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DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT CAPACITY IN RAW AND COOKED POTATO, SWEET POTATO AND TARO

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A project submitted as partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) form the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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LIST OF ABBREVIATIONS

	Abbreviations	Full form
1.	TPC	Total phenolic content
2.	TFC	Total flavonoid content
3.	FRAP	Ferric Reducing Antioxidant Power
4.	DPPH	2,2-diphenyl-1-picrylhydrazyl-hydrate
5.	BCB	Beta Carotene Bleaching Activity
6.	GAE	Gallic acid equivalent
7.	QE	Quercetin equivalent
8.	AOC	Antioxidant capacity
9.	TPTZ	2,4,6-Tripyridyl-S-triazine
10.	BHT	butylated hydroxytoluene
11.	HCl	Hydrochloric acid
12.	NaOH	Sodium hydroxide
13.	DW	Dried weight
14.	MRPs	Maillard reaction products
15.	DNA	Deoxyribonucleic acid
16.	ROS	Reactive Oxygen Species
17.	RNS	Reactive Nitrogen Species

Abstract

DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT CAPACITY IN RAW AND COOKED POTATO, SWEET POTATO AND TARO

Nur Diyana Binti Zolkiffly

Natural antioxidants are widely distributed in tubers and root crops. The effective proper assessment of antioxidants from food and medicinal plants is crucial to explore the potential antioxidant sources and promote the application in functional food study. Potatoes, sweet potatoes and taro contained compounds such as polyphenol which may have health-promoting effects. In this study, the flesh tissues of those three tubers and roots varieties were treated with the thermal process (frying, steaming and boiling). Higher levels of total phenolics, total flavonoids and antioxidant activity were found in the flesh of all tubers, depending on the thermal process applied. When the thermal process was applied, fried samples showed the highest values in all potato and taro, as well in antioxidant activity markedly higher in sweet potato variety. The total phenolic content in potato flesh was 18.88 - 66.58 mg/g gallic acid equivalents, 28.06 - 90.01 mg/g gallic acid equivalents in sweet potato and 8.22 - 40.22 mg/g gallic acid equivalents for total phenolics in taro flesh. Meanwhile, the total flavonoid content in potato was reported within 4.75 -15.75 mg/g catechin equivalents,9.25-18.75 mg/g catechin equivalents in sweet potato and the least total flavonoids were count in taro, ranging from 1.75-8.80 mg/g catechin equivalents. Strong positive correlations were found among total phenolics, total flavonoids and antioxidant activity but there is no significant result of these three sample using beta-carotene bleaching assay. Harvest process, storage and tubers maturity may also significantly affected the parameters studied. The present study provides a piece of idea and information on the determination of phenolic, flavonoid and antioxidant capacity in selective potato, sweet potato and taro.

Abstrak

PENETAPAN KANDUNGAN FENOLIK,FLAVONOID DAN AKTIVITI ANTIOKSIDAN DI DALAM KENTANG,KELEDEK DAN TARO MENTAH DAN SETELAH DIMASAK

Nur Diyana Binti Zolkiffly

Antioksidan semulajadi dijumpai secara meluas dalam tumbuhan jenis berubi. Penentuan kadar antioksidan dari tumbuhan makanan dan ubat-ubatan adalah penting untuk meneroka sumber-sumber antioksidan yang berpotensi dalam makanan harian. Kentang, ubi keledek dan ubi taro mengandungi sebatian seperti polifenol yang mungkin mempunyai kesan baik untuk kesihatan. Dalam kajian ini, tiga jenis ubi telah dirawat dengan proses termal (menggoreng, mengukus dan mendidih). Tahap total fenolik, flavonoid dan aktiviti antioksidan didapati dalam semua ubi, bergantung pada proses termal yang digunakan. Apabila proses termal digunakan, sampel goreng menunjukkan nilai tertinggi dalam sampel kentang, keledek dan taro, begitu juga dengan aktiviti antioksidan yang tinggi di dalam ketiga-tiga sampel. Kandungan fenolik dalam kentang adalah setara dengan 18.88 - 66.58 mg / g asid gallic, 28.06 - 90.01 mg / g asid gallic dalam ubi keledek dan 8.22 - 40.22 mg / g asid gallic untuk jumlah fenolik dalam ubi taro. Sementara itu, jumlah kandungan flavonoid dalam kentang dilaporkan dalam 4.75 -15.75 mg / g katekin, 9.25-18.75 mg / g katekin setara dalam ubi keledek dan jumlah flavonoid yang paling sedikit adalah jumlah dalam taro, antara 1.75-8.80 mg / g katekin. Korelasi positif yang kuat didapati di kalangan jumlah fenolik, jumlah flavonoid dan aktiviti antioksidan tetapi tidak terdapat hasil yang ketara dari ketiga-tiga sampel tersebut dengan menggunakan *beta-carotene bleaching assay*. Proses penuaian, penyimpanan dan tumbuhan matang juga mungkin memberi kesan ketara terhadap parameter yang dikaji. Kajian ini menyediakan satu idea dan maklumat tentang penentuan kapasiti fenolik, flavonoid dan antioksidan dalam kentang terpilih, keledek dan taro.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Vegetables and fruits are the primary sources of natural phytochemicals that promote the great quality of nutrition in the human diet (Prajapati et al., 2013). Despite vegetables and fruits, starchy tubers and roots are also greatly known as the contributor of phytochemicals in the human diet (Chandrasekara & Josheph Kumar, 2016). Potato (*Solanum tuberosum L.)* which belongs to the tubers group responsible as the functional foods for the population in any region worldwide (Zhang Hong, Xu Fen, Wu Yu & Hu Hong-Hai, 2017). The potatoes are categorized into a few main groups based on common characteristics, such as russet potatoes (rough brown skin), red potatoes, white potatoes, yellow potatoes and purple potatoes (Kita et al., 2013). Potato has medium to large-sized, oblong or slightly flattened and oval in shape with medium russet-brown netted colour skin and white to yellow pale flesh. Sweet potato also belongs to tubers crop with pale brown colour skin and have varieties of flesh colour depending on the cultivars (Jamal & Othman, 2014).In contrast to the tuber, root crop which is taro *(Colocasia esculenta)* is the most widely consumed in many regions of the world (Simsek & El, 2015). Taro also has a different genotype which differs in fleshed colour and the common characteristic is white to purple in colour with cylindrical to an almost round shape (M Alcantara, 2013). In this study, potato, sweet potato and taro were used as the sample as they were widely available in the Malaysian market.

Akyol et al. (2016) trace the evidence that different nutrients and bioactive compounds such as starch, dietary fibre, amino acids, minerals, vitamins, and phenolic compounds were found in potato. Several works showed that these potato compounds exhibited health-promoting effects in humans. It is generally accepted that many natural phytochemicals such as phenolic compound and vitamins have a mutual effect on antioxidant capacity in food (Hara et al., 2018). The primary phenolic compounds present in potatoes are phenolic acids and flavonoids. Galani et al. (2017) affirm that potato tubers contain high amounts of antioxidant compounds which phenolics contribute to 58%–82% of the total antioxidant activity. Meanwhile, the most abundant flavonoids found in potato are particularly catechin, epicatechin, and anthocyanidins (Ezekiel, Singh, Sharma, & Kaur, 2013).

Sweet potato is mainly grown in the tropical and subtropical regions, where the bulk of the crop is cultivated and consumed (Fu et al., 2016). In Malaysia, the demand for sweet potato meets the Malaysian preference especially as the main ingredients of making traditional kueh such as *keria, cucur badak* and *pengat*. In fact, FAO (2013) reported that China is the leading producer, with an annual production of 705 million tons (68.40% of the world's production). Sweet potato and its byproduct are reported rich in polyphenols, proteins, vitamins and minerals despite a good source of carbohydrates (M. Zhang & Mu, 2017). Indeed, the polyphenol content of many sweet potatoes cultivars was two to three times higher than some common green leafy vegetables such as spinach and kale (Sengul, Yildiz, & Kavaz, 2014). Due to this findings, many pharmaceutical studies revealed that sweet potato leaf polyphenols exhibited various health-promoting biological activities, such as antioxidant activity, anti-mutagenicity, anti-cancer, anti-carcinogenesis, anti-microbial, anti-diabetes, anti-inflammation (Chandrasekara & Josheph Kumar, 2016).

Taro *(Colocasia esculenta)* is an essential root crop in the humid tropics and a valuable source of essential mineral nutrients (Simsek & El, 2015). The same study added that taro corm represents a large underground stem that stores starch and other nutrients.

The corm consists of three main parts: the skin, the cortex and the core (the central part). Studies of taro nutritional composition suggest that it contains a range of important macronutrients and micronutrients. Some studies of the mineral compositions of taro corms suggest that potassium is the most abundant mineral followed by magnesium, phosphorus, and calcium (M Alcantara, 2013). In addition, data from the literature reveal that appreciable amounts of zinc are also present but from a nutritional standpoint, taro is rather low in iron and manganese. The nutritional composition of taro corms can vary widely and depends on the genotype, the growing conditions, and the interaction between the genotype, the environment and age of a plant (Mergedus et al., 2015).

The antioxidant contained in food is well appreciated. Antioxidant properties of tuber and roots crops are primarily due to the presence of plant phenolic (Lin et al., 2016). Through many interests in nutrition and health study and epidemiological data available, the increasing study reported on the health-promoting effect of antioxidant against oxidative stress, induced degenerative and age-related disease, combat cancer and delayed ageing and much more important role has gained renewed attention (Ji et al., 2012). The chemical-based assay is the most widely applied in the evaluation of the antioxidant properties of plant extract (Knezevic et al., 2011).

Few researchers have addressed the problem that multiple factors such as genotype difference, cultivation techniques, industrial treatment, domestic process, and postharvest storage condition may alter the stability of total phenolic content and antioxidant activity in tubers and roots crops (Galani et al., 2017). All type of tubers and roots must be cooked before consumption as the digestive enzyme, amylase needs to further convert the starch contained into glucose for nutrition (Awa & Eleazu, 2015). The processing step of potato and taro is crucial and that consists of various type of cooking methods such as boiling, frying, poaching, and baking. The most common forms of potato and taro consumption are mashed potatoes, fries or chips and ice cream (Ruiz et al., 2018).

In addition, all tubers and roots crop must be cooked before consuming due to the hard texture and in purpose to improve the palatability (Govela & Bornhorst, 2016). However, the thermal process applied with the various cooking method may interfere, either increase or decrease the bioactive constituents in the tubers and roots crop (Xu, Chen, Cao, Xia, & Jiang, 2016). A group of researcher agreed that domestic cooking induces adverse effects, such as the loss of important nutrients and formation of undesired compounds such as acrylamide or other toxic molecules (Tian et al., 2016). For instance, taro has the acridity properties, which result due to the presence of calcium oxalate (needle-like crystal) which can easily penetrate into soft skin and can cause irritation after consumption (Kumoro et al., 2014). Accordingly, the prolong heat applied through cooking may reduce the calcium oxalate and make the taro safer to eat. Furthermore, the functional components and antioxidant capacities being affected by different home cooking ways (Lewu, Adebola, & Afolayan, 2010). There are study proposed that such thermal processing can cause impairment of the functional compounds of sweet potato (Evelyn, Milani, & Silva, 2017). In contrast, there have been reports on the negative correlation between heat treatments applied on food and the effect on bioactive substances such as anthocyanins (De Aguiar Cipriano, Ekici, Barnes, Gomes, & Talcott, 2015).

1.2 Problem Statement

Most studies tended to focus only on the macronutrient present in potato. Despite this interest, there is still a need for concerning antioxidant capacity found in this tuber. The characteristics of phytochemicals and antioxidant capacity of cooked potato, sweet potato and taro have not been dealing with in-depth and remain unclear. In this context, very limited data studied on phytochemicals in the starchy tuber and roots crops. Despite that, the core problem statement in this study is on how the consumption of phytochemicals content in starchy tubers and roots crop and might help in preventing and reducing many metabolic diseases.

1.3 Significance of The Study

This study is conducted to mimic the industrial treatment and domestic process of potato, sweet potato and taro that are common practice in Malaysian industries and household. A striking feature on the effect of the cooking process applied on potato tubers may alter their phytochemicals properties and antioxidant capacity will be highlighted. This will contribute to an established evidence-based study for further research that may beneficial to community understanding and knowledge.

1.4 Objectives

General objective

To determine total phenolic content, total flavonoid content and antioxidant capacity in raw and cooked (fried, boiled & steamed) potato, sweet potato and taro.

Specific objectives

1. To determine and compare total phenolic content and total flavonoid content in raw and cooked (fried, boiled & steamed) potato, sweet potato and taro using the Folin-Ciocalteu's method and aluminium chloride colourimetric method.
2. To determine and compare antioxidant capacity in raw and cooked (fried, boiled & steamed) potato, sweet potato and taro using DPPH assay, FRAP assay and β-carotene bleaching assay
3. To determine the correlation between total phenolic content, total flavonoid content and antioxidant capacity in raw and cooked (fried, boiled & steamed) potato, sweet potato and taro.

1.5 Hypothesis

1. There are no significance differences among the means of total phenolic and flavonoid content in raw and cooked (fried, boiled & steamed) potato, sweet potato and taro.
2. There are no significance differences among the means of antioxidant capacity in raw and cooked (fried, boiled& steamed) potato, sweet potato and taro.
3. There are no significance associations between total phenolic content, total flavonoid content and antioxidant capacity in raw and cooked (fried, boiled & steamed) potato, sweet potato and taro.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Potato, sweet potato and taro: the story behind tubers and roots crops

In many Asian regions, tubers and starchy roots become the second most important staple energy sources cultivated after cereals (Singh & Saldana, 2011). They provide ample scale of the functional food supply in the world, range from animal feed to human consumption and extensive industrial used as the processed product (Ji et al., 2012). The common tubers and roots crops are potatoes, cassava, yam, taro, sweet potato, and jicama. In addition, all of the crops were easily propagated by vegetative parts which include tubers, stem cutting, vine cutting, side shoots, stolons, and cormheads, also the ability to growth in varies soil for bigger yield, adaptation to environmental temperature and also demands low agricultural budget (Chandrasekara & Josheph Kumar, 2016). In fact, tubers and roots crops promote numerous health benefits in the human diet as it can play vital roles in disease risk reduction such as antioxidative agent, antimicrobial and immunomodulatory activities (Tian, Chen, Ye, & Chen, 2016). The presence of bioactive constituents in the tubers and roots such as phenolic compounds, glycoalkaloids, saponins, and bioactive protein are the contributor for the health-desirable effect.

Potato, sweet potato and taro were the chosen tuber and roots crops in this study. Potato *(Solunum tuberosum)* is a tuber plant, originated from the Andes mountain of South America, specifically in Chile and Peru. They are reported as the world's largest crop after

maize, wheat, and rice, yet it becomes a staple food in many regions worldwide (Hong et al., 2017). Although the demands in the local market and food industry are high, none of the potatoes is cultivated and harvest in Malaysia and all of this crops were exported mainly from the United States in many grades (Ministry of Agriculture and Agro-Based Industry Malaysia, 2010). Food and Agriculture Organization of the United Nation reported that in align advancement of technologies of selective breeding, there are now more than 1,000 different types of potatoes, differ in size, skin and flesh colour as well as the phytochemical constituents that are available for human's consumption (Ahmed et al., 2018). The most common potato found in our market is Russet Burbank, Waxy Yukon Gold, and Red Bliss Potatoes. Potato has the earthy flavour and can be eaten only after undergoes the cooking process such as fry, boil and bake as it allows the digestibility of it In the daily diet, an exchange (per serving) for starch tubers approximately contains 15 grams carbohydrates, 3 grams protein and trace fat with 80 calories. A serving of potato if prepared by baked or steamed consist of 1 small size, about 3 oz meanwhile mashed potato required approximately ½ cup for carbohydrate exchange (Malaysian Diabetes Education Manual, 2016).

Despite potato, sweet potato *(Ipomoea batatas)* is also one of the prospective natural food belongs to tubers crops that have the potential of enriching the food with fibre and abundant content with nutrients (Selvakumaran et al., 2018). The same study reported that in the worldwide production, sweet potato has become the sixth most cultivated food crop and was planted in many regions as it served the purpose of food sources, animal feed and source for industrial raw material. Sweet potato is originated from Latin America and along with the globalization, this important economic crop has become staple foods crops in many Asians as well, especially in China and Japan (Rodrigues de Albuquerque, Sampaio, & Leite de Souza, 2018). Unlike the potato, the same study proposed that sweet potato is cultivated worldwide in both tropical and subtropical climates, also growing well all the year, hence, making it susceptible to grow in Malaysia. The cultivars of sweet potato are also diverse, as

obviously can be seen in the flesh colour, ranging from orange, yellow, white, red and purple (Hamouz et al., 2011). The difference in flesh colour also contributes to different bioactive constituents present in each cultivar (Chandrasekara & Josheph Kumar, 2016b). In our local market, sweet potato with orange, yellow and white flesh are more common. Packed numbers of simple fermentable sugars such as glucose, fructose and sucrose contribute to the natural strong sweet taste in sweet potato (Rodrigues de Albuquerque et al., 2018). A half cup of cooked sweet potato represents one serving of starches which relatively contains 15 grams of carbohydrates and contribute 80 calories per serving in the human diet.

In contrast, Taro or scientifically known as *Colocasia esculenta* is one of the starchy roots belongs to *the Araceae* family. FAO stated that Taro is also called by various names such as cocoyam, eddoe, and dasheen based on many regions in the world. Taro was cultivated in all over tropics and subtropics parts worldwide ranging from eastern India to the whole of Southeast Asia. Unlike the potato, this roots crop which is also known as 'Yam' or 'Keladi' can be cultivated in Malaysia and already being the iconic farm product in Kinta Valley, Perak (Tong, 2016). Thickening of the stem underground named corms and cormels is the most eaten part in Taro (Simsek & El, 2015). Researchers had found hundred species of taro, that vary in taste and appearance, flesh colours that range from white to yellowish, pink and purple. The taste varies from sweet, bitter to tasteless (Bhutia, 2017). The starchy corms of taro can be enjoyed through baked, roasted, boiled or either transformed into flour and chips and many more products. Consumption of 1/3 cup of cooked taro is equivalent to one exchange of carbohydrate per serving (Malaysian Diabetes Education Manual, 2016).

2.2 Nutritional composition in potato, sweet potato and taro

Nutritionally, tubers and roots have a high potential to supply dietary energy in the human diet. Potato is known to be high in carbohydrates but containing only a trace amount fat, has a fairly low content of protein, rich in vitamin C also a good source of several vitamin B and potassium (Camire, 2016). Despite the macronutrient availability discovered in the

same study, the potato was also reported as the good potential of contributing fibre, polyphenol, and carotenoids for daily consumption.

Presence of various vitamins, amino acids and minerals, tocopherol and beta-carotene, as well as rich source main nutritional material in sweet potato such as carbohydrates (starches and simple sugars), protein and fat has made sweet potato as one of the food with high nutritional value (Temesgen & Ratta, 2015). Krochmal-marczak et al. (2014) added that sweet potato with yellow flesh cultivars also contains significant amounts of carotenes. Research has found that yellow and orange sweet potatoes cultivars have carotenoids content such as β- carotene that primarily acts as the pigment molecule in the tubers, as well as the source of provitamin A that relates with vitamin A activity (Yu, Liu, Duan, Tang, & Yang, 2015). The same study proposed that carotenoids manifest a strong antioxidant capacity that has the ability to scavenging free radicals due to the presence of conjugated double bonds.

Compared to potato and sweet potato, taro has twice carbohydrate content which it yields 135 kcals per 100 g (Temesgen & Ratta, 2015). The same study also stated that taro has higher protein content compared to other tubers and root crop respectively, also containing nutrients such as minerals, vitamin C, thiamine, riboflavin, and niacin. Respectively, taro is also known as a good source of iron, phosphorus, iron, zinc, manganese, and copper (M Alcantara, 2013). Accordingly, the vitamin B-complex contains in taro is greater than the whole milk. Despite known with abundant macronutrient content, researchers have been developed a considerable interest in phytochemicals content in these tubers and roots crop. (Albishi et al., 2013).

2.3 Bioactives constituents in potato, sweet potato and taro

Phytochemicals in natural sources such as animals and plants, algae and microorganism have the rich bioactive compound that has the ability in defence mechanism in human (Pereira et al., 2015). A study reported that phytochemicals have primary and secondary compound, consist of protein, common sugars, and chlorophyll as primary

constituents and terpenoid, alkaloids and phenolics compound as the secondary constituents respectively (Wadood, 2013). Similar to other vegetables, tuber and roots crops are also rich in phytochemicals content that plays a major role in nutraceutical. Nutraceutical term is coined by Dr Stephen de Felice, was derived from the words nutrition and pharmaceuticals, which refers to any food products that have medical benefits (Prakash, Gupta, & Sharma, 2012). The mechanism happened is studied using in vitro systems,suggested that significant amount of phenolic compound is able to bind to cell plant wall and cell wall analogues, where then be delivered to large intestine, therefore, both dietary fibre and polyphenols exert positive effect on health by tackling microbial population (Han, Lin, Lin, & Hou, 2014). However, there are still lacking information available as in-depth research on phytochemicals content in tuber and roots has not been ascertained for many years.

In potato, phytochemicals present were particularly depending on the flesh colour, genotype and the storage condition (Ah-Hen et al., 2012). In the same study, the researcher proposed that potatoes which have white, yellow-orange pigmented potato are abundantly rich in carotenoid contents, such as lutein, zeaxanthin, violaxanthin, and antheraxanthin. Meanwhile, the phenolic compound that is found in reds, blue, or purple-fleshed potatoes is primarily known as anthocyanins (Zhang Hong, Xu Fen, Wu Yu, Hu Hong-Hai, 2017). These include monohydric phenol, coumarin, flavonoid, tannin, polyphenol, and lignin (Santos, Molina-Garcia, Cunha, & Casal, 2018). Also, the phenolic compound such as chlorogenic, ferulic, protocatechuic and p-coumaric as well as caffeic was reported has been identified in red- or purple- fleshed potatoes (Kita et al., 2013). There is also a little amount of flavonoid found such as quercetin, myricetin, naringenin, and kaempferol found (Mishra, Ojha, & Chaudhury, 2012). What makes up the most of 90 % of the total polyphenol content in potato is the phenolic acid, mainly chlorogenic acid in potatoes (Tian et al., 2016).

In a similar way with potato, different flesh colour of sweet potato also have a completely different chemical composition (A. Wang et al., 2018). Rodrigues de Albuquerque

et al., (2018) reported that the amount of bioactive compounds in sweet potato has renewed the interest of the researcher, focusing on the presence of phenolics, carotenoids, polysaccharides and peptides. It is then found that the major bioactive substances in sweet potato are phenolics and anthocyanins (Fu et al., 2016). Phenolics is one of the antioxidant molecules with at least one aromatic ring and one or more hydroxyl groups, while anthocyanins are known as one of the group in water-soluble flavonoids (Hamouz et al., 2011).

The phytochemicals present in taro may differ in colour variety as well. A group of researcher had previously addressed the phenolic composition of taro, such as anthocyanin (cyaniding and pelargonidin) and flavones (apigenin and luteolin) (Goncalves et al., 2013). In contrast to potato, one-quarter of the phenolic present on taro corm was composed of the flavonoid which is quercetin (Simsek & El, 2012). Alkaloids, thiamine riboflavin, carotenoids, thiamine, amylopectin was also the phytochemicals that being addressed in taro (Awa & Eleazu, 2015).

2.4 Total phenolic content and total flavonoid content in potato, sweet potato and taro

Phenolic compounds are pervasively found in plant species but very form available in the tissue, cellular and subcellular level. There are two types of phenolic which are insoluble and soluble. Soluble phenolic was found within the plant's vacuole while insoluble phenolic can be found in the cell walls which contribute to mechanical strength and regulates plant growth and morphogenesis (Knezevic et al., 2011). Phenolic content is not only mean for the nutritional aspect, but they are also essential for plant physiology such as pigmentation, pathogen resistance and pollination (Lin et al., 2016).

The phenolic compound present in the plant can be recognized through the presentation of a hydroxyl-substituted benzene ring within the structure and its differ the phenolic groups by

the number of phenol rings contained a structural element (Bhattacharya, Sood, & Citovsky, 2010).

For instance, in sweet potato, the individual compound was group into hydroxycinnamic acids, hydrobenzoic acids, flavonoids, flavonols and anthocyanins (Ruiz et al., 2018). Doutora, Maria, & Saraiva (2014) proposed that many studies found tubers with coloured flesh exhibit more anthocyanins composition compared to hydrocinnamic acids and insufficient result for other compounds were identified. In addition, phenolic acids, flavonoids, stilbenes and lignans were the primary polyphenols found in sweet potato (Kasote, Katyare, Hegde, & Bae, 2015). Ah-Hen et al., (2012) added that varieties of potatoes with the same flesh colour may also differ in total phenolic content, individual phenolic acid profiles and antioxidant activity.

Distinctions then are made between the flavonoid, phenolic acid, stilbenes and lignans as all of these compound occur in the conjugated form with one or more sugar residues liked to hydroxyl groups (Prakash et al., 2012). The same study also reported that the relationship with other a compound such as organic acids, amines, lipids and also other phen

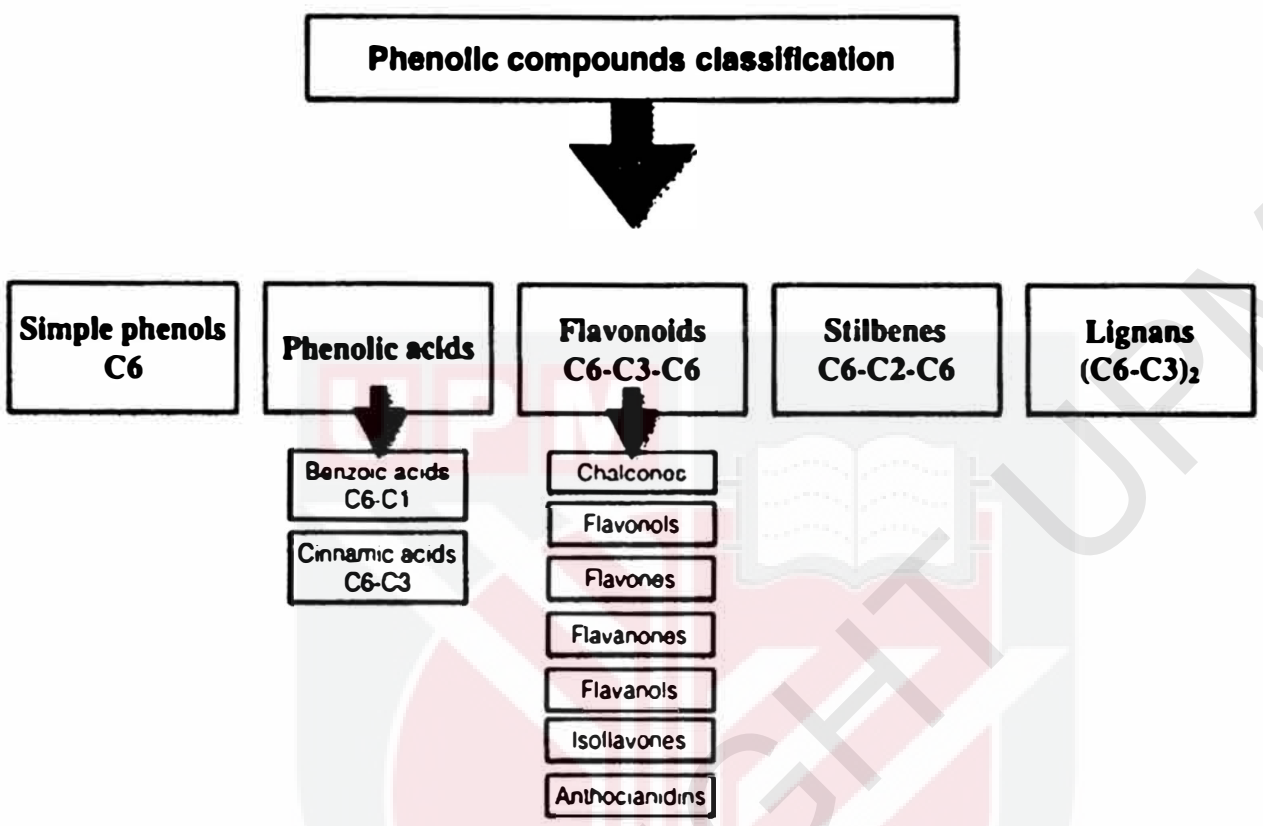


Figure 1: Schematic presentation of the main classes and subclasses of phenolic compounds and their respective chemical structure bone (Baiao et al., 2017).

The research found that the potato cortex and skin adjoining tissues content the highest phenolic compounds approximately about 50% as it decreases the concentration from the outside toward the centre of potato tubers (Ah-Hen et al., 2012). The quantification of phenolic was highly influenced by the chemical nature, extraction method, sample size particle, storage time and condition, the standard and practical assay used and presence of interfering substances such as wax and fats (Girard & Awika, 2018). In the laboratory advancement these days, several spectrophotometric methods have been developed to quantify the plant phenolic and many assays with different principles can be applied (Ruiz et al., 2018).

In potato, specifical potato with white-yellowish flesh colour, most of the phenolic content available is the substituted derivates of hydroxycinnamic acid in the free form and hydroxybenzoic acid in the bound form (Albishi, John, Al-Khalifa, & Shahidi, 2013). Researcher in the same field of the study added that the most common hydroxycinnamic acid derivatives were reported to be chlorogenic acid (50.31 %), caffeic acid (0.21 %) and ferulic acid. Meanwhile, the hydroxybenzoic acids present were gallic acid (41.67%), protocatechuic acid (7.81%) and many other derivates (Chellaram et al., 2014).

For total phenolic, individual phenolic acids such as chlorogenic acid,caffeic and dicaffeoylquinic as well as total anthocyanins are reported higher concentrations in purple flesh compared to yellow, white, red and orange varieties (A. Wang et al., 2018).In addition, Caffeoylquinic acid derivatives, including 5-caffeoylquinic acid (chlorogenic acid), 6-ocaffeoyl- β-D-fructofuranosyl-(2–1)-α-D-glucopyranoside, trans-4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, represent the major types of polyphenols in sweet potato; these compounds have shown to possess strong antioxidant and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities (Lebot, Michalet, & Legendre, 2016).In the same study, identification and quantification of polyphenols responsible for the antioxidant activity of sweet potato with different flesh colours showed that purple-fleshed presented the highest caffeoylquinic acid content (749.7 µg/g). Meanwhile,orange-fleshed sweet potato varieties presenting the highest total polyphenol contents (2.43 mg GAE/g) also presented the highest total antioxidant activities (4.12 mg TE/g) (Selvakumaran et al., 2018).

The active components in roots crop are saponin, phytosterols, glycans, doscoretine, diosbulins,paeonol and (+)-ß-eudesmol (Liu, Lin, Hu, & Yang, 2015). Specifically in taro, diosgenin saponin and dioscorine alkaloid is reported to be the most predominant phytochemicals (Senanayake, Ranaweera, Bamunuarachchi, & Gunaratne, 2013). Accordingly, dioscrine and diosgenis are considerable as toxic, that toxicity can be removed by proper food preparation of taro such as washing, boiling and cooking. The alkaloid

content also contributes the bitterness and toxicity in the roots crop. All parts of the plant have tiny sharp calcium oxalate that can cause irritation to the sensitive membranes lining such as mouth, skin, lips, and throat. The crystals are formed in bundles and released when the cell wall is broken. However, the longer cooking method will break the crystal chain, hence, make it edible and safe for human consumption. (Tong, 2016).

Flavonoid is made up through a combination of shikimic acid and acylpolymalonate pathways and its reported that flavonoid comprises most of the abundant class of plant polyphenols (Hua et al., 2016). Flavanoid shares a carbon skeleton of diphenyl propane, with two benzene rings joined by a linear three carbon chain. The central chain usually forms a closed pyran ring with one benzene rings (Awa & Eleazu, 2015). Based on the difference in heterocycle involved, flavonoid may be divided into six subclasses which are flavones, flavonols, flavanones, flavanols, anthocyanidins, and isoflavonoids (Baiao et al., 2017). The estimated consumption ranging from 20-40 mg per day in the Asian region (Chandrasekara & Josheph Kumar, 2016).

2.5 Antioxidant capacity in potato, sweet potato and taro

The first discovery on antioxidant properties in the plant is to believe started with the subsequent isolation of ascorbic acid from plants. As many interest growth, tubers and roots crop are the great exogenous sources of antioxidant which are primarily affected by the presence of phenolic compounds (Shad et al., 2014). Antioxidant from the plant may synergize with the endogenous antioxidant in the human body, therefore, protecting the body against reactive oxygen species (Albishi et al., 2013). Approximately, there are 19 *in vitro* and 10 *in vivo* method of assessing antioxidant activity in the plant (Chellaram et al., 2014).

Oxidative stress happens due to an imbalance between the production of free radicals and reactive oxygen species (ROS) and the elimination by a protective mechanism which is antioxidants (Han et al., 2014). The great deal to eliminate oxidative stress in the human body because it is proven it is a major causative agent of many life-threatening

diseases (Jin et al., 2018). In addition, oxidative stress may rupture the DNA in cells and tissues, also damaging lipids, proteins and carbohydrates, resulting in membrane damage and cell death (Ah-Hen et al., 2012). Many studies agreed that prolonged consequence of oxidative stress in the body will generate the development of the cardiovascular disease, diabetes, cancer, neurodegenerative disorder and autoimmune disorder (Prakash et al., 2012).

Here come the roles of antioxidant in against the oxidative stress. The antioxidant is known to delay, prevent and inhibit the oxidation of oxidizable substrate through the mechanism of scavenging free radicals and diminishing oxidative stress (Kasote et al., 2015). The same study also proposed that polyphenols play their role as the antioxidant by breaking the free radical chain reaction. The most important mechanism of antioxidant activity by the plant is the suppression of the free radical formation by regulation of enzyme activity or chelating metal ions involved in free radical production (Lin et al., 2016). The polyphenolic compound found in tubers and roots crops may also interact with other physiological antioxidants and this is another possible antioxidant pathway.

Since tubers and roots crop have abundant content of polyphenol content that contributes to major antioxidant activity in the human body, the peroxidase enzymes are also part of the antioxidant system in plants. The synergize happen between these compound and reported the most efficient in fresh tubers and roots (Wegener & Jansen, 2013). Data shown in the same study proposed that anthocyanin present in tubers and roots has four times greater compared to vitamins E and C in term of radical scavenging capacity. Accordingly, the antioxidant enzymes and anthocyanin may complement to sustain the antioxidant potential in the plant tissue, therefore, potato or taro that has coloured pigment may have an advantage for this properties (Kasote et al., 2015).

2.6 Anti-nutrient properties in potato, sweet potato and taro

Anti-nutrient is the compound that interferes the nutrient intake, digestion, absorption, and utilization of nutrient from food and might cause the adverse effect (Fekadu Gemede, 2014). Anti-nutrient is common in a raw plant that does not undergo adequate or effective processing. Being one of the staple food worldwide, toxicity related to potatoes is not a common issue. However, as part of the maturing process, potatoes will start to accumulate the number of alkaloids which later might cause discomfort due to the cholinesterase inhibition, teratogenicity, inflammation of the intestine and nausea (Deuber, Guignard, Hoffmann, & Evers, 2012). The main alkaloids in potato reported to be glycoalkaloids and the concentration that can pose risk to human health is above 200mg/kg fresh weight (Ji et al., 2012). In the same study, the researcher proposed that this anti-nutrient somehow could be removed almost in total through peeling (70%) and blanching (29%) and the domestic cooking can reduce the glycoalkaloids as well.

In contrast to the potato that commonly develops anti-nutrient molecules along the maturity process, breeding efforts may be the potential cause of sweet potato to develop undesirable nutritional composition (Mitiku & Teka, 2017). They added that tannin is found as one of the anti-nutrient in sweet potato, but somehow many present investigations supported that the tannin content in it is much lower than tannin present in taro corms. The presence of tannins is said may exert a negative effect on the bioavailability of proteins and mineral, especially iron and eventually it will affect the utilization of vitamins in plant-based food (Vladimir-Knežević et al., 2011). That is why food with a high level of tannins is categorized as food that is low in nutrient quality. On the other hand, phytic acid was also found in sweet potato but the value reported is still lower compared to other tubers and roots crop such as cassava and taro (Chandrasekara & Josheph Kumar, 2016). Indeed, the phytic acid will form insoluble salts with minerals elements such as calcium, iron and zinc hence preventing their bioavailability and succeeding utilization in the body, but the low content of

phytic acid and in an acceptable range of nutritional quality in sweet potato still allow the absorption of these elements (Mitiku & Teka, 2017).

Temesgen and Ratta (2015) proposed that there are also anti-nutrients present in taro which might have an adverse effect if consumed. Mucilage, oxalic acid, tannins, cyanide, lectins, alpha-amylase inhibitors and protease (trypsin and chymotrypsin) was reported to be the main anti-nutrient exist in taro.

2.7 Factors affecting phytochemicals content and changes of antioxidant capacity in potato, sweet potato and taro

Changes of antioxidant activity in the plant may be influenced by the phytochemicals content available and these properties can be observed under different environmental factors and stress condition (Kasote et al., 2015). Tubers and roots need to be processed and go through the desired cooking method to improve the digestibility of them. This process somehow will alter the biological, physical and chemical modification which later lead to nutritional, sensory and textural changes of food. (Tian et al., 2018). Accordingly, the changes occur can either beneficial or detrimental to human health.

2.7.1 Genetic diversity

For instance, the genetic diversity of the tubers and roots crop is the first factor that can cause changes in antioxidant capacity. Russet potatoes with white to yellowish flesh has different phytochemicals content that attributes to the antioxidant capacity compared to sweet potatoes or potato with oranges to purple flesh (Prajapati et al., 2013). The same idea was brought to taro with different genotype as well (Arici, 2016).

2.7.2 Food processing

The next factor is the preparation and cooking process applied. Many studies figured that potato has the richest phytochemical content the skin parts (Akyol, 2016). However, Malaysian often consume potato with the removed skin in any dishes preparation and eliminated the most nutritious part of the potato. A group of researcher agreed that domestic cooking induces adverse effects, such as the loss of important nutrients and formation of undesired compounds such as acrylamide or other toxic molecules (Tian et al., 2016). However, the purpose of cooking cannot be denied in improving digestibility, enhance the palatability and make root crop safer to eat especially in taro. Taro has the acridity properties, which result due to the presence of calcium oxalate (needle-like crystal) which can easily penetrate into soft skin and can cause irritation after consumption (Kumoro et al., 2014). Accordingly, the prolong heat applied through cooking may reduce the calcium oxalate and make the taro safer to eat.

Recent studies also supported that heat processing may not directly cause the reduction of quality nutrient , heat treatment produces no change and even improve the bioaccessibility of natural occurring antioxidant content in tubers as proposed by Alcantara (2013) , which shown that phytochemicals component in taro increase after drying , relatively 124.89% for phenols, 89.95% for saponins and 124.89% for flavonoids. Accordingly, the disruption of the cell wall during heat exposure may increase the phenolic content in taro.

Generally, sweet potato is cooked before consumption, it is either boiled, steamed, fried and roasted. Although the benefits of sweet potato are widely established through numerous studies, there is still limited information on how their functional components and antioxidant capacities being affected by different home cooking ways (Lewu et al., 2010). There are study proposed that such thermal processing can cause impairment of the functional compounds of sweet potato (Evelyn et al., 2017). In contrast, there have been reports on the negative correlation between heat treatments applied on food and the effect on bioactive substances such as anthocyanins (De Aguiar Cipriano et al., 2015). Heat

treatments widely used in tubers and roots crop preparation can induce changes in their chemical composition, impacting on the concentration and bioavailability of polyphenols. This is an important aspect because part of the polyphenols present in these crops are actually bound to fibre (bound form) and not in free form (Awa & Eleazu, 2015).

2.7.3 Environmental factor

In addition, the environmental factor such as cultivation area, storage temperature, and post-harvest treatment might alter the phytochemical composition (Yamdeu Galani et al., 2017). The cultivation area for tubers and root may resulting in geographic isolation for the crops and may differ them in the genetic base, thus, affecting the shape and flesh colour of crops hence vary in their phytochemical constituent (Muñoz-Cuervo, 2016). The proximal composition in these tubers and roots crop may also be affected by the storage temperature and post-harvest treatment. For tuber and roots crops that have a high content of starch, the formation of RS may cause the retrogradation of gelatinized starch and by cooling, the gelatinized starch become ordered and may resistant to digestive enzymes, consequently lowering the postprandial glycemia and benefits health of the consumer (Tian et al., 2016).

2.8 Principle and importance of antioxidant in disease prevention

The exogenous antioxidants are mainly derived from food and medicinal plants (Kasote et al., 2015). These natural antioxidants from plant materials are mainly polyphenols such as the phenolic acids, flavonoids, anthocyanins, lignans and stilbenes, carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) (Hamouz et al., 2011). As many studies reveal that these natural antioxidants exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-ageing and anticancer(Bontempo et al., 2013)

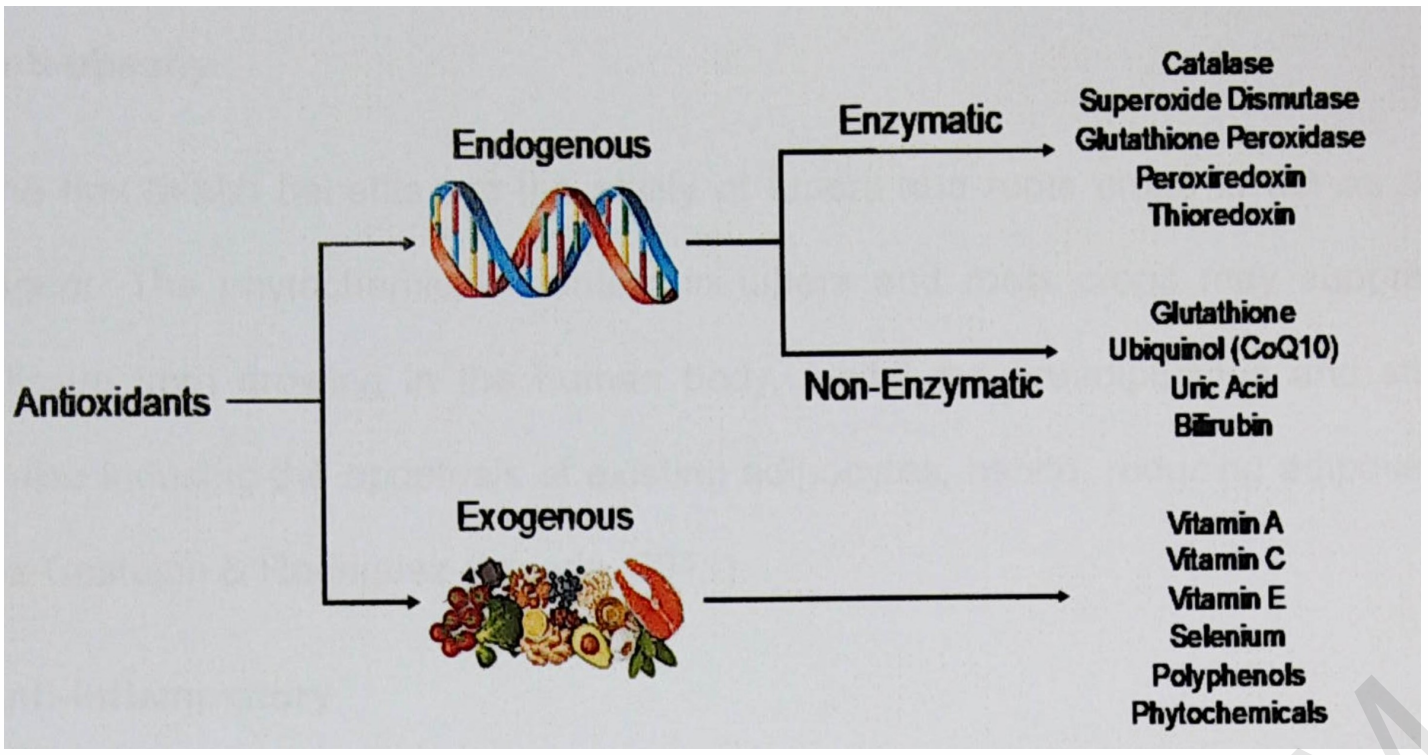


Figure 2: Type of Antioxidants (Kasote et al., 2015)

The in-depth study found that in our biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals can damage the DNA to the oxidation of lipid and proteins in cells(Kasote et al., 2015).Normally, the antioxidant system occurring in the human body can scavenge these radicals, which would keep the balance between oxidation and antioxidation. Nonetheless, the exposure of human to the environmental toxins such as cigarette smoking, alcohol or radiation may induce the production of excessive ROS and RNS, which disrupt the balance between oxidation and antioxidation and result in some chronic and degenerative diseases. The increment of intake of exogenous antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen and reducing agents.

2.9Health benefits of potato, sweet potato and taro

The current trends observed among the consumer were the demands on natural product supplementation that is believed to have a beneficial effect on health. Referred to the lifespan essential compound which is polyphenols, it is proven that these compound exhibit the capability in preventing the development of many metabolic diseases and maintaining human health (Silveira, Oyarzún, Sepúlveda, & Escalona, 2017).

2.9.1 Anti-obesity

The first health benefits are the ability of tubers and roots crops to act as an anti-obesity agent. The phytochemicals content in tubers and roots crops may suppress the adipose tissue from growing in the human body, inhibit the preadipocytes and stimulate lipolysis, also inducing the apoptosis of existing adipocytes, hence, reducing adipose tissue (González-Castejón & Rodriguez-Casado, 2011).

2.9.2 Anti-inflammatory

Anti-inflammatory properties of tubers and roots plant were also being studied as it is highly related to the total phenolic content found in these crops (S. Wang, Nie, & Zhu, 2016). However, the molecular mechanism behind these properties has been rarely reported. The same study reported that starchy crops exhibit the capacity in anti-hyperglycemic effect. Hyperglycemia is one of the diabetic complications that indicates exceptionally high blood glucose and the derived dietary materials in the starchy crops demonstrated the ability as the antidiabetic agent using the diabetic animal model and being generalized in the progression of human diabetes.

2.9.3 Anti-angiogenesis

Next, tubers and roots crop may act in Anti-angiogenesis. Angiogenesis is a complex process of the new blood vessel is formed from an existing blood vessel which can cause non-neoplastic disease letter as the degradation of extracellular matrices occur. Angiogenesis may also cause the proliferation of endothelial cells and tube formation in new blood vessels maturation which is not good for overall health (Chen et al., 2011).

2.9.4 Anti-cancer

There are still many health benefits that can be listed but antitumor and anticancer properties have been discussed in relation to phytochemicals from the starchy plant. Dietary materials such as anthocyanins, glycoalkaloids, and lection found in starchy crops have high

potential as antitumor agents (Zhang Hong, Xu Fen, Wu Yu, Hu Hong-Hai, 2017). Many epidemiological studies and clinical trials demonstrate that flavonoids have a chemopreventive effect on the cancerous cell (Hua et al., 2016). The same study also supported the justification that considering the intake of dietary flavonoids may reduce the risk of ovarian cancer and though the finding is still inconsistent and has controversial findings.

2.9.5 Immune booster

Results derived from many epidemiological studies suggest an inverse relationship between consumption of polyphenol-rich foods and the occurrence of various chronic disease (Mvitu Muaka, Longo-Mbenza, Tulomba Mona, & Nge Okwe, 2010). Similarly, the bioactive constituents in sweet potato may be impactful in maintaining and promoting human health (S. Wang et al., 2016). Singh et.al. (2017) summarised the bioactive compounds in sweet potato played a vital roles in health by improving immune function, reducing oxidative stress and free radical damages, as well as reducing the cardiovascular disease risk and suppress the cancer cell growth due to the ability of most favourable antioxidant status in it (Hara et al., 2018).

2.9.6 Anti-radical

In addition, chlorogenic acid is known for the capability of preventing hydroxyl radical formation, scavenging free radicals and avoiding oxidative activities, also exert antimutagenic and anti-carcinogenic effects in vitro and in vivo (González-Castejón & Rodriguez-Casado, 2011). However, the iron chelating function of chlorogenic acid may also cause reduced bioaccessibility of iron from sweet potato roots, which are considered moderate sources of dietary iron (approximately 8.5 µg/g) (Temesgen & Ratta, 2015).

2.9.7 Reduce the prevalence of developing metabolic disease

Despite the antioxidant status, consumption of tubers and roots crops may also help to reduce the metabolic disease prevalence due to the micronutrient content in it (Hou, Mu,

Ma, & Blecker, 2019). For instant, in sweet potato, the sodium to potassium ration (Na/K) is known as a great importance in controlling blood pressure because the value is less than 1, hence, regular consumption of sweet potato will be beneficial for hypertensive patients to control their high blood pressure (Krochmal-marczak et al., 2014). In addition, the ration of calcium to phosphorus (Ca/P) ranged from 3.72 to 4.88 (which is above 1), is considered as "good" food and help in maintaining good bone health (Lin et al., 2016). It is clearly concluded that consumption of tubers and roots crops warrants further and more intensive research in relation to the prevalence of the chronic disease.

CHAPTER 3

3.0 METHODOLOGY

3.1 Chemical and Reagent

All reagents were of high-class analytical grade. Folin-Ciocalteu reagent, Distilled water, 7.5% Sodium carbonate (Na_2CO_3), Gallic-Acid, 5% Sodium Nitrate ($NaNO_2$), 1M Sodium Hydroxide (NaOH), Catechin, DPPH solution, Butylated hydroxytoluene (BHT), Beta Carotene (BC), Chloroform, Linoleic Acid, Tween 40, Acetate Buffer, FRAP reagent, 2,4,6 – Tripyridyl-S-Triazine (TPTZ) Solution, Hydrochloride (HCL), $FeCl_3.6H_2O$, Iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$)

3.2 Apparatus

Filter paper, 96-well plate, Stovall belly dancer, micropipettes, microplate reader,test tube,conical flask,round bottom flask, waterbath, incubator, centrifuge, -20 freezer, freeze-dryer, test tube rack, filter funnel, refrigerator, desiccator, laboratory grinder, pH detector and rotary evaporator.

3.3 Sample Collection

All loose potatoes, sweet potatoes and taro were purchased at the Night market in Sri Serdang. The all-fresh tubers and roots are free from any mechanical or physiological damage. Samples were divided into four groups; raw (peeled skin), boiled (peeled skin), fried (peeled skin) and steamed (peeled skin) were used for the purpose of comparison. All skin was removed by 1 to 2 mm with the sharp knife to mimic what usually had been done in many households. The characteristics of potato, sweet potato and taro are reported in Table 1.0 and the external and internal appearances (transversal section) of the sample are shown in Figure 3.0.

Table 1: Characteristic of tubers and roots used in the study

Type	Maturity	Skin colour	Flesh colour	Tuber form	Cooking used
[1] Potato	Mature	Brown	Yellowish	Oval	Multiuse
[2] Sweet Potato	Mature	Pinkish Brown	Orange	Alternate heart-shaped	Multiuse
[3] Taro	Mature	Dark Brown	Whitish	Cylindrical	Multiuse

(Bellumori, Innocenti, Michelozzi, Cerretani, & Mulinacci, 2017)[1],(Rodrigues de Albuquerque et al., 2018)[2],(Temesgen & Ratta, 2015)[3]

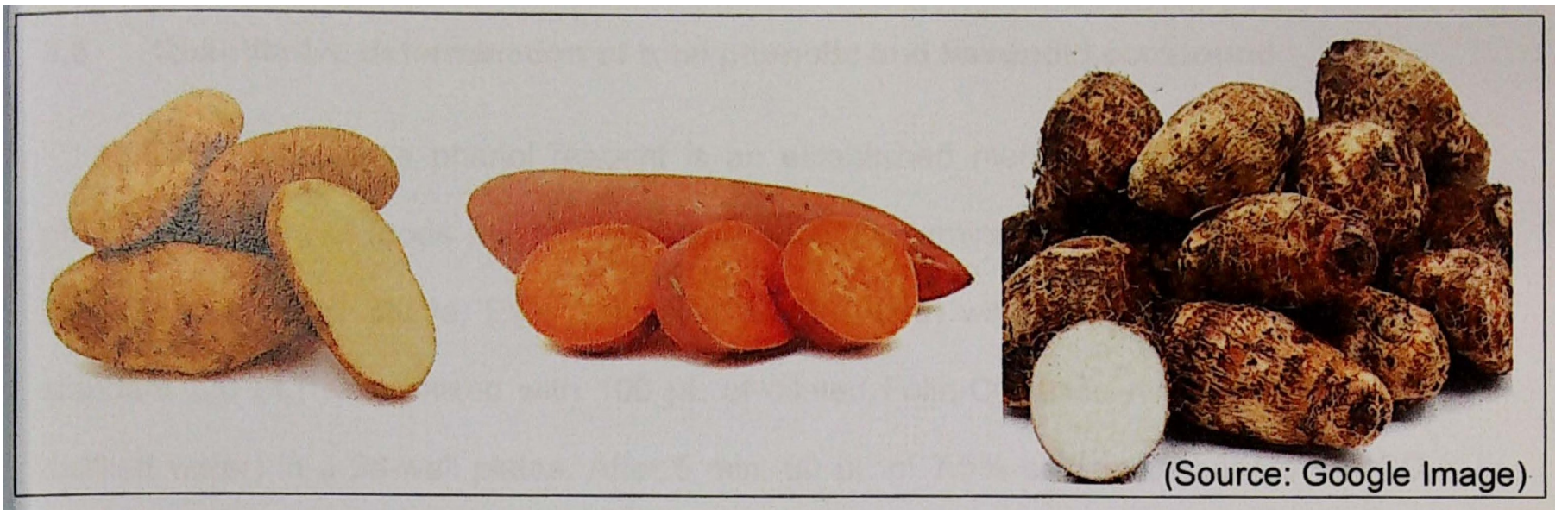


Figure 3: External and internal appearances (transversal section) of tubers and roots used in the study

3.4 Physical Treatment and Browning Control (Combined Treatment)

A representative amount of potatoes, sweet potatoes and taro (weight about 200g) were used for raw, frying, boiling and steaming process. However, the first crucial step was all of these tubers must be treated and immerse in water with NaCl (1%) and ascorbic acid for 10 minutes. The ratio of tuber/water was 1:4 w/v. This step is crucial and known as browning control as it rendering the susceptibility of tuber to wound. Processing operation carried out may cause injury to plant tissue, hence, increasing the respiration rate of the tubers that later will cause browning (Xie et al., 2017).

Sample Preparation

Sample powder was prepared as described by (Floegel, Kim, Chung, Koo, & Chun, 2011). All operations during sample preparation were performed very quickly by freeze-drying so as to avoid sample degradation. After freeze-drying and stabilization in a desiccator, samples were pulverized in a laboratory grinder. The mixture is then filtered through filter paper in a funnel and rinsed with distilled water or methanol depending on the standard use in the assay. The supernatant obtained was stored in -20 ^{o}C freezers. For each food item, dissolution was done in triplicate. All methodologies followed the recommendations of the Official Method of Analysis (AOAC, 1990) and all analyses were triplicated.

3.6 Qu nti ative determination of total phenolic and flavonoid compound

Folin–Ciocalteu's phenol reagent is an established method to determine the total phenolics content of foods extracts and beverages. Determination of TPC was carried out according to Ishak, Shafie, Esa, Bahari, & Ismail (2018) with modification. Sample or a standard (20 µL) were mixed with 100 µL of diluted Folin-Ciocalteu reagent (1:10, v/v in distilled water) in a 96-well plates. After 5 min, 80 µL of 7.5% sodium carbonate (Na_2CO_3) was added to each well. The plate was covered and left in the dark for 30 min on a Stovall belly dancer (Greensboro, NC, USA). The absorbance was measured at 765 nm against a reagent blank. A standard calibration curve using gallic acid (0.98–1000 µg/mL) was plotted, and the results were expressed as mg gallic acid equivalent (GAE)/g extract using the following formula: TPC per 1 g powder sample = [(TPC per mL sample x dilution factor x total sample volume used)/sample weight]. All samples were analyzed in triplicate.

The TFC was carried out according to Belguith-Hadriche *et al*. (2013) with modifications. Samples or a standard (25 µL) were pipetted into the 96-well plate. Then,100 µL of distilled water (dH_2O) and 7.5 µL of 5% sodium nitrite ($NaNO_2$) were added and incubated for 5 min. After that, 7.5 µL of 10% aluminium chloride hexahydrate ($AlCl_3.6H_2O$) was added and incubated for another 5 min. Then, 50 µL of 1 M sodium hydroxide (NaOH) and 60 µL of dH_2O were added and the absorbance was read using a microplate reader at 510 nm. A standard calibration curve using catechin (1.95–250 µg/mL) was plotted, and TFC were expressed as mg catechin equivalent (CE)/g extract using the formula: TFC per 1 g extract = [(TFC per mL sample x dilution factor x total sample volume used)/sample weight].

3.7 Antioxidant capacity measured by FRAP Assay (reducing capability)

FRAP assay is used to compare concentrations of reducing polyphenols in various extracts from natural antioxidants. In this research, The FRAP assay was carried out to determine the iron-reducing capacity of each extract according to the method by Benzie and Strain (1996), with modifications. Firstly, 300 mM acetate buffer (pH 3.6) and 40 mM HCl were prepared. FRAP reagent was freshly prepared by mixing the acetate buffer, 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mM HCl, and 20 mM FeCl3.6H2O at a ratio of 10:1:1 (v:v:v), and pre-warmed in a water bath at 37°C. Then, 20 µL of samples/standard/blank were mixed with 180 µL FRAP reagent in a 96-well plate and incubated at 37°C for 30 min. The absorbance was read at 593 nm. A standard calibration curve was plotted using iron (II) sulphate heptahydrate (FeSO4.7H2O) (0.1 – 1.0 mM) and the final results were expressed as mmol Fe2+/g dry weight.

The generation of the indigo Fe^{2+} -TPTZ complex in the FRA reagent from the clear Fe^{3+} -TPTZ complex can be easily observed at this stage. The absorbance of solutions containing extract and FRAP solution were measured at 595 nm after each Fe^{2+} addition increments. The absorbance and Beer-Lambert Law are used to determine the extinction coefficient. The proportion of Fe^{3+} reduced to Fe^{2+} concentration is then calculated. A standard calibration curve was plotted using iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$) (0.1 – 1.0 mM) and the final results were expressed as mmol Fe^{2+}/g extract (Ishak et al., 2018).

3.8 Antioxidant capacity measured by DPPH Assay (anti-radical activity)

In comparison with FRAP assay which highlights on the reducing agent properties, DPPH assay manifests the anti-radical activity from the sample. The method is based on the reduction of the relatively stable radical, DPPH, to the formation of a non-radical form in the presence of the hydrogen donating antioxidant. reduction of purple colour DPPH to the yellow colour diphenyl-picrylhydrazine derivatives indicated antioxidant activity. DPPH assay is geared for assessment of free radical scavenging potential of an antioxidant molecule and

considered as one of the standards and easy colourimetric methods for the evaluation of antioxidant properties of pure compounds.

This DPPH assay procedure used in this study was adapted from Zhang et al., (2013). Samples or a standard (50 µL) at various concentrations (0.98–1000 µg/mL) and 195 µL of 100 µM DPPH solution were mixed in a 96-well plate and left in the dark at room temperature for 30 min. The absorbance of the reaction mixture was read at 515 nm. Butylated hydroxytoluene (BHT) and gallic acid were used as standards.

The scavenging capacity was calculated (1 − As/Ac) × 100%, where Ac is the absorbance of the control and As is the absorbance of the tested sample after 30 min. Trolox was used as a standard. Free radical scavenging capacities of three tubers samples were expressed as mM Trolox equivalent and EC_{50} values (concentration of samples required to scavenge 50% of DPPH radicals).

3.9 Antioxidant capacity measured by Betacarotene Bleaching Assay

This beta carotene bleaching assay procedure used in this study was adopted from Lai, Lim, Sm, Blechnum, & Link (2011). The β-carotene (BC) working reagent was prepared by mixing 1 mL of BC (0.2 mg/mL in chloroform) with 20 µL linoleic acid and 200 µL Tween 40 in a 100 mL round bottom flask, and the mixture was evaporated using a rotary evaporator at 30°C for 1 min. Next, 50 mL of ultrapure water was added to the mixture and shaken vigorously to form an emulsion. An aliquot of 200 µL of the preheated emulsion (50°C) was transferred into a 96-well plate containing 20 µL of the sample/standard/blank. The zero- time absorbance was measured at 470 nm immediately, and the mixture was incubated at 50°C for 2 h. Blank samples (in the absence of β-carotene) were prepared for background subtraction, and BHT was used as the standard. All determinations were performed in triplicate. The antioxidant activity was calculated using the formula:

$$\text{Degradation rate (DR) of β-carotene} = \text{Ln}(A_{initial}/A_{sample})/60.$$

$$\text{Antioxidant activity (\%AOA)} = [(DR_{control} - DR_{sample})/DR_{control}] \times 100.$$

3.10 Statistical analysis

For all of the variables studied, an one way- ANOVA and Tukey's test was performed to test for the effect of the different potato, sweet potato and taro processing types. All of the data sets obtained were subjected to principal component analysis (PCA), and the correlation among the different variables was determined using the Pearson correlation coefficient. Statistical analyses were performed using the IBM SPSS Statistics software and considered statistically significant when $p < 0.05$ (Ruiz et al., 2018).

CHAPTER 4

4.0 RESULTS AND DISCUSSION

In all the assayed tubers and roots varieties, the fresh weight after cooking and freeze-dried, in comparison to the raw one, showed a decrease due to a water loss of the vegetable tissue. Therefore, to allow the comparison among varieties and cooking methods, all the results are expressed in terms of dry weight (DW). The nutritional and antioxidant quality of tubers and roots crops is associated with diverse factors such as harvest period, storage condition, the genotype of tubers and also the thermal effect applied. Thus, each of these parameters is discussed in detail below with the primary highlight on the thermal effect on each sample.

4.1 Total Antioxidant content

In this study, there were two assays that being carried out for determination of antioxidant content which were Folin-Ciocalteu method that using gallic acid as the standard for determination of total phenolic content meanwhile Aluminium Chloride Colorimetric method were tested for analysing the total flavonoid content in potato, sweet potato and taro.

4.2 Total Phenolic Content

Numerous studies have focused on the changes in phytochemical during domestic cooking, however, the findings are quite a contra compared to different studies. As the slight changes in the phytochemical will significantly affect our health, it is very important to retain the maximum phytochemical content during domestic cooking (Tian et al., 2016).

4.2.1 Total Phenolic Content of Potato

The results of the Folin–Ciocalteu assay for potato are shown in Table 2. The total phenolic contents among the different samples were expressed in terms of gallic acid equivalents using the standard curve equation $y = 0.0015x + 0.0217$, $R^2 = 0.997$. Raw potato showed the highest amount of total phenolic content with 66.58 GAE g/100 g DW. Among the potato that undergoes the different thermal process, the fried potato had the highest gallic acid equivalent (41.57 GAE g/100 g DW), followed by steamed and boiled samples (28.58 and 18.18 GAE g/100 g DW, respectively). In this study, the differences in the total phenolic contents were statistically significant between the raw and different cooked samples.

Table 2: Total phenolic content (TPC) of the potato sample with different thermal process applied

Thermal process	TPC value (mg GAE/g dried sample)
Raw	66.58 ±. 0.002[a]
Fried	41.57 ± 0.002[b]
Steamed	28.58 ± 0.005[c]
Boiled	18.18 ± 0.009[d]

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for TPC was 100 mg/ml.

A comparable level of total polyphenols was noted in potato cultivars reported by Akyol et al., (2016). However, since the potato cultivars used in this study was potato with pale yellow flesh colour, the total amount of phenols is much lower in comparison to the

content observed in red and violet varieties (Bellumori et al., 2017). Despite the genotypes itself, the decreasing phenolic content in potatoes that undergoes thermal process was expected as the findings were highlighted in many past studies (Faller & Fialho, 2009). This trend observed showed that the polyphenol synthesis has been shown to be stimulated under conditions of stress, such as temperature alterations in domestic cooking, UV exposure and pathogenic attack. Moreover, the degradation of TPC in the potato sample in the current study have concurred well with (Nemś & Pęksa, 2018).

For instance, the finding lends support to the very initial process of sample preparation such as cooking and storage of sample after cooking. The TPC Value for frying potato gives a shred of compelling evidence where the potato that in contact with cooking oil that is rich in fat has a significant influence on product quality as well as their bioactive constituents (Santos et al., 2018). It is fundamental to note that the storage changes occurring in fat absorbed by the potato slices after cooking may undergo the rancidity process induced mainly by hydrolysis and oxidation (Bontempo et al., 2013). This process somehow will degrade the phenolic in potato, primarily the chlorogenic acid that contributes the highest for the phenolics present in potato. This justification is supported by the Journal of Agricultural and Food Chemistry in the chlorogenic acid profiles, where the researches found that there is almost zero chlorogenic acids were found in fried potato.

Significant losses for phenolic content in steamed and boiled potato were observed. One study examining the effects of cooking procedures on mature potatoes showed that boiling in water for 30 min may decrease chlorogenic acid to only 35% of the total chlorogenic presence from the original amount (Rytel et al., 2014). The process of steaming and boiled cause great loss possibly due to leaching of ascorbic acid into the water as the medium of cooking. Chung, Seo, Ahn, & Kim(2011) reported that cooking treatments (boiling, baking and microwaving) reduced ascorbic and chlorogenic acid contents, total glycoalkaloids, a-chaconine and a-solanine with the exception of total anthocyanins in potato. These findings were also supported by the previous study that showed the significant

effects of cooking (microwave cooking, boiling, and steaming) on the phenolic contents of potatoes and the results showed that none of these methods decreased the content of chlorogenic, cryptochlorogenic, and neochlorogenic acids (Chandrasekara & Josheph Kumar, 2016).

4.2.2 Total Phenolic Content of Sweet Potato

The results of the Folin–Ciocalteu assay for sweet potato is shown in Table 3. The total phenolic contents among the different samples were expressed in terms of gallic acid equivalents using the standard curve equation $y = 0.0015x + 0.0217$, $R^2 = 0.997$. As presented in Table 3, the highest TPC values were retained in fried sweet potato samples (90.01 GAE g/100 g DW) while the TPC of the steamed and boiled samples declined distinctly (36.02 and 26.06 GAE g/100 g DW, respectively). The TPC of raw sweet potato (41.08 GAE g/100 g DW) and all samples were significantly different from each other.

Table 3: Total phenolic content (TPC) of the sweet potato sample with different thermal process applied

Thermal process	TPC value (mg GAE/g dried sample)
Raw	41.08 ±. 0.012[a]
Fried	90.01 ± 0.037[b]
Steamed	36.02 ± 0.105[c]
Boiled	28.06 ± 0.099[d]

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for TPC was 100 mg/ml.

In contrast to potato and taro, polyphenols and flavonoids have in sweet potato shown certain stability when exposed to high temperatures, a quality that reflected in the preservation of their antioxidant capacity (Bradbury, Bradshaw, Jealous, Holloway, & Phimpisane, 1988). Studies performed on the previous study towards different vegetables after cooking showed that the total polyphenol content and antioxidant capacity could be either higher or lower in comparison to the fresh food (Tang, Cai, & Xu, 2015).

Unlike the potato, sweet potato manifests the highest significant TPC Value in fried sweet potato. From the literature, this trend may occur due to the bioactive constituents found in the sweet potato which is pelargonidin (Kita et al., 2013). The same study revealed that pelargonidin derivatives were more stable during frying than petunidin and malvidin derivatives. Moreover, a study conducted by Koutsidis (2019) proposed that when vegetables are treated at low temperature, prooxidants are generated, whereas treating at high temperature decreases the prooxidants and increases antioxidant properties due to the production of Maillard's Reaction Products (MRPs). Such antioxidant activity of the MRPs comes from the high molecular weight brown compounds that are formed in the advanced stages of the reaction. However, it should be mentioned here that MRPs can also exhibit prooxidant properties (Tamanna & Mahmood, 2015). This phenomenon indicated a correlation between the Maillard reaction with total phenolic contents in sweet potato. At high pH and temperature, the Maillard reaction rate increases and the formation of MRPs also increases. Thus, there is the possibility that the phenolic group was produced from MRPs during frying the sweet potato.

All the other potato that undergoes different cooking process were found to be significantly different from each other in terms of phenolic content. Antioxidant activity values also depend strongly on the preparation of the sample, in this case, the lyophilisation process (Lebot et al., 2016). This may confirm that zero contact with cooking process causes the higher TPC Value in raw sweet potato. Although steam blanching was employed primarily to deactivate degradative enzymes while minimizing losses of phenolic substances due to leaching, extended steaming may result in losses of phenolic compounds due to their susceptibility to leaching from the plant tissue and degradation of heat sensitive phenolic substances also cites the tendency of phenolic compounds to accumulate at the peel as the cause of low phenolic content in the flesh of potatoes (Doutora et al., 2014). This may explain the lower TPC value in both steamed and boiled sweet potato in the current study.

4.2.3 Total Phenolic Content of Taro

The results of the Folin–Ciocalteu assay for taro are shown in Table 4. The total phenolic contents among the different samples were expressed in terms of gallic acid equivalents using the standard curve equation y = 0.0015x + 0.0217, R^2 =0.997. As illustrated in Table 3, the least phenolic content was reserved in boiled taro,8.22 GAE g/100 g DW in comparison to other thermal processed samples. All samples were significantly different from each other. Showing a similar pattern like potato, raw taro had the highest TPC value, 40.22 ±. 0.103, followed by fried,steamed and boiled, 14.48 ± 0.097,12.35 ± 0.108,8.22 ± 0.102, respectively.

Table 4: Total phenolic content (TPC) of the taro sample with different thermal process applied

Thermal process	TPC value (mg GAE/g dried sample)
Raw	40.22 ±. 0.103[a]
Fried	14.48 ± 0.097[b]
Steamed	12.35 ± 0.108[c]
Boiled	8.22 ± 0.102[d]

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for TPC was 100 mg/ml.

Few studies have previously addressed the phenolic composition of taro, anthocyanins (cyanidin and pelargonidin derivatives) and flavones (apigenin and luteolin derivatives) being already described in different varieties (Goncalves et al., 2013). The reduction of TPC Value in taro may be due to various factor such as genotype, preharvest treatment, flesh colour and also the thermal process applied (Tang, Cai, & Xu, 2015). As current study focusing on the heating treatment to improve the digestibility and palatability of taro corm, the decreasing TPC Value in taro with the total loss approximately 40% in each cooked sample become the major concern. In many past studies, the anthocyanins derivates which are cyaniding are susceptible to degrade when exposed to a prolonged heat treatment

and the trigger effect of high hydrostatic pressure to hydrolyse cyanidin-3-glucoside (Cy3gl) (De Aguiar Cipriano et al., 2015).

Moreover, the pelargonidin was also to be said as relatively unstable and easily susceptible to degradation during processing and storage (Sun et al., 2016). The same study convinces that the factors affecting anthocyanins include light, oxygen, temperature, pH, structure and concentration of the anthocyanins, and the presence of other compounds, including other flavonoids and phenolics. This may explain why degradation occurs among the cooked sample. Both anthocyanins derivatives present in the taro corm sample may not give overall purplish colour to the taro used in the current study, but they did appear in abundant small circle dot which was purple in colour.

4.3 Total Flavonoid Content

As antioxidants, flavonoid has been reported to be able to interfere with the biochemical pathway involved in the generation of reactive oxygen species (ROS), in addition to quenching of free radicals and chelating of redox active metals (Hue, Boyce, & Somasundram, 2012). Results illustrate those total phenolics, flavonoids and flavanols are present in significant amount in tubers and roots crop tested in the current study. Their presences in addition to their multifaceted actions make the crops as a potent candidate for exploring as an antioxidant.

4.3.1 Total Flavonoid Content in Potato

The results of the Aluminium Chloride assay for potato are shown in Table 5. The total flavonoid contents among the different samples were expressed in terms of catechin equivalents using the standard curve equation $y = 0.001x + 0.0095$, $R^2 = 0.9986$. It was observed that the flavonoids contents ranged between 4.75 and 15.75 mg/g catechin equivalents. The raw potato sample has the highest flavonoids content at 15.75 ± 0.056 mg/g followed by the fry potato sample, whereas the steamed and boiled varieties had almost similar total flavonoids contents whereas boiled potato contained the lowest flavonoids content compared to the other potato that undergoes different thermal process at 4.75 ± 0.102 µg/g. All samples were significantly different from each other.

Table 5: Total flavonoid content (TFC) of the potato sample with different thermal process applied

Thermal process	TFC value (mg CE/g dried sample)
Raw	15.75 ± 0.056^{a}
Fried	10.75 ± 0.095^{b}
Steamed	6.25 ± 0.088^{c}
Boiled	4.75 ± 0.102^{d}

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for TFC was 100 mg/ml.

A significant reduction TFC from raw to the cooked potato was observed. The least TFC were found is boiled potato, probably due to the boiling process itself, where it helps thin out the cell walls and cause the leaching of bioactive constituents into the boiling water (Bellumori et al., 2017). In addition, a previous study by Naeem (2011.) offered indisputable evidence for the total loss of flavonoid found in the current study. Analysis of fresh potatoes revealed that they were always a better source of flavonoids. However, when potatoes samples were deeply fried it was observed that loss of flavonoids was 73-74 %.

There is a satisfactory agreement between the flavonoid found in potatoes such as quercetin, myricetin, naringenin, and kaempferol and its effect towards high temperature. For instance, many studies reported that these flavonoid derivatives will undergo great degradation in foods treated at 100° C (A. Wang et al., 2018). Despite the heat applied, different processing methods such as peeling and blanching result in a great loss of flavonoids (X. Chen et al., 2017). One study reveals that the pilot plant peeling and blanching as what was applied in the current study may significantly reduce the flavonoid content to approximately one-half of the starting levels; this is probably because of the loss of the outer flavonoid-rich potato peel layer (Rytel et al., 2014).

The results indicated that heating at different temperatures resulted in fluctuation in the content of the individual flavonoids, but the overall flavonoid content was retained. One downside of thermal cooking applied in potato sample with relation to TFC content, heating is accountable for the oxidation, thermal degradation, and leaching of bioactive compounds from fresh samples (Nurdjanah, 2017).

4.3.2 Total flavonoid content in Sweet Potato

The results of the Aluminium Chloride assay for sweet potato are shown in Table 6. The total flavonoid contents among the different samples were expressed in terms of catechin equivalents using the standard curve equation y = 0.001x + 0.0095, R^2 =0.9986. It was observed that the flavonoids contents ranged between 9.25 and 18.75 mg/g catechin equivalents. The fried sweet potato sample has the highest flavonoids content at 18.75 ± 0.113 mg/g, whereas the steamed and boiled varieties had almost similar total flavonoids contents whereas boiled sweet potato contained the lowest flavonoids content compared to the others sweet potato that undergoes the different thermal process at 9.25 ± 0.110 µg/g. All samples were significantly different from each other.

Table 6: Total flavonoid content (TFC) of the sweet potato sample with different thermal process applied

Thermal process	TFC value (mg CE/g dried sample)
Raw	12.25 ± 0.098^{a}
Fried	18.75 ± 0.113^{b}
Steamed	13.75 ± 0.109^{c}
Boiled	9.25 ± 0.110^{d}

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for TFC was 100 mg/ml.

The colour of the raw sample of sweet potato used in this study was orange. The flavonoid or being called Vitamin P itself is the pigment that contributes to the plant's colour. From the previous study that being broadly discussed, anthocyanin is the most flavonoid found in sweet potato with purple coloured flesh (Cai et al., 2018). Even though these sweet potato samples differ from some published studies, they are consistent with those result reported by Lebot et al.,(2016). For orange-fleshed, the phytonutrients that obligate to give orange colour in sweet potato fleshed is the carotenoids. To be specific, the sweet potato belongs to beta-

carotene in the Carotenoid Phytochemical Sub-classes while it also promotes the pro-vitamin A as the benefits to consumers.

Yellow varieties of sweet potatoes and yams are good sources of carotenoids. It has been demonstrated that zeaxanthin and lutein are stable throughout artificial digestion, whereas β-carotene and all-trans lycopene are degraded in the jejunal and ileal compartments (Muller, Frohlich, & Bohm, 2011). However, in this case, the isomerisation and stability of β-carotene at different temperatures were examined. The highest TFC Value was found in fried sweet potato, probably due to the vegetable cooking oils used were a carrier for vitamin A fortification.

Despite the micronutrients that eventually contribute to the flavonoid level due to the synergize effect, proanthocyanidins are flavonoids that have also been reported to be found in sweet potato (Renard, Watrelot, & Le Bourvellec, 2017). The stability of this flavonoids somehow is also an issue. Its fortified in foods can be lost due to degradation and epimerisation during thermal processing (Hue et al., 2012). Since that, precautions are necessary when sweet potatoes undergo any thermally processed or cooked. The flavonoid has also been found to be very unstable in alkaline solutions Thus, the sample preparation starting from blanching, steaming and boiled especially foods in liquid form, having high pH values could lead to degradation of the during processing. In order to maintain the bioactive constituents in these foods, acids may have to be added to reduce their pH but not practical in order to mimic household practise, although can be applied in food industry processing.

4.3.3 Total flavonoid content in Taro

The results of the Aluminium Chloride assay for taro are shown in Table 7. The total flavonoid contents among the different samples were expressed in terms of catechin equivalents using the standard curve equation $y = 0.001x + 0.0095$, $R^2 = 0.9986$. It was observed that the flavonoids contents ranged between 4.75 and 15.75 mg/g catechin equivalents. The raw potato sample has the highest flavonoids content at 15.75 ± 0.056 mg/g followed by the fry potato sample, whereas the steamed and boiled varieties had almost similar total flavonoids contents whereas boiled potato contained the lowest flavonoids content compared to the other potato that undergoes different thermal process at 4.75 ± 0.102 µg/g. All samples were significantly different from each other.

Table 7: Total flavonoid content (TFC) of the taro sample with different thermal process applied

Thermal process	TFC value (mg CE/g dried sample)
Raw	8.80 ± 0.127^{a}
Fried	6.75 ± 0.103^{b}
Steamed	5.25 ± 0.119^{c}
Boiled	1.75 ± 0.106^{d}

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for TFC was 100 mg/ml.

The differences in flavonoid content in taro observed in the could due to the cooking process applied, which have been shown to have an effect on the accumulation of flavonoid compounds presence (Goncalves et al., 2013). As the major bioactive constituents that presence in taro corm is quercetin (Cuervo et al., 2016), the reduction of TFC value in relation with these two bioactive compounds and its effect on heat process were discussed in depth.

There is a huge reduction of TFC Value from raw taro corm in comparison to taro that undergoes frying, steaming and boiling process. The processing of taro powder involves several steps such as storage, pre-treatment, drying, and cooking where these steps affect

the composition of the bioactive components of the taro as reported by Arici et al.,(2016). The reason for the fluctuation could be because of the heating time required at a particular temperature for the breakdown of cellular constituents to release the individual flavonols. The quercetin conjugates in the taro corm are resistant to thermal degradation. However, during food processing autolytical changes can influence the flavonol composition in taro, hence, reducing the total flavonoid content in it (Cuervo et al., 2016),(Bradbury et al., 1988).

4.4 Total Antioxidant Capacity

Assessment of the antioxidant capacity of natural products has been regarded as a basis for ranking the antioxidant plants and recommending best antioxidant foods for consumption. The evaluation of the antioxidant activity of food and medicinal plants can be performed using chemical-based assays and cellular-based assays, however, a current study focusing the evaluation via chemical-based assay which is ferric ion reducing antioxidant power (FRAP), DPPH radical scavenging activity assay and Beta Carotene bleaching assay.

4.4.1 FRAP Reducing Power (FRAP assay)

FRAP Assay measures the change in absorbance at 593 nm due to the formation of a blue coloured Fe^{2+}- tripyridyltriazine compound from colourless oxidized Fe^{3+} form by the action of electron donating antioxidants. The ability of the organic sample to reduce Fe^{3+} to Fe^{2+} were explained in the previous study (Bolanos De La Torre, Henderson, Nigam & Apenten, 2015).

4.4.2 FRAP Assay in Potato

The results of the FRAP assay for potato are shown in Table 8. The total FRAP value among the different samples was expressed in terms of $FeSO_4$ equivalents using the standard curve equation $y = 0.012x - 0.0105$, $R^2 = 0.9983$. The lyophilized raw sample showed the strongest reducing power (7.94 ± 0.137), which was significantly higher than that of other samples that undergo the thermal process. This was followed by samples treated by the frying process(5.81 ± 0.111) and steaming process (3.15 ± 0.125). However, boiled lyophilization treated sample demonstrated the lowest reducing power (2.95 ± 0.109). Aqueous solutions of known Fe (II) concentration, ($FeSO_4$) were used for obtaining the calibration curve.

Table 8: FRAP Value for raw potato with different thermal process applied

Thermal process	FRAP value (mm Fe^{2+}/g dried sample)
Raw	7.94 ± 0.137^{a}
Fried	5.81 ± 0.111^{b}
Steamed	3.15 ± 0.125^{c}
Boiled	2.95 ± 0.109^{d}

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for FRAP assay was 100 mg/ml.

Direct raw lyophilization treatment, which was without heat showed much stronger antioxidant activity than other processing method treated samples. This could explain the relation between phenolic and flavonoid content reported in earlier subtopics with the antioxidant capacity in potato. In addition, phenolic present in potato is well known as a strong antioxidant (Ruiz et al., 2018).

The presence of antioxidant compound presence in potato leads to the disappearance colour of absorbance reading generated in chemical method of antioxidant detection are the stable free radicals (Bolanos De La Torre et al., 2015).In this case, chlorogenic acid in potato able and potent to donate hydrogen and electrons(Jin et al., 2018). Stabilization of ascorbic is often stabilized in the biological sample by the addition of strong acid that is used in this current study, may significantly increase the antioxidant content (Jamal & Othman, 2014).

As reported by Fu et al.,(2016), the quercetin may appear as the most active compound and were 3.02 times more active than Trolox,hence, give the highest contribution to the antioxidant capacity in potato.

4.4.3 FRAP Assay in Sweet Potato

The results of the FRAP assay for sweet potato are shown in Table 9. The total FRAP value among the different samples was expressed in terms of $FeSO_4$ equivalents using the standard curve equation y = 0.012x - 0.0105, R^2 =0.9983. The fried sample showed the strongest reducing power (8.01 ± 0.091), which was significantly higher than that of other samples that undergo the thermal process. This was followed by samples treated by the steamed process (4.21 ± 0.105) and boiling process (3.01 ± 0.078). The raw sample demonstrated a moderate reducing power (4.08 ± 0.117). Aqueous solutions of known Fe (II) concentration, ($FeSO_4$) were used for obtaining the calibration curve.

Table 9: FRAP Value for raw sweet potato with different thermal process applied

Thermal process	FRAP value (mm Fe^{2+}/g dried sample)
Raw	4.08 ± 0.117^{a}
Fried	8.01 ± 0.091^{b}
Steamed	4.21 ± 0.105^{c}
Boiled	3.01 ± 0.078^{d}

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for FRAP assay was 100 mg/ml.

The polyphenol present in sweet potatoes such as pelargonidin, petunidin, malvidin and peonidin is susceptible to hydration, which may cause the colour loss in FRAP Assay (Awa & Eleazu, 2015). The availability of those components may help in chelation between bioactive components and metal cation and can increase the stability as well, hence, the discolouration that occurs in sweet potato sample may explain this (Kadiroglu, Aydemir, & Akcakaya, 2018). In addition, the reduction of the phenolics present in sweet potato that undergoes the steaming and boiling process. The stability of those polyphenols is also to be said as strongly influenced by pH, light, heat and mechanical stress (N. Chen, Zhao, Niepceron, Nicolai, & Chassenieux, 2017). Thus, an important attribute of these pigments is

that they are a potent antioxidant in human diet, however, may be significantly reduced depending on the type of thermal process applied.

4.4.4 FRAP Assay in Taro

The results of the FRAP assay for taro are shown in Table 10. The total FRAP value among the different samples was expressed in terms of $FeSO_4$ equivalents using the standard curve equation y = 0.012x - 0.0105, R^2 =0.9983. A similar trend was observed for taro and potato. The raw sample showed the strongest reducing power (4.21 ± 0.156), which was significantly higher than that of other samples that undergo the thermal process. This was followed by fried samples (2.84 ± 0.131) and steamed process (1.34 ± 0.165). The least reducing power is demonstrated in the boiled sample (1.21 ± 0.149). Aqueous solutions of known Fe (II) concentration, ($FeSO_4$) were used for obtaining the calibration curve. The comparative activity of taro to the standard used suggests the potential antioxidant activity of the sample.

Table 10: FRAP Value for raw taro with different thermal process applied

Thermal process	FRAP value (mm Fe^{2+}/g dried sample)
Raw	4.21 ± 0.156[a]
Fried	2.84 ± 0.131[b]
Steamed	1.34 ± 0.165[c]
Boiled	1.21 ± 0.149[d]

Data are expressed as mean ± SD (n = 3), based on dried weigh. In each column, different small letters indicate significant differences ($p<0.05$) among different samples. The concentration used for FRAP assay was 100 mg/ml.

Studies on the chemical constituents of taro have reported the presence of pelargonidin-3-glucoside, cyanidin-3-rhamnoside, cyanidin-3-glucoside, orientin, isoorientin, isovitexin, vitexin X-O-glucoside and luteolin 7-O-glucoside (De Aguiar Cipriano et al., 2015). However, the thermal process applied may reduce or increase those constituents, depending on its properties. In taro, degradation of reducing power when the thermal

process was applied especially in the sample with water as a cooking medium were observed.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Kasote et al., 2015). In addition, those compound have metal chelating potential (Wu et al., 2015). The same study convinces that all these reactions mechanism allow phenolic compounds to act as preventive or chain-breaking antioxidants. This may explain the relation of reducing polyphenol with the degradation of reducing, due to the effected stability polyphenols presence in processed taro sample (Pereira, Silva, Verícimo, Paschoalin, & Teixeira, 2015). It can be said that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

4.5 DPPH Radical Scavenging Activity

According to Chellaram et al. (2014), the ability of phenolic compounds to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity assay. Activity is measured as the relative decrease in absorbance at 517 nm as the reaction between DPPH and antioxidant progress. DPPH radical scavenging activity is plotted as a function of µg per mL sample concentration. Per cent activity was observed to increase with sample concentration between 20 and 100 µl per mL.

4.5.1 DPPH Radical Scavenging Activity in potato

The scavenging activity of potato with different treatments was in the order of steamed,fried and boiled > raw flour (Figure 4). For the raw potato, scavenging DPPH radical effects were superior to the other three samples. The scavenging effects were all above 80% at 100 mg/ml. The potato sample possessed a similar DPPH scavenging activity to those of BHT that being used as standard.

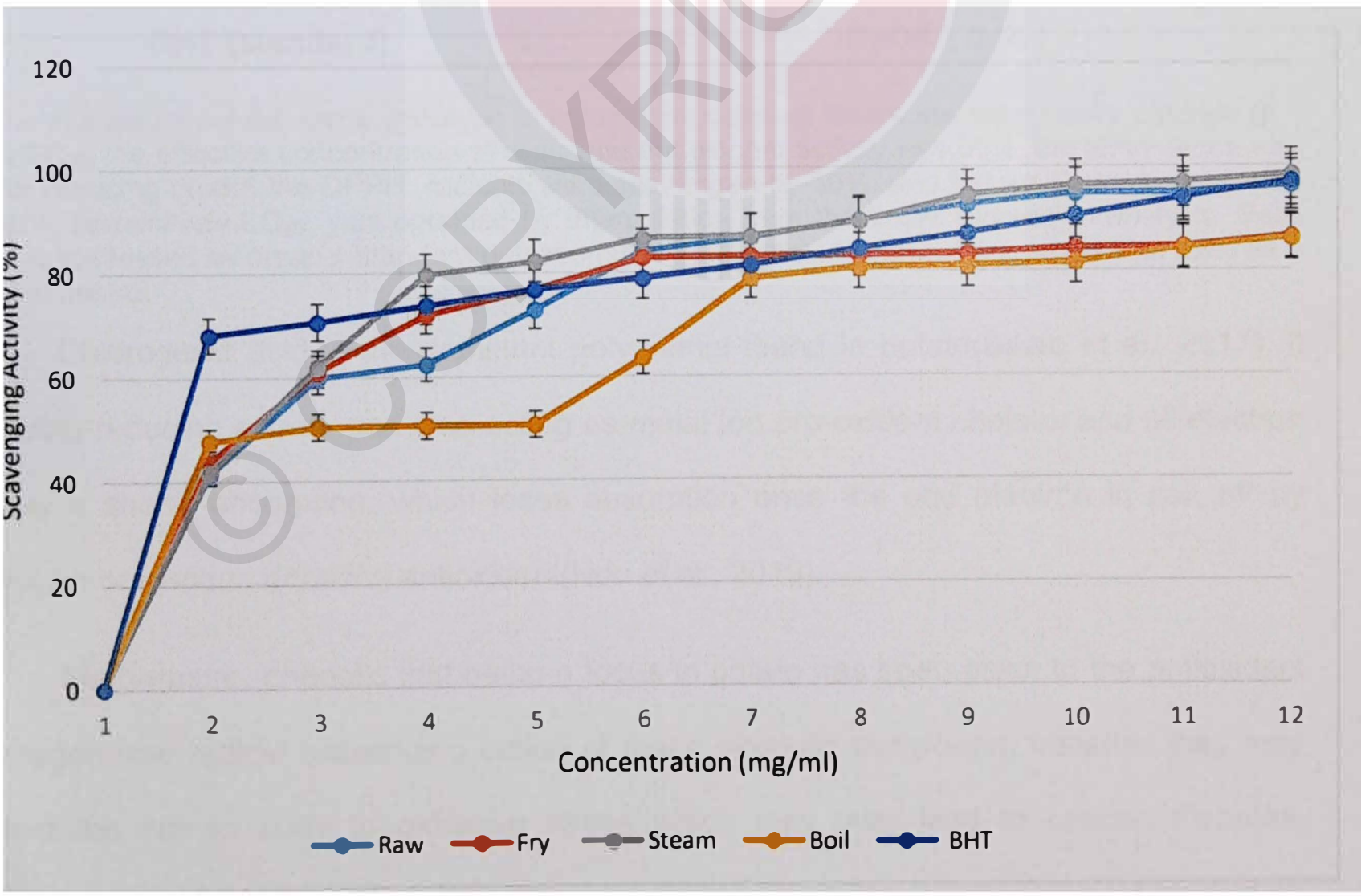


Figure 4: Scavenging DPPH radical effects of raw potato with different thermal process applied

EC_{50} values for scavenging DPPH radicals of potato were ranged from 2.214 – 4.342 mg/ml (Table 11). As the EC_{50} indicates the effective concentration at which antioxidant activity was formed by 50%, the raw potato has the significant lowest EC_{50}, which means, it has higher antioxidant potential. The potato also showed potent of both free radical scavenging activity in inhibitory activity. The concentration increase linearly with the scavenging potential.

Table 11: EC_{50} in antioxidant properties from raw potato and different thermal process applied

Thermal process	EC_{50} value
Raw	2.214±0.03[a]
Fried	2.864±0.06[b]
Steamed	2.525±0.18[c]
Boiled	4.342±0.04[d]
BHT (standard)	0.908 ± 0.381

Means in a column of the same genotype of potato with different letters are significantly different ($p < 0.05$).EC_{50}, the effective concentration at which the antioxidant activity was 50%, the absorbance was 0.5 for reducing power, the DPPH radicals were scavenged by 50%, and ferrous ions were chelated by 50%, respectively.EC_{50} was obtained by interpolation from the linear regression analysis. Each value is expressed as mean ± standard deviation (n = 3). Butylated hydroxytoluene (BHT)is used as a positive control.

Chlorogenic acid is the dominant polyphenol found in potato(Baiao et al., 2017). It providing reducing activity and also acting as metal ion pro-oxidant chelator.and all electron display a strong absorption, which loses absorption once the odd electron in pair off by hydrogen or electron donating antioxidant(Hou et al., 2019).

Furthermore, phenolic that being a focus in potato has been given to the antioxidant or oxygen free radical scavenging action of these phenolic compound, because they may protect the human body to oxidative stress which may later lead to cancer, diabetes, coronary heart disease and Alzheimer(Manach, 2004). Thus, the chlorogenic acid production is known to have potent scavenging radical activity (Jin et al., 2018), and might present abundantly in the potato genotype present in the current study. Therefore, the consumption

of an adequate amount of potato that undergoes frying, steaming and boiling daily maybe be able to protect the human body from such condition.

4.5.2 DPPH Radical Scavenging Activity in sweet potato

Radical scavenging activity of sweet potato was compared with the activity of synthetic antioxidant, BHT (Figure 5).In this investigation, the activity of the sample and BHT increased linearly with concentration. Although the activities of the boiled sample were lower than the activity of BHT, all sample demonstrated notable antioxidant properties. In fact, many previous studies reported that the purple-fleshed sweet potato cultivar has a higher radical-scavenging or antioxidant activity than those with white, yellow or orange flesh (Hara et al., 2018). Furthermore, phenols were the major compounds for DPPH radical-scavengers in sweet potato(Manach, 2004). Similar results were found in this study although the sweet potato used is orange, whereas it still showed a potent scavenging activity.

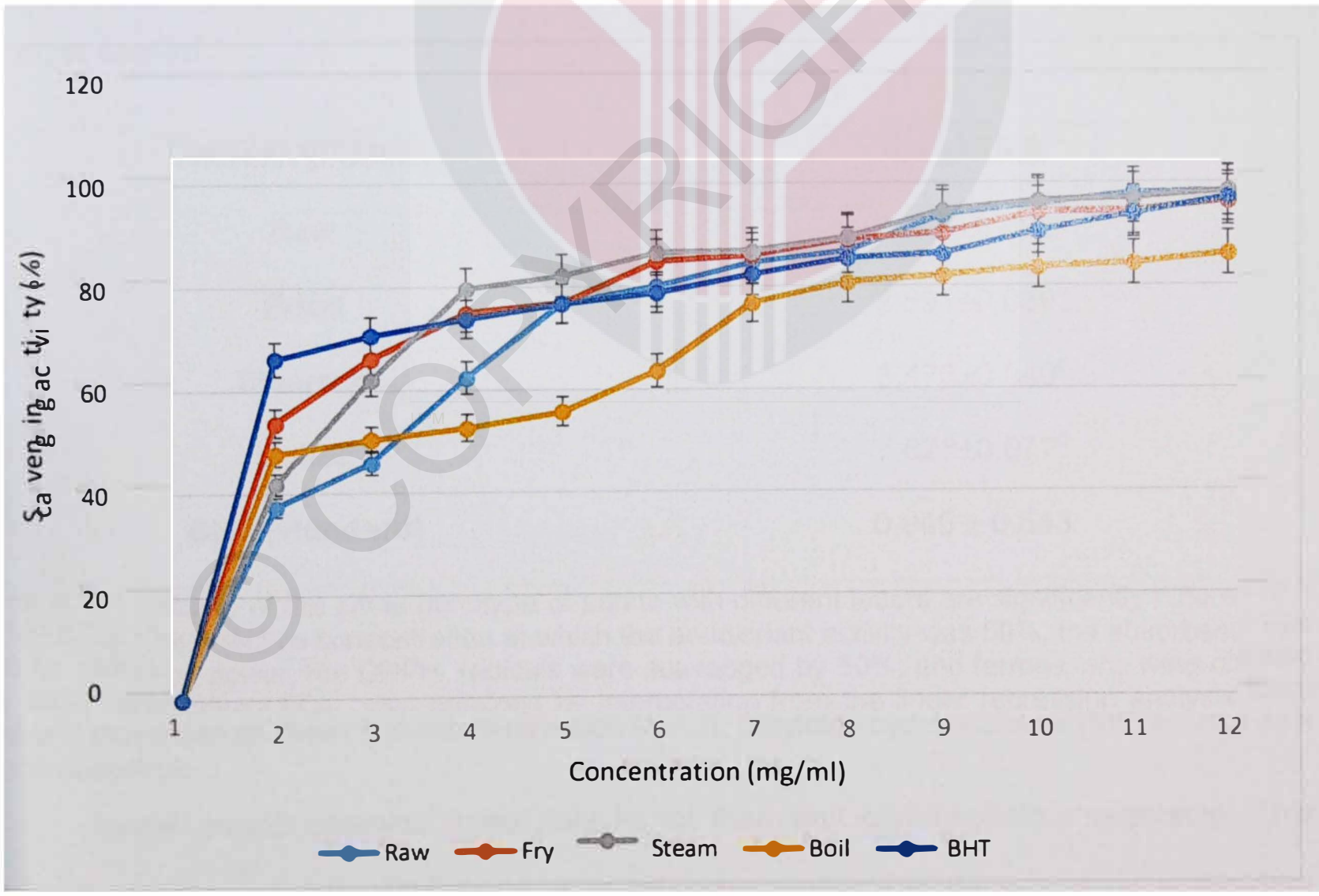


Figure 5: Scavenging DPPH radical effects of raw sweet potato with different thermal process applied

The previous study reported that the yields from cooked sweet potato flours were 3–4 times higher than that from the corresponding raw sample (Wegener & Jansen, 2013). This implied that cell structure damage and/or partial hydrolysis of biopolymers might occur during cooking treatments. In contrast to the yield production, the EC50 in fried sweet potato was the lowest among all thermal process and raw sweet potato. This has been discussed earlier that sweet potato which has demonstrated Maillard model system consisting of cysteine and glucose at low and high pH and resulted that a high total phenolic content was found in alkali conditions than in the neutral and acidic conditions (Tamanna & Mahmood, 2015). Our study also showed the same phenomenon where the total phenolics increased with increasing flavonoids and the end point, hence, increasing the antioxidant capacity potential in fried sweet potato (Oladejo et al., 2017).

Table 12: EC_{50} in antioxidant properties from raw sweet potato and different thermal process applied

Thermal process	C_{50} value
Raw	3.556± 022[a]
Fried	2.891±0.039[b]
Steamed	6.473±0.040[c]
Boiled	7.628±0.077[d]
BHT (standard)	0.945 ± 0.543

Means in a column of the same genotype of potato with different letters are significantly different ($p < 0.05$).EC_{50}, the effective concentration at which the antioxidant activity was 50%, the absorbance was 0.5 for reducing power, the DPPH radicals were scavenged by 50%, and ferrous ions were chelated by 50%, respectively.EC_{50} was obtained by interpolation from the linear regression analysis. Each value is expressed as mean ± standard deviation (n = 3). Butylated hydroxytoluene (BHT)is used as a positive control.

Sweet potato contains more polyphenol than any commercialize vegetable. This phytochemical in sweet potato possess multifaceted action and these include antioxidant properties(Teow et al., 2007). As phenolic acid was generally present in sweet potato, beta carotene is also known as one of the terpenoids with a strongly coloured red-orange pigment available in the plant(Wadood, 2013). Related flavonoid and the phenolic compound may

scavenger free radical reactive oxygen species inside the cell (Knezevic et al., 2011). Thus, the antioxidant capacity of sweet potato is correlated with the total polyphenol content.in contrast, many studies also reported that stronger antioxidant content founds in the peel of white and purple varieties as well as the skin of sweet potato itself(Lebot et al., 2016).

Moreover, zeaxanthin and lutein present in sweet potato are also as well known phytochemicals that help in decreasing the formation of products of oxidation damage to biological molecules by scavenging superoxide and hydroxyl radicals (Cuervo et al., 2016). The mechanism of antioxidant of melandonins has been attributed to affinities that include scavenging hydroxyl and peroxy radicals in sweet potato, respectively, breaking the radical chain by donation of hydrogen and also chelating pro-oxidant transition metal ions (Kadiroglu et al., 2018).

4.5.3 DPPH Radical Scavenging Activity in taro

In comparison with the BHT as standard, all samples manifest linear trends corresponding with the higher concentration, the higher the scavenging activity, All samples were relatively scavenging up to 90%, respectively (Figure 6).

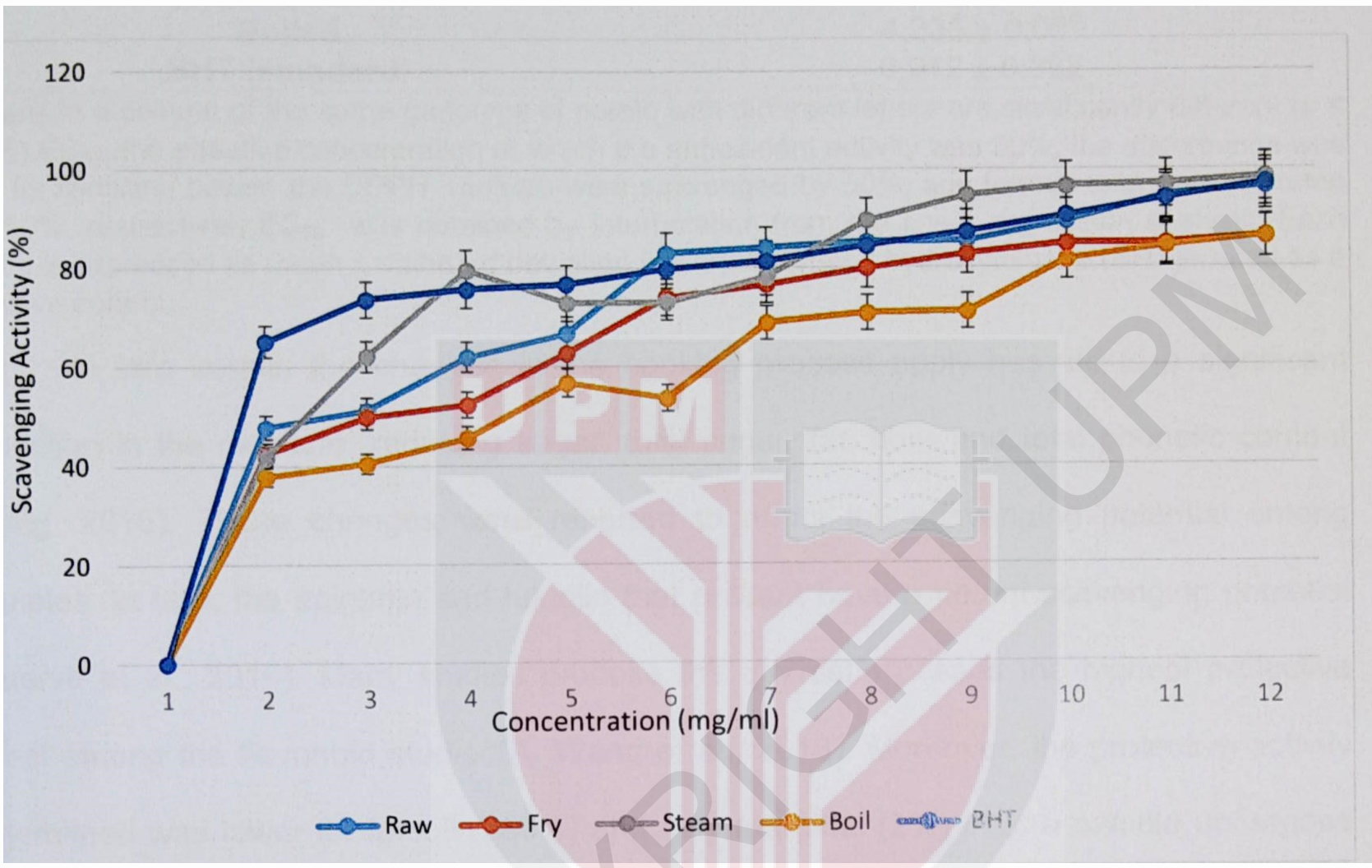


Figure 6: Scavenging DPPH radical effects of raw taro with different thermal process applied

Despite that, as what has been discussed earlier, the raw taro had the highest phenolic content, hence, it also has the highest DPPH radical scavenging activity with an EC_{50} value of 2.608±0,05mg/ml. Among the taro that undergoes the thermal process, it was observed that fried taro has the EC50 of 3.604 ± 0.03, followed by steamed (4.652 ± 0.08) and boiled taro (4.335 ± 0.05).

Table 13: EC_{50} in antioxidant properties from raw taro and different thermal process applied

Thermal process	EC_{50} value
Raw	2.608 ± 0.05^{a}
Fried	3.604 ± 0.03^{b}
Steamed	4.652 ± 0.08^{c}
Boiled	4.335 ± 0.05^{d}
BHT (standard)	0.912 ± 0.362

Means in a column of the same genotype of potato with different letters are significantly different ($p < 0.05$).EC_{50}, the effective concentration at which the antioxidant activity was 50%, the absorbance was 0.5 for reducing power, the DPPH radicals were scavenged by 50%, and ferrous ions were chelated by 50%, respectively.EC_{50} was obtained by interpolation from the linear regression analysis. Each value is expressed as mean ± standard deviation (n = 3). Butylated hydroxytoluene (BHT)is used as a positive control.

In taro corms, the changes in the cooking process apply has led to a significant reduction in the moisture, reducing sugar, total sugar, fat, fibre and total phenolic content (Tong, 2016). These changes were reported to affect the scavenging potential among samples. In taro, the apigenin and luteolin that present have a potent scavenging potential (Cuervo et al., 2016). Many studies propose the quercetin posses the highest protective effect among the flavonoid studied(A. Wang et al., 2018). Moreover, the protective activity determined was lower for luteolin (40%) and only marginal (2%) after a sample undergoes heat treatment(Mergedus et al., 2015), explaining the trend of the current study. On the other hand, a higher concentration may also increase apigenin(Cuervo et al., 2016). This flavonoid somehow may induce DNA single-strand break. This indicates the ability of apigenin to serve as a prooxidant, hence, reduce the scavenging capability in process taro. Although this scavenging pattern may not be higher as potato and sweet potato, it still contributes to many health benefits as it offers anticancer properties, controlling blood sugar and may reduce the risk of heart disease.

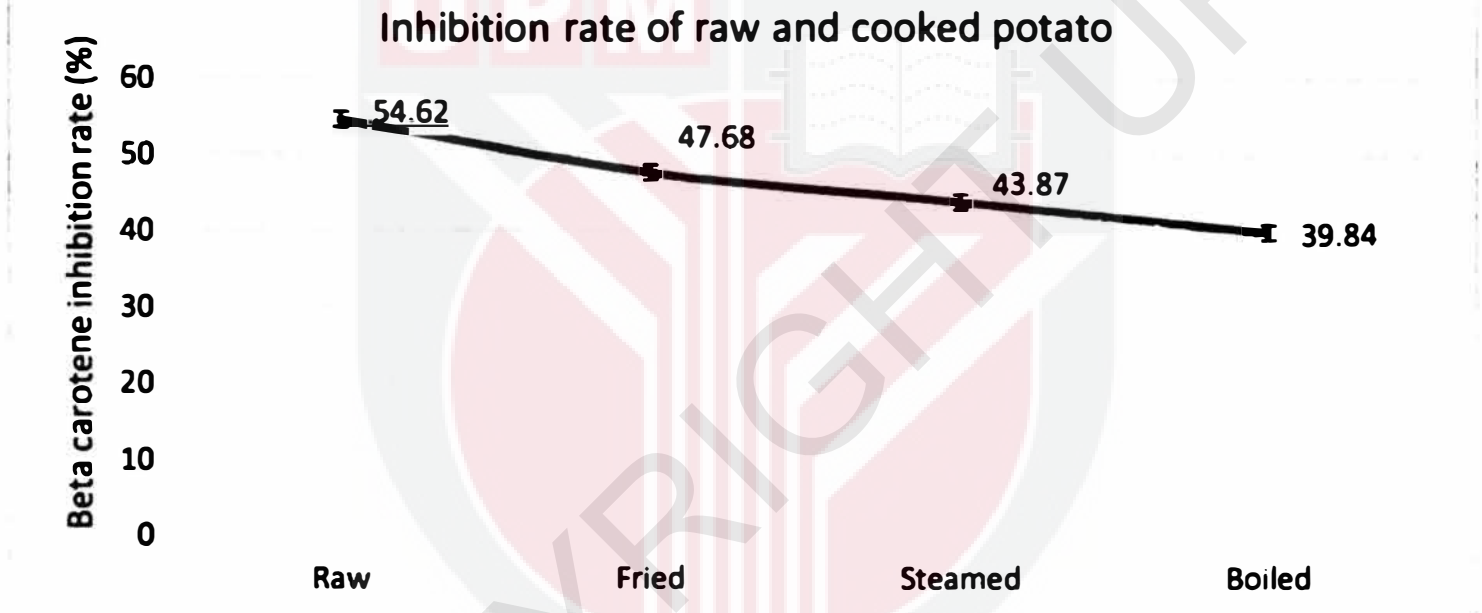
4.6 Beta Carotene Bleaching Activity

The β-carotene bleaching assay for evaluating antioxidant activity is one of the common methods used in the field of food chemistry (Reyes et al., 2016). The same study suggests the principle of the method is based on the discolouration of yellowish colour of a β-carotene solution due to the breaking of π-conjugation by addition reaction of lipid or lipid peroxyl radical (or) to a C=C double bond of β-carotene. The radical species is generated from the autoxidation of linoleic acid by heating under air atmosphere.

When the appropriate antioxidant is added to the solution, the discolouration can be retarded by competing for the reaction between β-carotene and antioxidant with the subjected radicals. The structural similarity between fullerenes and β-carotene, such as highly π-conjugated molecules, enables the accurate evaluation of antioxidant activity by this β-carotene bleaching assay in contrast to other methods like DPPH radical assay.

4.6.1 Beta Carotene bleaching activity in potato

β-Carotene bleaching inhibition showed that maximum antioxidant activity was observed in the raw potato followed by the fried, steamed and boiled. It was also clear that raw potato parts have significantly high inhibited antioxidant activity compared to potatoes that undergo the thermal process. The antioxidant effect responsible for each pharmacological activity is found to inhibit ROS (Kasote et al., 2015). Those compound mainly chlorogenic acid as it may help in anti-microbial, blood coagulating and insecticidal properties.



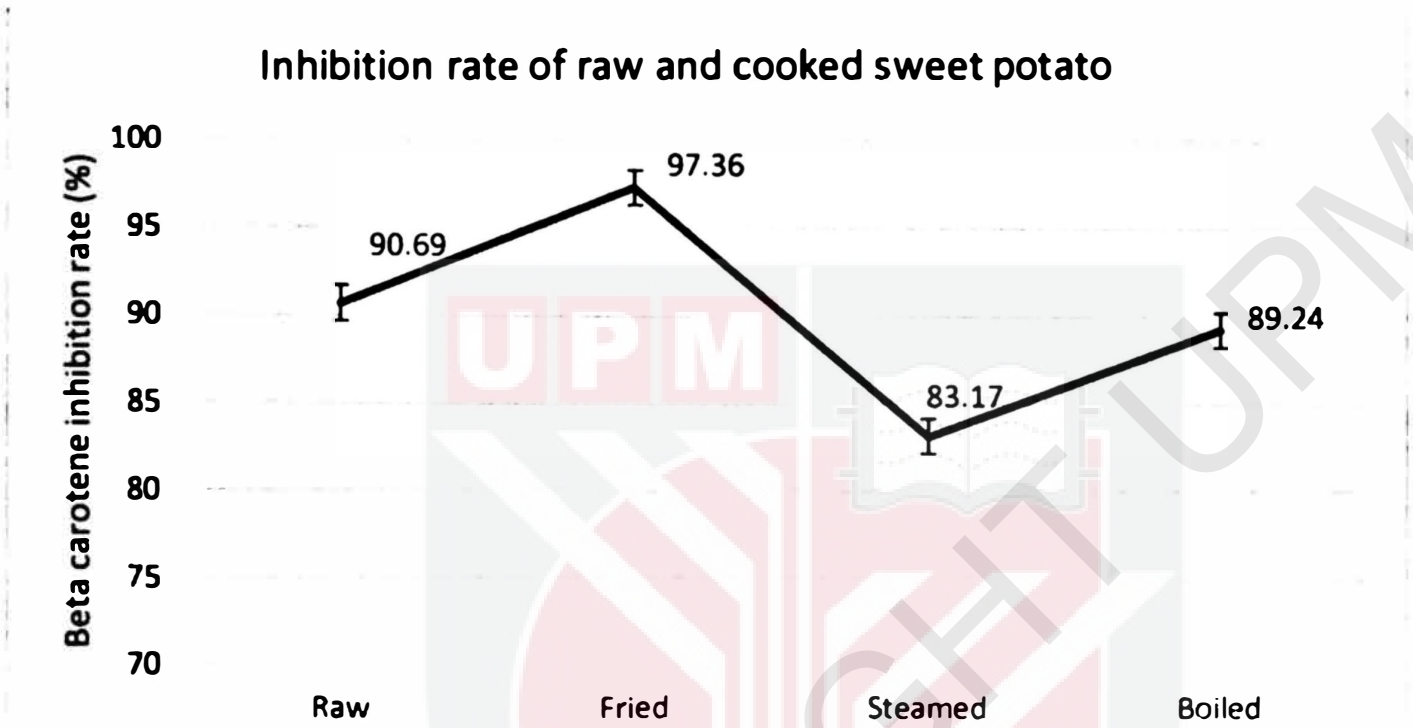
β-Carotene bleaching activity was determined in different thermal process in the potato. The bars represent standard deviations of three independent experiments. The antioxidant activities of potato parts using the β-carotene bleaching assay were evaluated by measuring the inhibition of potato.

Figure 7: Inhibition rate of raw and cooked potato

Moreover,the antioxidant activity of the antioxidant and positive control demonstrate the dose-respond relationship. Other previous studies agree that chlorogenic acid expresses a relation with 20.8 – 0.3 % antioxidant activity at the concentration of 0.125 mg/ml (Singh & Saldana, 2011). Furthermore, the rate of beta carotene bleaching can be delayed with the present of antioxidant explained in a previous study (Prajapati et al., 2013). However, it needs to be noted that phenolic acid present when being exposed to prolong heat may tum into pro-oxidant, hence, induce ROS or inhibit antioxidant system and eventually will affect the total antioxidant capacity (Kasote et al., 2015).

4.6.2 Beta Carotene bleaching activity in sweet potato

In sweet potato, the β-Carotene bleaching inhibition showed that maximum antioxidant activity was observed in the fried sweet potato followed by the boiled and steam. Although all taro sample including the raw one posses more than 80% inhibition rate, this was explained by the antioxidant content in the sample itself, as discussed in the earlier section.



β-Carotene bleaching activity was determined in different thermal process in the sweet potato. The bars represent standard deviations of three independent experiments. The antioxidant activities of sweet potato parts using the β-carotene bleaching assay were evaluated by measuring the inhibition of sweet potato.

Figure 8: Inhibition rate of raw and cooked sweet potato

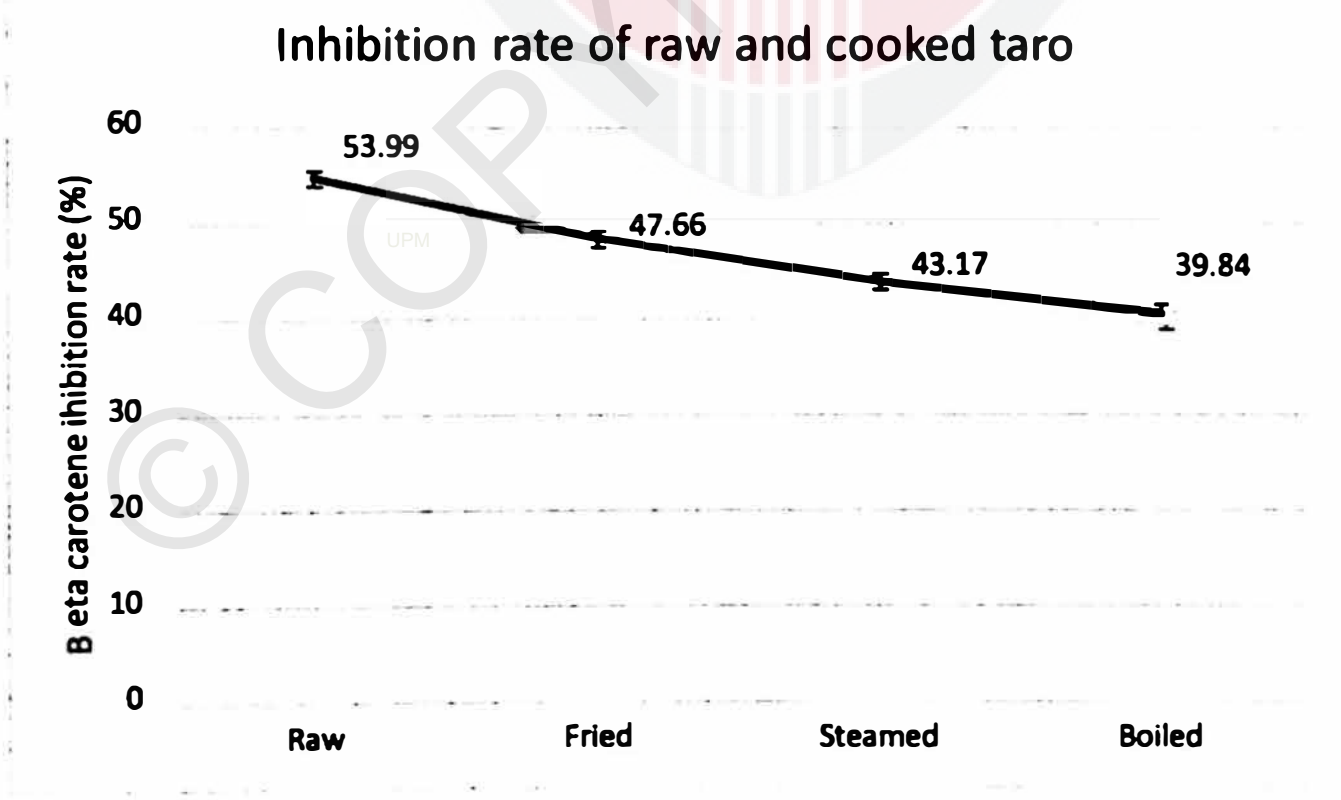
It was also clear that raw sweet potato has significantly high inhibited antioxidant activity and comparable with sweet potatoes that undergo the thermal process. The antioxidant effect that responsible for each pharmacological activity are found in taro were responsible to inhibit ROS(Kasote et al., 2015). Those compound mainly chlorogenic acid as it may help in anti-microbial, blood coagulating and insecticidal properties.

Sweet potato with orange flesh is believed to have abundant carotenoid(Teow et al., 2007). Thus, this may explain the activity of various BC isomers in protecting against cancer and cardiovascular disease and its inhibition rate that explain the antioxidant capacity in sweet potato (Mvitu Muaka et al., 2010). As the carotenoid and lutein were respectively reported to decrease when it undergoes the thermal process, it may also influence to reduce

the inhibition capacity (Sun, Mu, Xi, Zhang, & Chen, 2014). However, in fried sweet potato, lycopene may attribute to a different pattern, whereas after prolong heat were applied, the concentration may increase in the flesh but dramatically reduce in the skin(-36 °C) (Krochmal-marczak et al., 2014). Although lycopene is not the most easily found in sweet potato, these phytochemicals present may slightly attribute to the amount of antioxidant as well.

4.6.3 Beta Carotene bleaching activity in taro

For taro, the β-Carotene bleaching inhibition showed that minimum antioxidant activity in the boiled sample, followed by steamed and fried, in comparison to the raw taro which manifests the highest inhibition rate. However, the inhibition activity was quite low (does not exceed 60%) which is reduced almost half from antioxidant capacity showed in potato and sweet potato. This was explained by the antioxidant content in the sample itself, as probably not only the selective thermal process applied influence this pattern, but also from the storage, sample preparation and selection of taro genotype itself.



β-Carotene bleaching activity was determined in different thermal process in the taro. The bars represent standard deviations of three independent experiments. The antioxidant activities of taro parts using the β-carotene bleaching assay were evaluated by measuring the inhibition of taro.

Figure 9: Inhibition rate of raw and cooked taro

As explained in the antioxidant content part of taro earlier, there were also slightly low compared to potato and sweet potato even in a raw sample. However, it still manifests the inhibition rate at moderate range (Figure 9). This may be due to the polyphenols present that are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications (Prakash et al., 2012).

Due to its inhibition rate in taro, many studies agreed with their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled and their capacity to modulate key cellular enzyme functions. The polyphenols present may also attribute as a potent inhibitor for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase (Camargo et al., 2017).

4.7 Correlation Between Total Phenolic Content,Total Flavonoid Content And Antioxidant Capacities

4.7.1 Total phenolic content, Total flavonoid content and Antioxidant capacities

The correlation coefficients (r) between TPC and antioxidant capacities of potato, sweet potato and taro are shown in Table 14. In all sample in arrangement of potato,sweet potato and taro,TPC revealed a very strong positive correlation with the FRAP assay (r = 0.999),(r = 1.000) and (r =0.994).

Table 14: Pearson correlation analysis of the antioxidant contents (TPC) and antioxidants activities of raw potato, sweet potato and taro with different thermal process applied

		FRAP	DPPH	BCB
Potato	**TPC**	0.999**	-0.352**	-0.476
Sweet Potato		1.000**	-0.507**	-0.433
Taro		0.994**	0.399**	0.098

In addition, the correlation coefficients (r) between TFC and antioxidant capacities of potato,sweet potato and taro are shown in Table 15. In all sample in arrangement of potato,sweet potato and taro,TFC revealed a very strong positive correlation with the FRAP assay (r = 0.992),(r = 0.979) and (r =0.892). Table 15 tabulate the strong negative correlation with the DPPH assay in potato (r = -0.329), sweet potato (r = -0.313) and positive correlation with the taro(r = 0.232) assay.

Table 15: Pearson correlation analysis of the antioxidant contents (TFC) and antioxidants activities of raw potato, sweet potato and taro with different thermal process applied

		FRAP	DPPH	BCB
Potato	**TFC**	0.992**	-0.329**	-0.509
Sweet Potato		0.979**	-0.313**	-0.302
Taro		0.829**	0.232**	0.158

**Correlation is significant at the 0.01 level (2-tailed). TPC,Total phenolic contents;TFC,Total flavonoid contents;DPPH, 2,2-Diphenyl-1-picrylhydrazyl; BCB,Beta carotene bleaching assay; FRAP, ferric reducing antioxidant power

The correlation between total phenolic content and DPPH radical scavenging activity and FRAP assay indicates that phenolic compounds are responsible for the antiradical activity. Antioxidant activity values also depend strongly on the preparation of sample (leaching, extended steaming, boiling and frying as well as the lyophilisation) (Chellaram et al., 2014). The EC_{50} for three potato extracts were significantly different. Genotype and growth conditions, such as water availability, light quality and temperature, affect the synthesis and accumulation of phenolic compounds in some parts of the plant, and consequently, antioxidant activity (Omar et al., 2012).

A significant negative correlation for phenolic content and EC_{50} values was observed in potato, sweet potato and taro varieties, indicating that these phenolic compounds may contribute directly to the radical scavenging activity and ability to reduce the power of antioxidant. Insignificant correlation between total phenolic content and antioxidant activity of the potato extracts shows the presence of other non-phenolic constituents with antioxidative activity such as ascorbic acid in some varieties of potato, sweet potato and taro in beta-carotene bleaching assay.

CHAPTER 5

5.0 CONCLUSION

The total polyphenol contents and antioxidant capacity of the flesh of potato, sweet potato and taro were shown to be variety dependent. According to the results obtained by the Folin-Ciocalteu method and aluminium chloride assay, all samples were chosen as the materials for antioxidant evaluation by the DPPH Assay, beta carotene bleaching assay and FRAP methods. The results demonstrated that the extract all sample has potent antioxidant activity and that the main bioactive compounds for the antioxidant activity of roots and tubers crop are polyphenols with different variations depending on the flesh colour.

However, all samples need to undergo the thermal process in order to improve palatability, thus, heating or thermal process applied is accountable for the oxidation, thermal degradation, and leaching of bioactive compounds from fresh samples especially in tubers and roots crops studied in the current study. Depending on the morphology and nutritional properties of vegetables, the positive and negative effects of heating have been reported. Different heating conditions (e.g., heating method, medium used, duration and temperatures) have different effects on the antioxidant properties of roots and tubers crops.

From the current study, raw sample showed the highest phenolic, flavonoid and antioxidant capacity in potato and taro, respectively, followed by fried, steamed and boiled sample. In contrast, the antioxidant component exerts higher antioxidant activity in fried steam potato compared to steamed, boiled and raw due to the combination of prolonged contact with frying medium and alter the polyphenol present in sweet potato.

In addition, the application of domestic cooking methods (boiling, frying and steaming) of fresh tubers and roots crop can affect the nutritional values and functional

properties of foods at different degrees. Although thermal processing partially results in the degradation of intrinsic phytochemicals and the denaturation of endogenous antioxidants, the nutritional dietary phytochemicals and their biological properties are well retained and protected with the appropriate cooking methods. In particular, numerous studies have been devoted to the effects of domestic cooking methods on phytochemicals and antioxidant activities. However, the effect of structural changes with the release of nutrients and phytochemicals during cooking was mostly ignored. As the changes in bioactive compounds can be beneficial or detrimental depending on the processing conditions, an assessment of their various physiological effects is necessary to be determined whether they should be preserved or eliminated.

Meanwhile, the gentlest cooking methods for preserving the nutrients should still be investigated in future studies as consuming fried food in bulk still in controversial discussion for health. Since cooking is an essential part of our daily life, the mechanism of action by which various cooking processes exert their effects on phytochemicals which are related to human health merits further investigations in long-term operation. Based on profound research interest in food processing with dietary phytochemicals, the above directions in the future could be pursued.

On the other hand, tubers and roots of different varieties possess a range of compounds (e.g., polyphenols, vitamins, proteins and polysaccharides) capable of exerting a key role in health promotion and prevention of diseases related to human nutrition and life-style. However, the nutritional composition and presence and amounts of bioactive compounds in tubers and roots crop are variety-dependent. Returning to the problem statements highlighted, the potato, sweet potato and taro should be considered a healthy food option for use by consumers in different domestic meal preparations as well as for use by the food industry as an ingredient for the formulation of added-value functional food products.

5.1 LIMITATION

The current study was limited by the limited findings on the actual bioactive components in all potato, taro and tubers that contributes to the total antioxidant content. The understanding of this concept may help in determining the side contributor to the antioxidant content pattern exert in the study, not only due to the phenolic and flavonoid presence. In addition, the specific cooking method used in the study might not be generalized into all fresh vegetables.

5.2 RECOMMENDATION

Since the current study focusing on the fresh sample, various method of extraction should be done in future to compare. In addition, exploring post-harvest process treatment such as understanding the tuber's maturity and storage condition and its effect on the antioxidant capacity can be done specifically, not only on the thermal process applied. Further study should be carried out to elucidate the degraded plant materials, composition and structure, besides, exploration of the potential application of bioactive components in plant protection, antimicrobial, anticancer, packaging, drug delivery material and prebiotic should be carried out. Despite the abundant antioxidant content, many recent studies suggest the consumption of tubers and roots crop may have the relation with enhancing fertility in human and this debate may be tested in the laboratory in purpose to make wide use of tubers and roots consumption, especially for food supplementation. Lastly, an extended study should also focus on the total bioactive content analysis in tubers and roots crop, not only focusing on the flavonoid and phenolic, in order to give a better understanding on the antioxidant content and capacity.

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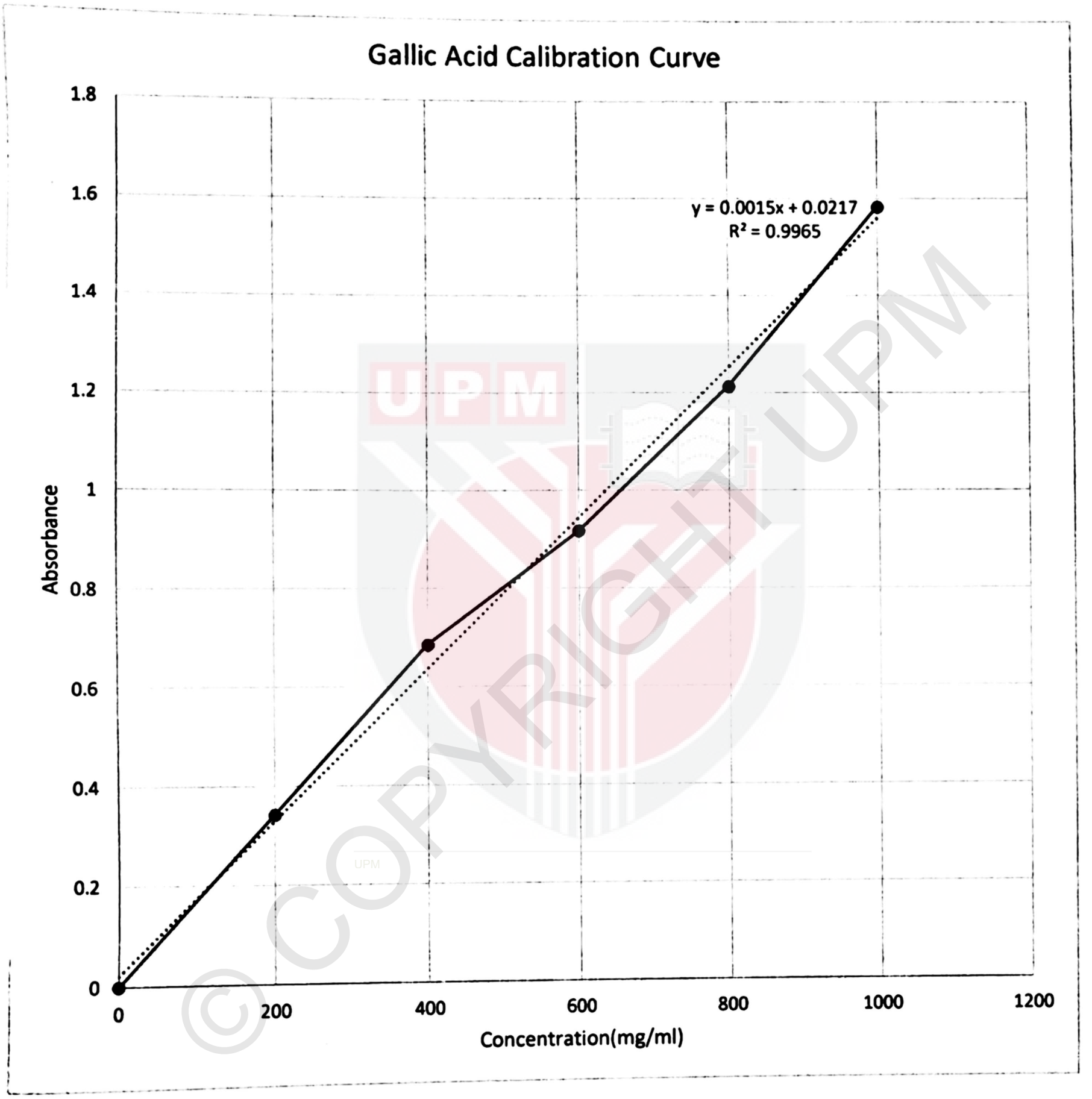
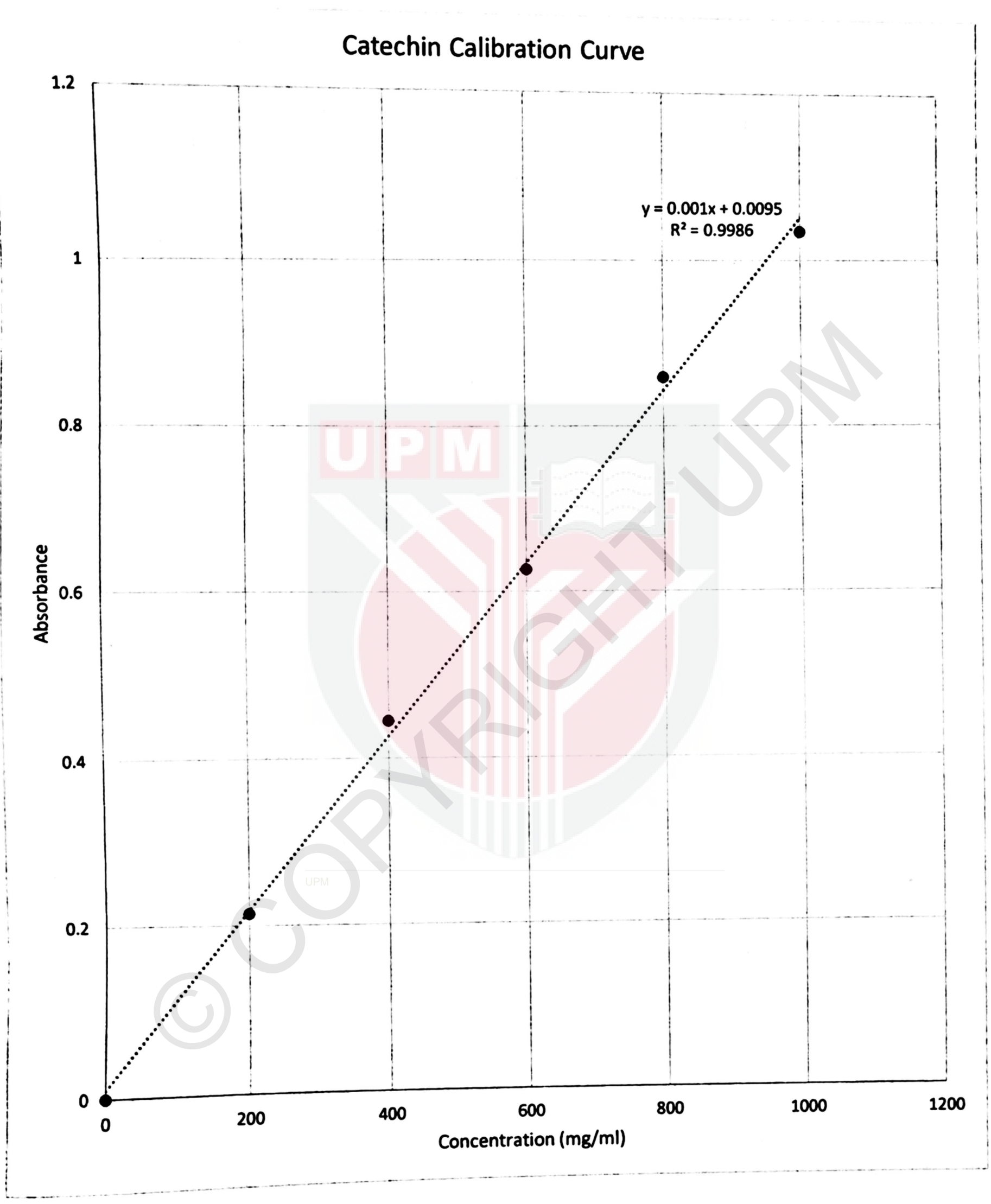
APPENDICES

Gallic Acid Calibration Curve



Catechin Calibration Curve



$FeSO_4$ Calibration Curve