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UDUL: Antioxidant Activity, Total Phenolic and Vitamin C Content of Selected Dried and Fresh Fruit Peels From Sabah

JAZAH: saujana Muda Sains Makanan dan Penokoran dengan Kegunaan


Bayar: MOO AUN KHENG

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ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND VITAMIN C CONTENTS OF SELECTED FRESH AND DRIED FRUIT PEELS FROM SABAH

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SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2009
DECLARATION

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

17 APRIL 2009

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ABSTRACT

Five tropical fruits (bambangan, tarap, salak, dragon fruit, pomelo (flavedo and albedo) from Sabah were selected in this study to determine their antioxidant activity, total phenolic and vitamin C contents in the form of fresh and dried peels. All samples were obtained from local market in Kota Kinabalu, Sabah. Samples were extracted using methanol solution for measuring antioxidant activity by FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging and β-carotene bleaching assays and total phenolic content by Folin-Ciocalteu method. Vitamin C content of samples was measured by UV-vis spectrophotometry of 2,6-dichlorophenolindophenol (DCIP). Fresh bambangan and dried tarap peel displayed the highest free radical scavenging (EC₅₀ values of 0.18 ± 0.16 mg/ml and 0.04 ± 0.00 mg/ml, respectively), ferric reducing (15.23 ± 0.62 mg Trolox/g extract and 11.00 ± 3.81 mg Trolox/g extract, respectively) and linoleic acid scavenging activities (EC₅₀ values of 1.56 ± 0.02 mg/ml and 0.80 ± 0.01 mg/ml, respectively). Both fresh and dried flavedo of pomelo contained the highest vitamin C content. The antioxidant activity of fruit peels, except β-carotene bleaching, were significantly (p < 0.05) correlated with the total phenolic content. However, those assays were not correlated with vitamin C content. This study indicated that phenolics were the major contributor for antioxidant activity while vitamin C was only a minor contributor. Overall, fresh bambangan and dried tarap peels had the highest potential as natural source of antioxidant among the selected samples.
ABSTRAK


Lima jenis buah-buahan tropical (bambangan, tarap, salak, buah naga, limau betawi (albedo dan flavedo) dari Sabah dipilih dalam kajian ini untuk mengkaji aktiviti antioksidan, jumlah kandungan fenolik dan kandungan vitamin C dalam bentuk kulit segar dan kulit kering. Semua sampel telah dibeli dari pasar tempatan di Kota Kinabalu, Sabah. Sampel-sampel diekstrak dengan larutan metanol untuk menguji aktiviti antioksidant dengan ujian FRAP (Kuasa Antioxidan Penurunan Ferik), ujian DPPH (2,2-diphenyl-1-picrylhydrazyl) pengurai radikel bebas serta ujian pelunturan β-karoten dan jumlah kandungan fenolik dengan ujian Folin-Ciocalteu. Kandungan vitamin C sampel diuji dengan kaedah 2,6-diklorofenolindofenol (DCIP) UV-vis spektrofotometrik. Kulit bambangan segar dan kulit tarap kering menunjukkan aktiviti-aktiviti penguraian radikel bebas (nilai-nilai EC₅₀ ialah 0.18 ± 0.16 mg/ml dan 0.04 ± 0.00 mg/ml masing-masing), penurunan ferik (15.23 ± 0.62 mg Trolox/g ekstrak dan 11.00 ± 3.81 mg Trolox/g ekstrak masing-masing) dan penguraian asid linoleat (nilai-nilai EC₅₀ ialah 1.56 ± 0.02 mg/ml dan 0.80 ± 0.01 mg/ml masing-masing) yang tertinggi. Flavedo bagi limau betawi dalam bentuk segar dan kering mengandungi kandungan vitamin C yang tertinggi. Aktiviti antioksidan bagi kulit buah-buahan berhubung kait dengan jumlah kandungan fenolik secara nyata (p < 0.05). Manakala, ujian-ujian ini tidak berhubung kait dengan kandungan vitamin C. Kajian ini telah menunjukkan fenolik adalah penyumbang utama bagi aktiviti antioksidan tetapi vitamin C adalah hanya penyumbang yang kecil. Secara keseluruhan, kulit bambangan segar dan kulit tarap kering adalah berpotensi yang tertinggi sebagai sumber antioksidan semulajadi di kalangan sampel yang terpilih.
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<td>AEAC</td>
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CHAPTER 1

INTRODUCTION

1.1 Background

Many clinical trials and epidemiological studies have been done in these past few years strongly suggested that long-term consumption of diets rich in plant foods give the protection against chronic diseases especially cancers (Lipkin, 1985). Thus, the word “antioxidant” has received much attention due to its health-protecting factor. In food science, antioxidants in food may be defined as any substance which is capable of delaying, retarding or preventing the development in food of rancidity or other flavor deterioration due to oxidation (Gordan, 2001). In the aspect of health, antioxidants may define as any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell & Gutteridge, 1999; Halliwell, 1990; Halliwell, 1995). The term “oxidizable substrate” includes every type of molecule found in vivo.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are potentially harmful free radicals, are also so-called “oxidizable substrate”, which are generated in vivo and results in oxidative stress when these substrates are over-produced (Guo et al., 2003). Free radicals in the body are produced from two sources, endogenous and exogenous sources. Nutrient metabolism and the aging process are the sources for endogenous; whereas, exogenous sources are pollutants, cigarette smoke, chemicals, and environment toxins may enhance the production of
reactive species (Elsayed, 2001; Lachance et al., 2001; Patthamakanokpom et al., 2007; Wang, 2007).

According to numerous studies such antioxidant activity appears to be closely related with the prevention of degenerative illnesses, such as the different types of cancer, cardiovascular and neurological diseases, cataracts and oxidative stress dysfunctions (Frei, 1994; Grey et al., 1991; Mackerras, 1995; Riemersma, 1994; Schwartz, 1996). In this situation, antioxidants become scavenger of free radicals (Alonso et al., 2002) and serve as inhibitors to inactivate or remove these free radicals to prevent generation of ROS, to destroy potential oxidants and to scavenge ROS. Thus, oxidative stress-induced tissue damage is minimized (Benzie & Strain, 1996).

Food products which are high in lipid or lipid-containing foods often develop an undesired flavor and rancidity due to the process of lipid peroxidation (Singh et al., 2002). Deterioration processes in foods which are mainly caused by oxidation reactions and decomposition of oxidation products. These reactions are initiated by several factors. These factors include excess of oxygen, high temperature, the activation of enzymes catalyzing oxidation, high oxygen pressure and unsuitable packaging (Pokorny et al., 2001). With the reasons above, synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are usually the compounds that are added into food products for the above purposes. However, these synthetic antioxidants, BHA and BHT, have restricted use in foods as these synthetics antioxidants are suspected to be toxic and carcinogenic (Madhavi & Salunkhe, 1995). Therefore, the interest on natural antioxidant has increased as the alternative solution to prevent lipid peroxidation in food products and also to reduce the health problems such as cardiovascular diseases, cancers, neurodegenerative diseases and atherosclerosis, in human (Halliwell, 1994).

Fruits are considered as excellent dietary sources of high antioxidants. Many studies have been done on fruits related to the antioxidant activities. Compounds such as phenolics, vitamin C, carotenoids and flavonoids are the most abundant types of antioxidants in fruits that have been studied. A number of functions can be performed well by these antioxidants, including free radical scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors and synergists
Recently, peels, which are considered waste or by-products of various fruits, have received much attention with a view being used or recycled rather than discharged (Okonogi et al., 2006). Several studies have shown that peels and seeds of some fruits have higher antioxidant activity than the pulp (Jayaprakasha et al., 2001). According to previous studies, the antioxidant activity in fruit peels, such as mangosteen, rambutan, banana, coconut, passion fruit, dragon fruits (Okonogi, 2006), pear fruit (Rizzolo et al., 2001), pomegranate peels (Sigh et al., 2002; Li et al., 2005) and guava peels (Lim et al., 2006), vary greatly. However, all these fruit peels have the potential as naturally occurring antioxidants for the market of functional food ingredients and nutraceutical antioxidants (Shui & Leong, 2006).

Sabah has the climate and soils to grow a great variety of quality fruits. Fruits production is mainly for domestic although a moderate but growing quantity of high quality fresh fruits and fruit products are exported. As the fruit products are being processed, very large amounts of byproduct wastes, such as peels are formed every year. Thus, in order to discard these peels as a waste and becoming a source of pollution, these residues have the potential to be used as natural source of antioxidant to promote health and to replace the synthetic food additives. However, there are limited resources and studies have been done on the local fruits in Sabah, especially the fruit peels, and the nutritional values of these local fruits are still under studies. In this study, aqueous methanolic extraction of fresh and dried fruit peels from Sabah is used to determine total antioxidant activity, phenolic and vitamin C contents. Many fruits grow wild in Sabah are not found in Peninsular Malaysia. Although these fruits are not planted commercially, these local fruits have their great market values. Bambangan, tarap and salak are the fruits grow wild in Sabah, however, are commonly consumed by local people. Whereas, dragon fruits and pomelo are commercially planted in Sabah and both fruits are widely used in processing food products. Because of these fruits are considered to have high market values, especially in the markets of functional foods and nutraceuticals, these five fruits were chosen for this study.

The importance of this study is to determine the antioxidant activities in peels of the local fruits from Sabah. This study will provide information on potential use of peels of local fruits in Sabah as potential source of natural oxidant for the food industries of their potential as natural antioxidant sources and also for those health-
concerned consumers who are concerned with health aspect of the food products in the market. In addition, this study will provide the data for further studies on nutritional value of the peels of local fruits from Sabah. All these information may trigger and are beneficial to the market of functional foods and nutraceuticals in Sabah as well as the development of economy in Sabah.

1.2 Objectives

i. To determine and compare the antioxidant activity using FRAP, DPPH and β-carotene bleaching assays, total phenolic using Folin-Ciocalteau assay and vitamin C content of selected fresh and dried fruit peels from Sabah.

ii. To evaluate the correlations of antioxidant activities among the different antioxidant assays with total phenolic and vitamin C of selected fresh and dried fruit peels from Sabah.
CHAPTER 2

LITERATURE REVIEW

2.1 Free Radicals and Antioxidants

2.1.1 Free Radicals and Reactive Species

In the world of chemistry, the most stable molecules are the paired electrons. This means that the orbitals contain two electrons having opposite spins and bond with covalent bonds or may be in non bonded pairs, such as oxygen atom of water (Larson, 1997). However, free radicals are molecules having an unpaired electron in the outer orbit. They are generally unstable and very reactive (Fang et al., 2002). Normally, a free radical can be formed whenever a covalent single bond between two atoms is cleaved and left at least one electron in an unpaired state. An equal or slightly greater input of energy or is so-called “bond dissociation energy” is required to break the bond. This process is known as homolysis. This typical reaction is the cleavage, by heat or UV light, of the O-O bond in peroxides (Larson, 1997).

In human body, free radicals are formed either as an essential mediator in essential processes including neurotransmission and inflammatory reactions, or as a byproduct that does not have a role in the actual process. In aerobic life forms, the reduction of oxygen is of special concern. This reduction comprises binding of most of the oxygen to hydrogen to give water, a process involved in the oxidative phosphorylation. However, a small part of the oxygen (approximately 1-3%) is only partly reduced during those redox reactions (Magder, 2006). As a result, free radicals or other reactive species, that can either oxidize other compounds or easily form
radicals, will arise. These partly reduced forms of oxygen are collectively described as reactive oxygen species (ROS). Similarly, reactive nitrogen species (RNS) are continuously produced (Boots et al., 2008). Physiological important ROS include singlet oxygen (\( ^1\text{O}_2 \)), superoxide (\( \text{O}_2^- \)), hydrogen peroxide (H\(_2\text{O}_2\)), hydroxyl radical (\( ^\cdot\text{OH} \)), ozone (O\(_3\)) and hypochlorous acid (HOCI) (Boots et al., 2008). The importance physiological of RNS are nitric oxide (NO\(^+\)) and peroxynitrite (ONOO\(^-\)). It should be noted that not all ROS and RNS are equally reactive. Non-radical ROS such as H\(_2\text{O}_2\) might pass biological membranes and in that way spread their reactivity and possible toxicity (Boots et al., 2008).

ROS and RNS can be either beneficial or damaging in human body. ROS and RNS react readily with practically all bio-molecules, including DNA, RNA, proteins, carbohydrates and lipids, thereby damaging the attacked molecule (Boots et al., 2008). For example, hydroxyl radical, one of the most reactive free radicals, can be formed during the exposure of living organisms to ionizing radiation causes fission of O-H bonds in water. Many harmful effects of excess exposure to ionizing radiation upon living organisms are thought to be initiated by attack of \(^\cdot\text{OH} \) upon protein, carbohydrate, DNA and lipids (Halliwell, 1994). Besides that, these active oxygen species may play a causative role in a variety of diseases including heart disease and cancer, and aging (Noguchi & Niki, 1999). In contrast, ROS and RNS also play important roles in several crucial physiological functions in human body, including production of energy, synthesis of biologically essential compounds, and phagocytosis, a critical process of immune activities by attacking foreign material as virus particles, bacteria, fungi and other invading micro-organisms (Noguchi & Niki, 1999; Karlsson, 1997; Boots et al., 2008). Nitric oxide and superoxide are the good examples in biological life that both radicals are beneficial in the immune and aseptic activities of white blood cells. They possess the roles of killing parasites by macrophages in some mammalian species and inactivating viruses and bacteria (Karlsson, 1997; Halliwell, 1994).

Most foods contain lipids with the major classes being triglycerides, are also known as tricylglycerols. Lipids occur in fat storage cells of plants and animals; and phospholipids, which occur in biological membranes. In the presence of oxygen in atmosphere, a spontaneous reaction can occur with lipids, known as autoxidation or so-called lipid peroxidation, is the most common process leading to oxidative
deterioration. Hydroperoxides, are the components formed in the initial stage of lipid peroxidation. These radicals are involatile and odourless; they are relatively unstable compounds and decompose either spontaneously or in catalysed reactions to form volatile aroma compounds, which are perceived as off-flavours (Gordon, 2001).

Oxidative deterioration of lipids may cause the bleaching of foods due to the reaction of pigments, especially carotenoids, with the reactive intermediates, termed free radicals, which are formed during lipid oxidation. Additionally, the reduction of nutritional quality by reaction of free radicals with vitamins may occur, especially vitamin E, which is lost from foods during its action as an antioxidant. The concentration of free radicals is much more dependent to the temperature. Higher level of concentration of free radicals in frying oils as the higher temperature are used (Gordon, 2001).

2.1.2 Antioxidants

Many definitions have been defined for the term ‘antioxidants’ which carries different means in different fields. In the aspect of health, antioxidants may define as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990; Halliwell, 1995). As mentioned in introduction, the word ‘antioxidant’ uses in food related fields refers as any substance which is capable of delaying, retarding or preventing the development in food of rancidity or other flavor deterioration due to oxidation (Gordan, 2001). However, according to the Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board, “dietary antioxidant” is proposed as a substance in food that significantly decreases the adverse effects of reactive oxygen species (ROS), reactive nitrogen species (RNS) or both on normal physiological function in human (Comelli, 2009). Thus, the term “antioxidant” often related to chain-breaking inhibitors of lipid peroxidation both in vivo and in vitro (Halliwell, 1995).

According to Noguchi & Niki (1999), antioxidants play different functions in human defense system. In the first dense line, preventive antioxidants suppress the formation of free radicals and active oxygen species. In the second defense line, radical scavenging antioxidants are responsible to inhibit chain initiation and/or
REFERENCES


