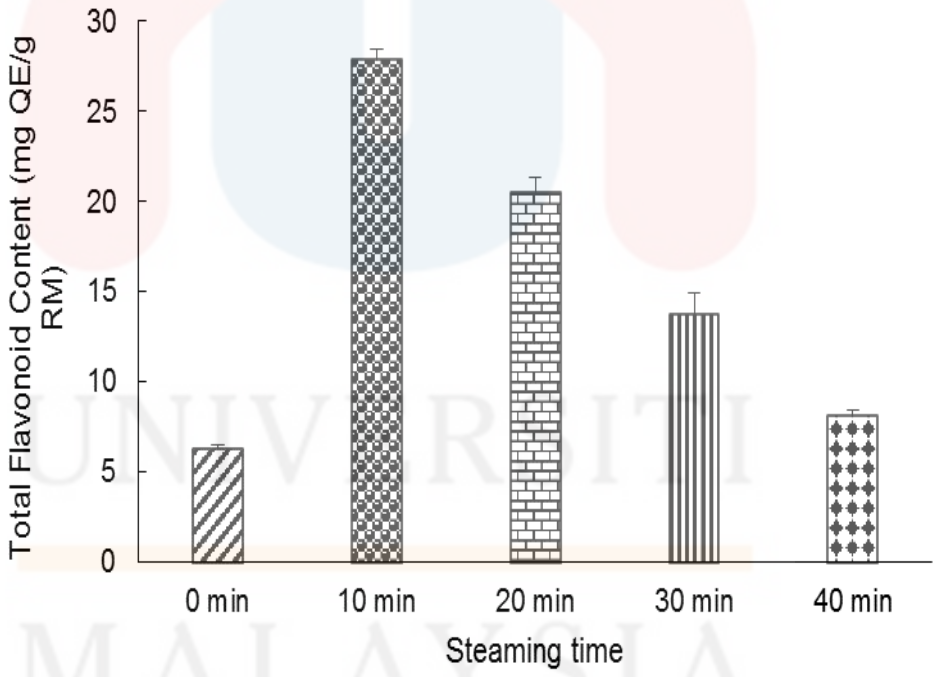

Figure 4.6: Total Flavonoid Content (TFC) in steamed *Ipomoea batatas* root extracts

Based on the results in Figure 4.6, it can be conclude that initial steaming increase the flavonoid content but with further increase of steaming at 40 min degraded the total flavonoid content which indicate that some flavonoid were probably destroyed. However, among non-steamed with steamed samples, it showed that flavonoid content has significant increased after steaming. According to Manach et al., (2004) in most fruits and vegetables, flavonoids contain C-glycosidase bonds and the industrial processing such as heating, boiling or steaming results in the formation of monomers by the hydrolysis of C-glycosides bonds.

The degradation of flavonoid depends on structural solidity therefore a double bonds need more energy in order to be degraded thus modification of structure such as steaming lead to changes in antioxidant activity (Da Costa et al., 2002). According to Chaaban et al., (2017), degradation product synthesized can decrease antioxidant activity which mean that degradation products have lower antioxidant activity or remain constant antioxidant activity indicating that degradation products have the same antioxidant activity as native flavonoid or antioxidant activity can increase which mean degradation products have a higher antioxidant activity.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Changes in antioxidant activity in orange-fleshed *Ipomoea batatas* has significant effect on different steaming times. This findings indicate that mild and short steaming process enhances phenolic, flavonoid and total antioxidant activity. The steamed samples show higher antioxidant activity, total phenolic as well as total flavonoid content. However, further steaming time up to 40 min might destroyed the antioxidant compound hence decrease the total phenolic and flavonoid content in orange-fleshed *Ipomoea batatas*.

5.2 Recommendation

Orange-fleshed *Ipomoea batatas* has high antioxidant, known as beta-carotene which converts to vitamin A once consumed. Therefore, further research on heat stable

antioxidant need to be conducted using various thermal treatment due to different thermal treatment such as boiling and microwave may have different antioxidants effect. In addition, DPPH assay alone cannot proven the antioxidant activity thus more antioxidant assays such as ABTS and FRAP can be conducted to strengthen the antioxidant claim in orange-fleshed *Ipomoea batatas*. Potential antioxidant in other parts of *Ipomoea batatas* such as in leaves can be investigate as it may contribute to medicinal health functions, functional food as well as neutraceutical application.

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APPENDICES

APPENDIX A

Table A.1 Percentage DPPH free radical scavenging in sample extracts

Steaming time	R1	R2	R3	Mean ± STD
0 min	16.768	16.037	15.854	16.220±0.484
10 min	14.756	15.122	14.207	14.695±0.460
20 min	17.683	17.317	17.561	17.561±0.211
30 min	14.207	14.024	14.451	14.451±0.588
40 min	15.671	16.220	15.610	15.610±0.642

Table A.2 Total Phenolic content in sample extracts

steaming time	R1	R2	R3	Mean ± STD
0 min	7.211	7.331	7.353	7.299±0.076
10 min	12.300	12.283	12.291	12.291±0.008
20 min	16.144	16.768	17.494	16.802±0.676
30 min	8.652	8.764	8.756	8.724±0.063
40 min	10.437	10.496	10.512	10.482±0.039

Table A.3 Total flavonoid content in sample extracts

Steaming time	R1	R2	R3	Mean ± STD
0 min	6.204	6.481	6.2037037	6.296±0.160
10 min	27.164	28.060	28.358209	27.861±0.621
20 min	21.493	20.000	20.000	20.498±0.862
30 min	13.115	15.082	13.115	13.770±1.136
40 min	7.870	8.148	8.426	8.148±0.278

APPENDIX B

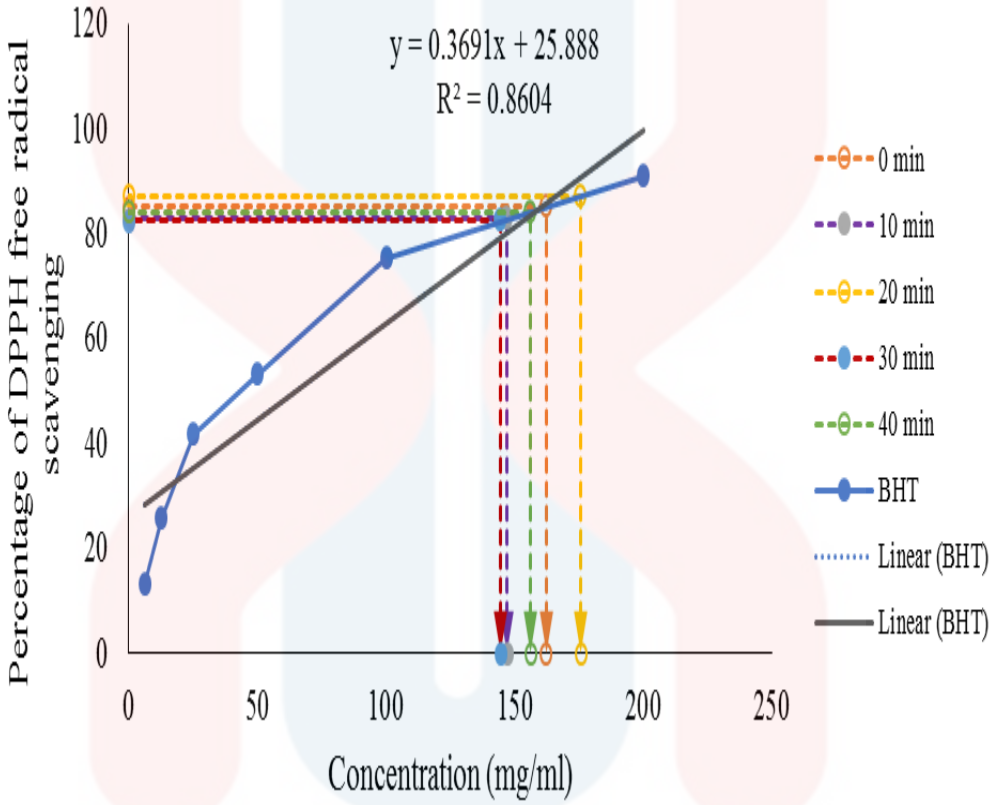


Figure B.1: Equivalent graph of BHT

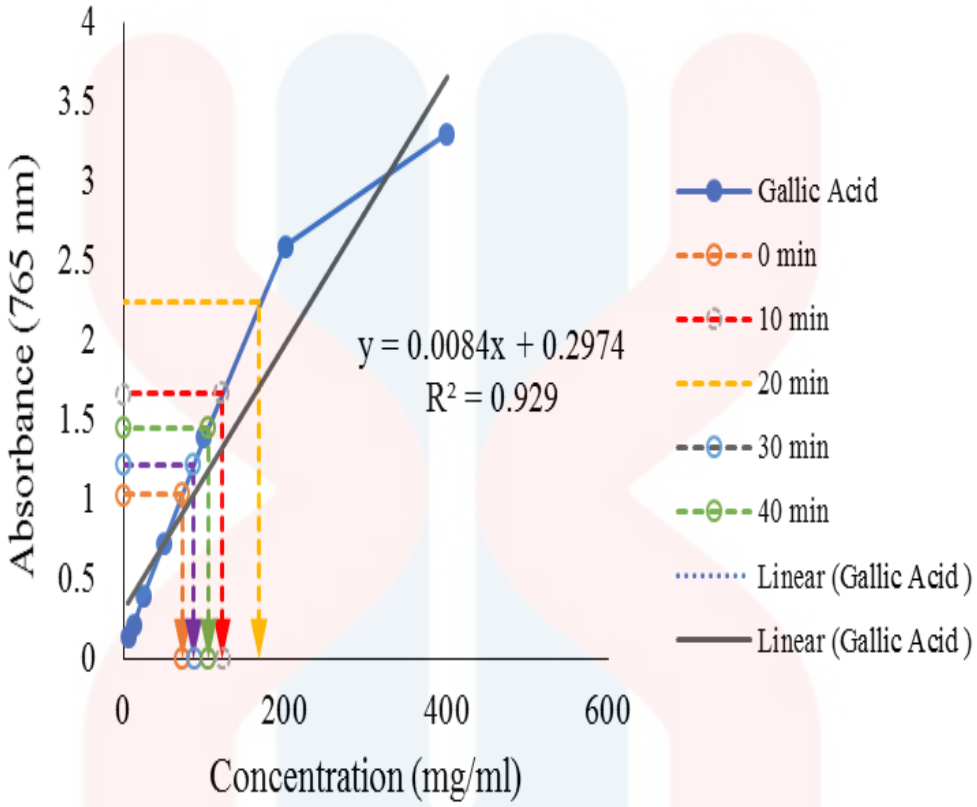


Figure B.2: Equivalent graph of gallic acid

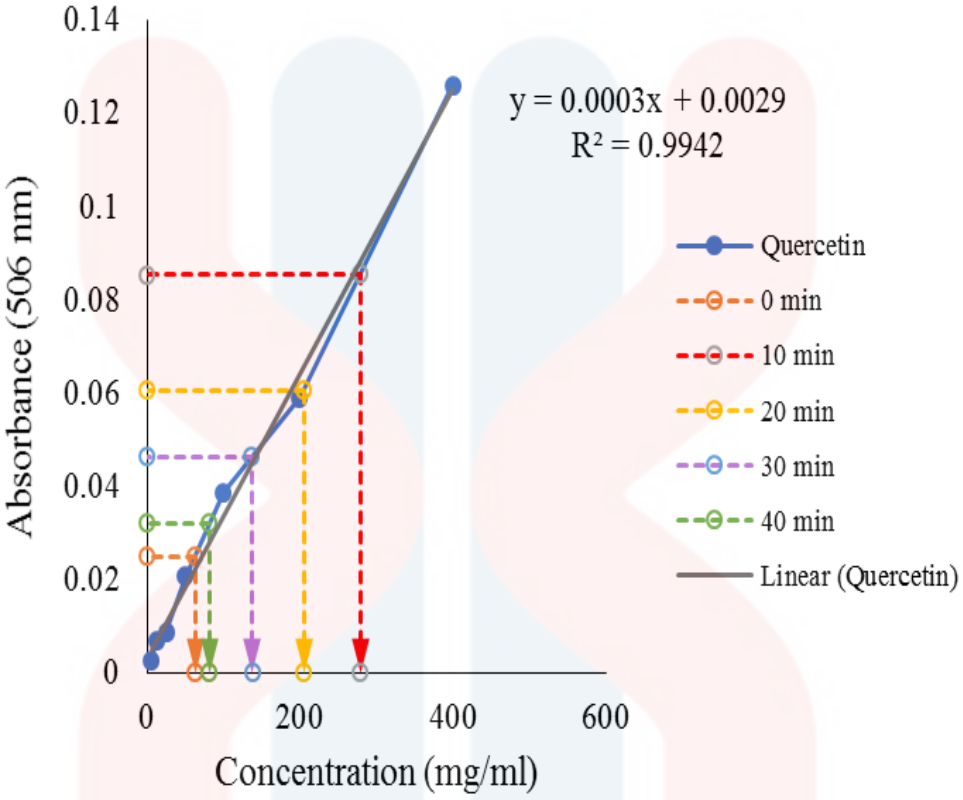


Figure B.3: Equivalent graph of quercetin

APPENDIX C

Table C.1 T-test analysis for DPPH, TPC and TFC assay

Sample	*p*-value		
	DPPH	TPC	TFC
0 min vs 10 min	0.021	4.60×10^{-5}	1.28×10^{-4}
0 min vs 20 min	0.018	6.78×10^{-4}	7.47×10^{-4}
0 min vs 30	0.041	3.20×10^{-5}	2.81×10^{-3}
0 min vs 40	0.133	2.24×10^{-5}	4.93×10^{-3}
10 min vs 20 min	0.008	3.74×10^{-2}	6.54×10^{-3}
10 min vs 30 min	0.361	6.44×10^{-5}	1.07×10^{-3}
10 min vs 40 min	0.007	1.08×10^{-4}	5.64×10^{-5}
20 min vs 30 min	0.004	9.98×10^{-4}	1.07×10^{-2}
20 min vs 40 min	0.027	1.69×10^{-3}	1.34×10^{-3}
30 min vs 40 min	0.121	3.87×10^{-5}	7.06×10^{-3}

APPENDIX D

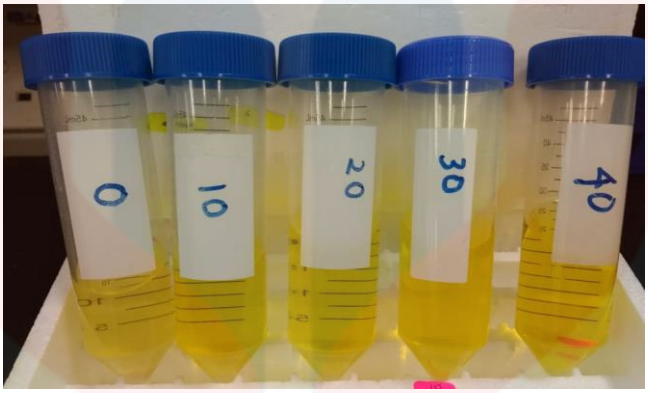


Figure D.1: Colour of *Ipomoea batatas* extract with different steaming time

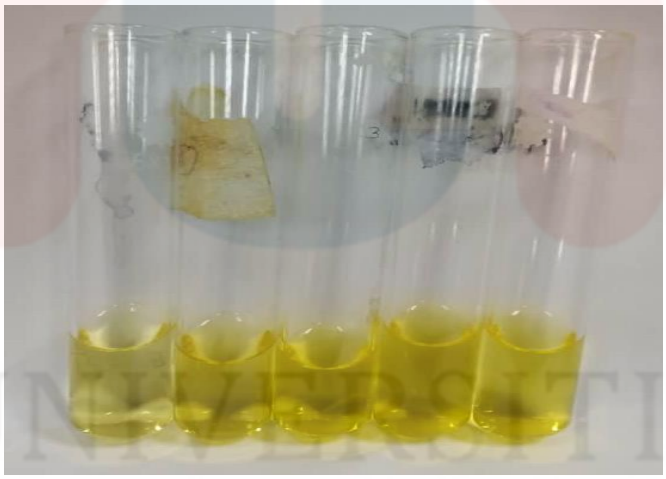


Figure D.2: DPPH reagent with sample extract

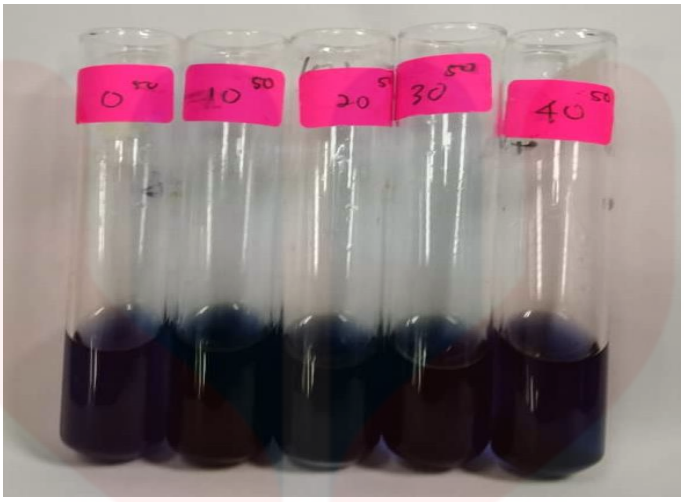
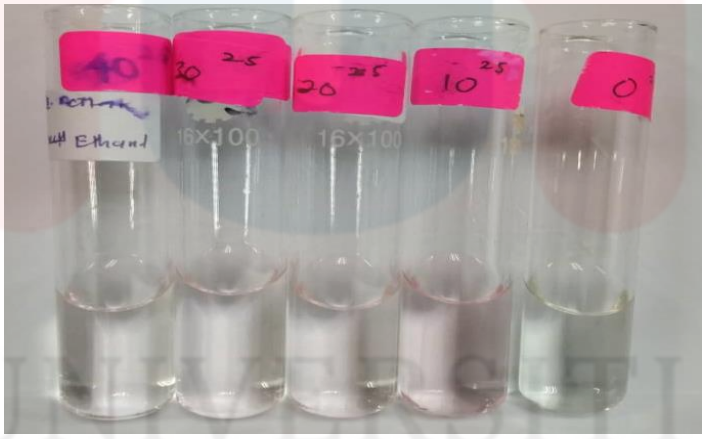


Figure D.3: Folin-Ciocalteu reagent with sample extract



D.4: Aluminium Chloride hexahydrate reagent with sample extract