THE CHANGES IN THE ANTIOXIDANT CAPACITY OF SELECTED TROPICAL FRUITS UPON TREATMENT WITH GASEOUS OZONE AND ULTRAVIOLET C RADIATION

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The Changes in Antioxidant Capacity of Selected Tropical Fruits Upon Treatment With Gaseous Ozone and Ultraviolet C Radiation

by

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LIST OF ABBREVIATIONS / SYMBOLS

LDL	Low Density Lipoprotein
HACCP	Hazard Analysis and Critical Control Points
ISO	International Organization for Standardization
FRAP	Ferric reducing/antioxidant power assay
DPPH	1,1-diphenyl-2-picrylhydrazyl
CVDs	Cardiovascular diseases
WHO	World Health Organization
WCRF	World Cancer Research Fund
AICR	American Institute for Cancer Research
AIDS	Acquired Immune Deficiency Syndrome
FAO	Food and Agriculture Organization
MT	Metric ton
UV	Ultraviolet radiation
K_a	Acid constant
TPTZ	2,4,6-tris (1-pyridyl)-5-triazine
pH	Hydrogen exponential
MAP	Modified Atmosphere Packaging
PEF	Pulsed Electric Fields
HHP	High Hydrostatic Pressure
MPa	Mega Pascal
kV/cm	Kilo volt per centimeter
MeV	Million electron volt
Co	Cobalt

Cs	Cesium
IAEA	International Atomic Energy Agency.
kGy	Kilogrey's
FDA	U.S. Food and Drug Administration
ppm	Part per million
rpm	Revolutions per minute
TP	Total phenol content
mg GAE/100 g FW	Milligram gallic acid equivalent per 100 gram fresh weight
TF	Total flavonoid content
mg CEQ/100 g FW	Milligram catechin equivalent per 100 gram fresh weight
DCIP	2,6-dichloroindophenol
AOAC	Association of Official Analytical Chemists' methods
PAL	Phenylalanine ammonia lyase
ABTS	2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt
$kJ.m^{-2}$	Kilojoule per square meter
mg AA/ g FW	Milligram ascorbic acid per gram fresh weight
ROS	Reactive oxygen species

PERUBAHAN DALAM KAPASITI ANTIOKSIDAN BAGI BUAH-BUAHAN TROPIKA TERPILIH YANG DIOLAH DENGAN GAS OZON DAN SINARAN ULTRA UNGU

ABSTRAK

Kandungan fenol dan kapasiti antioksidan bagi tiga jenis isi buah tropika iaitu nenas madu, pisang mas dan jambu batu tanpa biji Thai telah diuji. Tiga jenis sistem pelarut (metanol, etanol dan aseton) dengan kepekatan yang berbeza (50, 70 dan 90%) dan 100% air suling telah digunakan. Kesan rawatan UV-C dan gas ozon pada kandungan fenol, flavonoid, vitamin C, dan kapasiti antioksidan buah yang baru dipotong telah dikaji secara berasingan. Semasa rawatan UV-C, ekstrak buah telah diiradiasi selama 0, 10, 20 dan 30 min. Bagi rawatan gas ozon, ekstrak buah telah didedahkan kepada gas ozon dengan kadar aliran 8 ± 0.2 ml/s selama 0, 10, 20 dan 30 min. Kandungan fenol ditentukan dengan menggunakan kaedah Folin-Ciocalteau manakala kandungan flavonoid menggunakan kaedah aluminium klorida kolorimetrik. Aktiviti antioksidan bagi ekstrak buah telah dinilai menggunakan kaedah *ferric reduce antioxidant power* (FRAP) dan kesan radikal bebas terhadap penentuan radikal DPPH. Tahap keberkesanan sistem pelarut mengekstrak fenol daripada tiga jenis buah tersebut adalah berbeza. Nilai kandungan fenol (berdasarkan berat basah) untuk jambu batu adalah antara 123.21 ± 4.45 dan 190.58 ± 4.35 asid galic setara/100 g (GAE/100 g) manakala pisang antara 24.37 ± 2.45 dan 72.21 ± 2.03 GAE/100 g dan 34.65 ± 3.44 dan 54.68 ± 1.79 GAE/100 g untuk nenas. Kandungan fenol yang tinggi adalah berkadar secara signifikan dengan kapasiti antioksidan yang tinggi. Kedua-dua rawatan UV-C dan gas ozon didapati

menyebabkan perubahan yang signifikan terhadap kandungan fenol, flavonoid dan vitamin C buah pada tahap yang berbeza bergantung kepada jenis buah dan masa rawatan. Jumlah fenol dan flavonoid jambu dan pisang meningkat dengan signifikan dengan peningkatan masa rawatan UV-C, manakala kandungan fenol buah nenas adalah tidak signifikan berbeza. Kandungan flavonoid buah nenas meningkat dengan signifikan hanya selapas 10 min rawatan UV-C. Bagi rawatan gas ozon, kedua-dua kandungan fenol dan flavonoid buah nenas dan pisang meningkat dengan signifikan dengan peningkatan masa rawatan sehingga ke masa rawatan 20 min. Walau begaimanapun, buah jambu menunjukkan penurunan dengan peningkatan masa rawatan. Kapasiti antioksidan buah dipengaruhi secara signifikan oleh rawatan UV-C dan rawatan gas ozon bergantung kepada jenis buah dan masa rawatan. Kedua-dua rawatan UV-C dan gas ozon menurunkan kandungan vitamin C ketiga-tiga jenis buah secara signifikan.

THE CHANGES IN THE ANTIOXIDANT CAPACITY OF SELECTED TROPICAL FRUITS UPON TREATMENT WITH GASEOUS OZONE AND ULTRAVIOLET C RADIATION

ABSTRACT

The phenol content and antioxidant capacity of three tropical fruits pulps, honey pineapple (*Ananas comosus* Merr), a local type of banana (*Musa paradasiaca*) locally called *pisang mas*, and Thai seedless guava (*Psidium guajava* L.) was studied. Three solvent systems were used (methanol, ethanol, and acetone) with three different concentrations (50, 70, and 90%) and 100% distilled water. The effects of UV-C and gaseous ozone treatments on total phenol, flavonoid, vitamin C contents, and the antioxidant capacity of fresh-cut of the three fruits have been investigated separately. During UV-C treatment, the fresh-cut fruits were irradiated for 0, 10, 20, and 30 min. The fresh-cut fruits were exposed to gaseous ozone with the flow rate of 8 ± 0.2 ml/sec for 0, 10, 20, and 30 min. Total phenol and total flavonoid contents of the fruits were measured using Folin-Ciocalteau and aluminum chloride colorimetric methods respectively. The antioxidant activity of the fruit extracts was evaluated using ferric reducing/antioxidant power (FRAP) assay and the free-radical-scavenging effect on the DPPH radical assays. The efficiency of solvents used to extract phenols from the three fruits varied considerably. Values of total phenol content (on fresh weight basis) ranged from123.21 ± 4.45 to 190.58 ± 4.35 gallic acid equivalents / 100 g (GAE/100 g) for guava fruits, from24.37 ± 2.45 to 72.21 ± 2.03 GAE/100 g for banana and from 34.65 ± 3.44 to 54.68 ± 1.79 GAE/100 g for pineapple. High phenol content was significantly correlated with higher antioxidant capacity. Both UV-C and gaseous ozone treatments were found to cause significant changes in the total phenol, flavonoids, and vitamin C contents of the fruits with different extents based on the type of fruit and treatment time. Total phenol and flavonoid contents of guava and banana increased significantly with the increase in UV-C treatment time, while the increase was insignificant in the total phenol of pineapple fruits. Flavonoid content of pineapple increased significantly only after 10 min UV-C treatment. For gaseous ozone treatment, both total phenol and flavonoid contents of pineapple and banana increased significantly with the increase in treatment time up to 20 min, while it decreased in guava fruits with the increase in treatment time. The antioxidant capacity of the fruits was affected significantly with the UV-C and gaseous ozone treatments based also on the type of fruit and treatment time. Both UV-C and gaseous ozone treatments decreased the vitamin C content of the three fruits significantly.

CHAPTER 1

INTRODUCTION

1.1. Background

Fruits are major food products and considered as key ingredients in many processed food. Eating fruits and vegetables has long been associated with health benefits. Recent studies indicate that the frequent consumption of fruits is associated with lower risk of stroke and cancer (Beecher, 1999).

There is a strong evidence that free radicals are responsible for the damage of lipids, proteins and nucleic acids in cells (Leong & Shui, 2002). Some of the ways in which fruits enhance health have only become clear in recent decades. The protective effect of frequent fruits consumption is related to the plant antioxidant phytochemicals contained in the plant parts.

Phytochemicals are very diversified chemical compounds, which are derived from phenylalanine and tyrosine (Shahidi & Naczk, 2004). Plant phytochemicals have a variety of functions such as pigmentation, antioxidation, protection against UV light (Shahidi & Naczk, 2004). They are widely distributed in the plant kingdom with different structures at the tissue, cellular, and sub-cellular levels. Under certain conditions, the concentration of plant phytochemicals may increase. These conditions include the exposure to radiation sources, wounding, storage on low temperatures, and/or exposure to extreme temperatures (Zobel, 1997).

It is evident that different fruits would exhibit different antioxidant capacity according to their polyphenol content, vitamin C, E, carotenoids and flavonoids (Saura-Calixto & Goni, 2006).

Recently, there is considerable attention to study the polyphenols flavonoids from different plant sources. Flavonoids are polyphenols with diphenylpropanes ($C_6C_3C_6$) skeletons. They are considered to be the largest group of naturally occurring phenols and it is estimated that 2% of all the carbon photosynthesised by plants is converted into flavonoids (Vijayakumar, et al., 2008). Flavonoids are widely distributed in plant kingdom with a huge diversity of structures (Iwashina, 2000). They are shown to be very effective in the protection against cardiovascular diseases by reducing the oxidation of LDL.

The traditional objective of any food preservation technique is to control the growth of pathogenic and spoilage microorganisms in food and food products. This concept has witnessed a radical modification in the current century.

Newer innovations and techniques have been introduced and explored in the field of food preservation. These innovations are a result of the ever-increasing demand from consumers' world-over for high quality foods with emphasis laid on its safety. Some of the objectives of the emerging food preservation technologies include: minimally processed, high nutritional-low energy natural foods with no or minimal incurrence towards chemical preservatives. However, as each step during food processing will affect their natural properties to various extents, HACCP and ISO standards have been implemented by the food authorities to overcome these hindrances. Hence, in order to cope with this revolutional development in food industry, there should be a complete and thorough understanding of every single step the food goes through from farm to fork.

As mentioned earlier, there is a general tendency lately to increase the consumption of fruits and vegetables as much fresh as it can be, by applying the so called minimal processing technologies, to increase the intake of those bioactive antioxidants.

As a result, the minimally processed food can be defined as that food that is microbially safe after being exposed to a certain processing technique which (i.e., the processing technique) should not cause serious alternation in the nutritional value, the flavour, and the colour, and consuming low energy with the complete absence of chemical preservatives.

There are many immerging preserving technologies have been introduced and explored in the food industry. Of those, the ultraviolet irradiation, ultrasound, ozone, membrane filtration, electron beam, pulsed electric field, etc.

Excellent studies and monographs have been carried out to probe the impact of those new technologies on the preservation of fresh plant produce, especially, fruits and vegetables. Most of those researches have been focusing on the microbial safety and the enhancing of the shelf life of the plant produce. However, there is paucity in information's pertaining towards the effect of those technologies on the antioxidant properties of fruits and vegetables.

1.2. Research Hypothesis

Tropical fruits are well known for their diversity and their high content of antioxidants. Frequent consumption of fruits is associated with health promoting effects. This fact is attributed to the fruit content of antioxidants.

Different processing techniques have been shown to have various impacts on the nutritional value of food in different extents depending mainly on the technique conditions besides the type of food itself.

The current study raises the question about the impact of ultraviolet C and gaseous ozone treatments on the antioxidant status of selected tropical fruits namely honey pineapple, a local cultivar of banana called *'pisang mass'*, and the Thai seedless guava. It assumes that these treatments will cause changes in each of the total phenol, total flavonoid, and vitamin C contents in addition to changes in the antioxidant capacity of these fruits

1.3.Objectives

The present study was conducted with the aim of contributing to knowledge of the effect of new emerging technologies on the chemical composition of selected tropical fruits.

Objectives of this study were:

1. To study the effect of different new technologies namely ultraviolet light and gaseous ozone at different treatment times on the chemical composition of three common tropical fruits in Malaysia namely honey pineapple (*Ananas comosus* Merr); a local cultivar of banana (*Musa paradasiaca*) called pisang mas; and the Thai seedless cultivar of guava (*Psidium guajava* L.). The study highlighted the changes in each of total phenolic compounds content, total flavonoids content, and vitamin C content of the three fruits.

2. To study the changes in the antioxidant capacity of the fruits measured by two colorimetric methods: ferric reducing/antioxidant power (FRAP) assay and the free-radical-scavenging effect on the DPPH radical assays.

CHAPTER 2

LITERATURE REVIEW

2.1. Health Benefits of Fruit Consumption

It is strongly established that fruits are rich source of the antioxidant vitamins β-carotene (pro-vitamin A), α-tochopherol (vitamin E), and acorbic acid (vitamin C). The high intake of those vitamins is frequently associated with reduced disease risk. Many studies revealed that those individuals who practice frequent consumption of fruits and vegetables tend to suffer the least of diseases like cancers, cardiovascular diseases (CVDs), and several inflammations (Greenwald et al., 2001; Key et al., 2002; Kris-Etherton et al., 2002; Temple and Gladwin, 2003). For this reason, fruit consumption is considered recently one of the main factors that obviously affecting healthy life style beside exercise and genetic impact.

Recent studies suggest that all those vitamins have a minor role in disease prevention. This fact was further discussed by recent and thorough work on vitamin C (Frei, 2003), and vitamin E (Upston et al., 2003) with exception of folic acid (vitamin B9) which can be largely derived from sources other than fruits and vegetables (e.g., meat, and grain) (Ashfield-Watt et al., 2001, Ferguson et al., 2004).

These studies approved that the beneficial effect of the consumption of plant material is not only associated with vitamins and other nutrients, but also with other components within the plant tissue such as sulfur and selenium (Ferguson et al., 2004), phenolics like flavonoids, stilbenes, and ellagic acid in addition to some non-vitamin carotenoids like lycopene and lutein (Fraser and Bramley, 2004).

During the last two decades there was an increasing global interest to urge individuals to increase fruits and vegetables consumption in the raw, processed form, and/or by incorporating high amounts of both in their meals. In 1990, The World Health Organization (WHO) recommended a goal of at least 400 g of vegetables and fruits daily. This amount included at least 30 g of legumes, nuts and seeds (WHO, 1990). The World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) also recommended that diets should be based primarily on foods of plant origin because such diets are nutritionally adequate and widely diversified (WCRF and AICR, 1997). These reports and many others have been translated into a recommendation for the consumption of at least five portions of fruits and vegetables per day.

Although several studies have been conducted to demonstrate the relationship between fruit intake and the prevention or fighting chronic disease, The specificity of a certain fruit type towards a specific disease remained uncertain. In 1995, the incidence of cancer (rather than lung and skin cancers) was 30% less in Greece than in Iceland (Bray et al., 2002) where fruits and vegetables are consumed as much as 7 times more than that in Iceland (Riboli and Norat, 2001). Another study associated a ~ 40% lower death rate from stroke with a threefold increase in fruit and vegetable consumption (Bazzano et al., 2002).

Plant cells produce a wide range of redox-active secondary metabolites (i.e., antioxidants) such as ascorbic acid, phenolic compounds, carotenoids, and enzymes with antioxidant activity. The production of antioxidants in animal cells is much more limited (Halvorsen et al., 2006).

There is a constant production of free radicals in the body as a result of oxidative metabolism. The generation of free radicals will be promoted by the incidence of having certain diseases, smoking, alcohol, exposure to ionizing radiation, and environmental poisons. This will affect the adequacy of the antioxidant defense against the oxidative stress in the organism (Blomhoff et al., 2006). Many studies have highlighted a close link between oxidative stress and development of different diseases such as inflammatory conditions, cancer, AIDS, gastric ulcers, and cardiovascular diseases (Hegde et al., 2005; Papaharalambus & Griendling, 2007).

It has been postulated that consuming diets rich in antioxidants with different chemical properties would work synergistically to protect the cells from damage by strengthening the human antioxidant defense system (Blomhoff at al., 2006).

As mentioned earlier, three common tropical fruits in Malaysia were used in this study namely honey pineapple (*Ananas comosus* Merr); a local cultivar of banana (*Musa paradasiaca*) called pisang mas; and the Thai seedless cultivar of guava (*Psidium guajava* L.) Table 2.1 shows the chemical composition of the three fruits used in this study.

Table 2.1 Chemical composition of pineapple, banana, and guava fruits (grams per 100 g of edible portion) (Hui, 2006)

Fruit	Water	Carbohydrates	Protein	Fat	Fiber	Vitamin C (mg)
pineapple	84	12.0	1.2	ND	1.2	36.2
Banana	75	20	1.2	0.3	3.4	8.7
Guava	82	15.7	1.1	0.4	5.3	183.5

The ripe pineapple fruit contains sugars, free organic acid, a trace of vanillin, and the enzyme bromelin (Perry and Metzger, 1980). The fruit is used to treat various women's health conditions in the Dominican Republic (Andreana et al., 2002). The fruit or its juices is shown to have the potential in promoting digestion as well as being useful in treating throat troubles and it has been also used in the treatment of uterine fibroids and menorrhagia. According to Food and Agriculture Organization of the United Nations (FAO), it is estimated that the annual production of pineapple in Malaysia was 340,000 metric ton (MT) in 2005.

Banana is commonly eaten raw and it is also dried and made into flour. Banana contains vitamin A, B, C, E, serotonin, noradrenaline, dopamine, and muscarin (Chang, 2000). The high potassium content makes the banana useful in the treatment of high blood pressure (Duke and duCellier, 1993). The peel and pulp of fully ripe bananas were found to contain antifungal and antibiotic principles (Morton, 1987). It was reported that banana lipids did not affect the concentration of serum cholesterol despite of the fact that feeding of dopamine, n-epinephrine, and serotonin is usually tends to raise the concentration of serum cholesterol (Horigone et al., 1992). Banana can be used to treat ailments of the skin, back, and blood; headaches, fever, and flu; and diarrhea and constipation (Rieger, 2006). According to FAO, it is estimated that the annual production of banana in Malaysia was 530,000 MT in 2005.

Guava is a good source of minerals like iron, calcium, and phosphorus as well as many vitamins like ascorbic acid, pantothenic acid, vitamin A, and niacin (Paull and Goo, 1983). It is also reported to be rich in antioxidants like phenolics and carotene (Luximon-Ramma et al., 2003). Fresh guava leaves have shown a certain effectiveness in treating mild and moderately chronic cases, reducing blood glucose and urine glucose, and the frequency of urination (Perry, 1980; Duke and Ayensu, 1985). It is estimated that the annual production of guava in Malaysia was 30,000 MT (Hui, 2006).

2.2. Plant phytochemicals

Phytochemicals are bioactive nonnutrient plant compounds in fruits, vegetables, grains, and other plant food (Liu, 2003). Plant parts contain different types and varying levels of phytochemicals. Those phytochemicals have been proved to have health promoting properties. Despite of the fact that around 5000 types of phytochemicals could be identified, a large percentage still remains unknown (Shahidi and Naczk, 1995).

Cells in human and other organisms are constantly exposed to a variety of oxidizing agents. Maintaining the balance between oxidants and antioxidants will sustain optimal physiological conditions in the body. This balance can be compromised by the overproduction of oxidants in the cell resulting in oxidative stress. This status is common in the case of chronic bacterial, viral, and parasitic infections (Liu and Hotchkiss, 1995).

Fruits contain a huge diversity of antioxidant compounds (phytochemicals). Of those, phenolics and carotenoids have been shown to protect the cellular systems from oxidative damage induced by free radicals and to lower the risk of chronic diseases like CVDs, and cancers. Health joint comities have been recommending individuals to increase the amount of phytochemicals through the frequent comsumption of fruits and vegetables.

2.3. Phenolic compounds

Phenolic compounds represent a large group of secondary metabolites that are produced and widely distributed in plants. They posses one or more phenolic hydroxyl group attached to one or more benzene rings. Phenolic compounds group contains heterogeneous chemical structures with various molecular weights. The group includes simple phenols, flavonoids, lignin, lignans, stilbenes, and condensed tannins. They are derived from phenylalanine and tyrosine (Shahidi and Naczk, 2004). Table 2.2 shows an example of phenolic compounds from fruit source.

An increasing interest in phenolic compounds is being witnessed due to their antioxidant activity as well as their inhibitory effect on certain deleterious enzyme systems. The antioxidant activity of phenolic compounds is related to their chemical structures which involving particularly the substitution on the aromatic ring and the structure of the side chain (Shahidi and Wanasundara, 1992). These rather varied substances are involved in too many biological functions in the plant.

Phenolic compounds are essential for the growth and reproduction of plants and also acting as antifeedant and antipathogens (Butler, 1992). Phenolic compounds are responsible for the pigmentation of plant foods and they work as natural pesticides, protective agents against ultraviolet (UV) light, and as structural materials for plant stability (Shahidi and Naczk, 2004).

Phenolic compounds, such as quercetin, rutin, narigin, catechins, caffeic acid, gallic acid and chlorogenic acid, are very important plant constituents because of their antioxidant activities (Croft, 1998; Paganga et al., 1999).

Table 2.2 Antioxidative polyphenols in fruits (Naczk and Shahidi, 2006; Pokorny et al., 2001).

Fruit	**Phenolic compounds**
Apple	Chlorogenic acid
Grapefruit	Naringin
Grapes	Anthocyanins, flavonols, flavanonols, stilbenes.
Peach	Chlorogenic acid, neochlorogenic acid
Pear	Chlorogenic acid, hydroxicinnamic acid, condensed tannins, arbutin
Mangosteen	Xanthones
Apricots	Hydroxycinnamic acids
Cherries	Anthocyanins,
Berries (blueberries, cranberries, bilberries)	Hydroxycinnamic acids, hydroxybenzoic acids, anthocyanins, flavanols, hydrolyzable tannins.
Citrus fruits	Hydroxycinnamic acids, flavanones, flavones

2.3.1. Classification of Phenolic Compounds

Although phenolic compounds are having a common stigma represented by the phenolic hydroxyl group, still their chemical structures vary widely. This variation make it possible to classify the phenolic compounds in a number of ways. Swain and Bate-Smith (1962) classified the phenols in plants as “common” and “less common” categories. Harborne and Simmonds (1964) came up with an alternative classification by dividing the phenolic compounds into groups depending on the number of carbons in the molecule. They started with simple phenolics (C_6), through betacyanins (C_{18}), ending with carbon polymers and oligomers like lignins and tannins.

Another classification was done by Ribéreau-Gayon (1972). This classification was more specific about the common and less common phenols where they were categorized into three groups as follow:

1. Widely distributed phenols: This groups contains phenols that are ubiquitous to all plants or of importance in a specific plant.
2. Phenols that are less widely distributed
3. Phenolic constituents: These are present as polymers.

Table 2.3 shows some of the widely distributed phenolic compounds classified according to the number of carbons.

Table 2.3 Widely distributed phenolic compounds (Vermerris and Nicholson, 2006)

Carbon	**Class**	**Example**
C_6	Simple phenolics	Resorcinol (1,3-dihydroxybenzene)
C_6-C_1	Phenolic acids	Gallic acid, vanillic acid, salicylic acid
C_6-C_3	Cinnamic acids, coumarins, isocoumarins	*p*-coumaric acid, ferulic acid, caffeic acid, berginin, umbelliferone.
C_{15} (Flavonoids) (C_6-C_3-C_6)	Flavans, flavonones, anthocyanidin, anthocyanins, flavones.	Catechin, gallocatechin, kaemferol, quercitin, cyanidin, peonidin, petanin.
C_6-C_1-C_6, C_6-C_2-C_6	Xanthones, stilbenes	Xanthone, stilbene resveratol, pinosylvin.
C_{18}	Betacyanins	Betanidin
Oligomers	Lignans	(+)-pinoresinol, (+)-sesamin,
Polymers	Lignin	
Oligomers and polymers (tannins)	Condensed, complex, and hydrolysable tannins.	Procyanidin B_2, Gallotannins, Acutissimin

2.3.2. Chemical Properties of Phenolic Compounds

Phenol is a hydroxyl group with benzene ring. The reactivity of phenol and phenolic compounds are highly related in a way or another to the chemical properties of benzene ring. Benzene is acidic because it can release a proton (H^+) while in a solution; hence phenolic compounds are, in general, acting as weak acids. The acidity of phenolic compounds varies according to the overall structure of the whole molecule in addition to the substituents on the aromatic ring(s). Fig. 2.1. shows the basic arrangement of carbon skeleton of plant phenols.

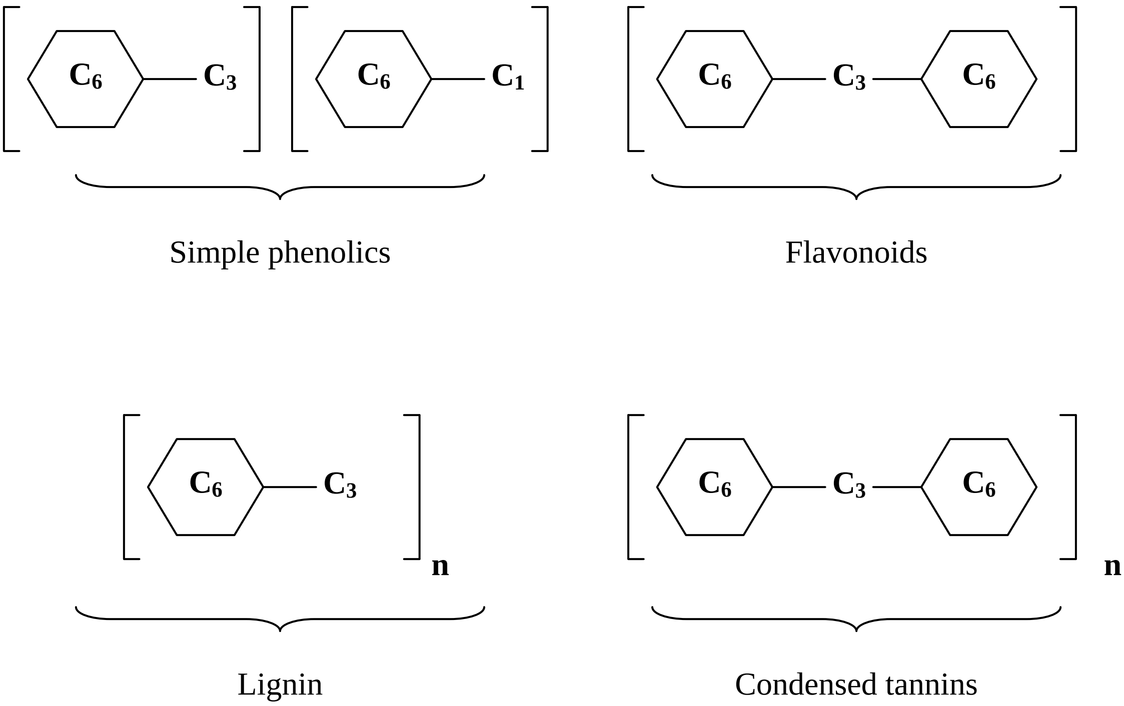


Fig. 2.1. The basic structures of the principal classes of plant phenols (Taiz and Zeiger, 1991).

The acid constant (K_a) of a certain phenolic compound is important because it affects the way this particular phenolic compound is extracted. The less acidic phenolic compounds do not lose H^+ and tend to be free phenols and they are not soluble in water. These compounds are extracted with organic solvent such as acetone, ethanol (Vermerris and Nicholson, 2006).

Oxidation of phenolic compounds is one of the most important aspects of those compounds in biology. The oxidation of phenolic compounds can result in tissue browning and also in the production of some toxic metabolites with a toxic nature to plants and animals. Those metabolites can lead to food spoilage in processing.

Phenols and polyphenolic compounds are considered to be amphiphatic molecules. This is due to the presence of both hydroxyl groups (hydrophilic) and the aromatic rings (i.e., the benzene rings which are hydrophobic). The extent of the presence of hydroxyl groups in phenolic compounds will affect both the physical and chemical properties of the compound. It makes the molecule capable of forming hydrogen bonds (H-bond) with other compounds which, in turn, explains the complexity of phenolic compounds with different molecules as well as the variation in the degree of extractability of those compounds (Handique and Brauah, 2002).

Plant polyphenols are considered as multifunctional molecules. They can act as reducing agents, as hydrogen atom-donating antioxidants, and as singlet oxygen quenchers. Some polyphenols also act as antioxidants by metal ion chelation properties (Brown et al., 1998).

2.3.3. Extraction of Phenolic Compounds

Soluble phenolic compounds can be extracted easily from plant tissue using methanol or methanol acidified with 0.1% (v/v) HCl. Phenolic compounds can easily break down; therefore, the isolation process should be carried out in the dark and under cold conditions (Vermerris and Nicholson, 2006).

There are several methods established for the extraction of polyphenols from plant tissue. Those methods vary in solvents and conditions used. The extraction method is essential for the accurate quantification of antioxidant content and activity. This fact makes it hard to compare data from literature reports due to the reason mentioned earlier (Santas et al., 2008).

Naczk and Shahidi (2006) identified a group of factors that influence the quantification of phenolics in plant materials. The chemical nature of the phenolic compounds, the extraction method employed as well as the assay method were some of those factors.

Some of the organic solvents used for the extraction of phenolic compounds are believed to be toxic and the modification of extraction conditions would result in a wide fluctuation in the extraction yield.

Li et al. (2006) found that the total phenol content of different citrus peels were affected by the method of peel preparation (frozen or dried), the type and the concentration of the solvent (ethanol), the extraction temperature, and the type of citrus peel used.

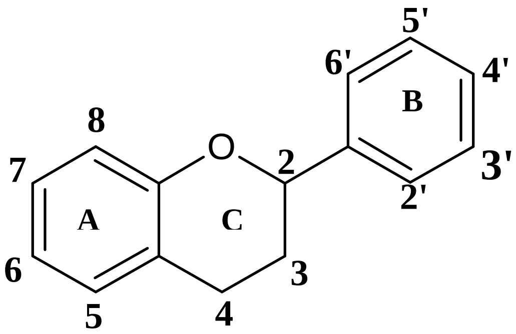
Durling et al. (2007) studied different parameters for the extraction of phenolic compounds and essential oils from dried sage *Salvia officinalis*. They found that particle size (diameter), extraction temperature, extraction time, solvent-to-sage ratio, and solvent concentration all affected the extraction efficiency of three classes of compounds: rosmarinic acid, carnosic acid (and related compounds), and essential oils from dried sage.

2.3.4. Flavonoids

Flavonoids are phenolic compounds formed as secondary plant metabolites that include C_6-C_3-C_6 carbon framework. Flavonoids are derived from two biosynthetic pathways, namely the shekimate and the acetate pathways (Harborne, 1989). Flavonoids compose the largest and the most studied group of plant phenols and they can be divided into 13 different classes according to the heterocycle structure and its degree of hydroxylation.

According to Cadenas and Packer (2002), the molecular structure of flavonoids consists of an aromatic ring A, condensed to heterocyclic ring C, attached to a second aromatic ring B (Fig. 2.2). They contain several phenolic hydroxyl groups attached to the aromatic rings. These groups contribute to the potent antioxidant activity of flavonoids.

Flavonoids in plants exist primarily as 3-*O*-glycosides and polymers. Flavonoids have a large compositional differences exist between different types of plants and even between different parts of the same plant (Hammerstone et al., 2000).



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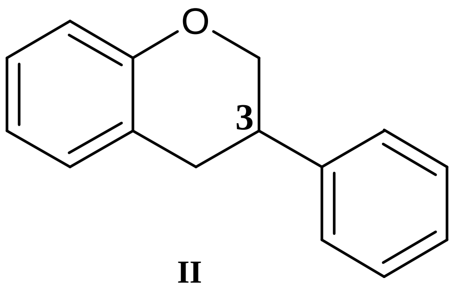
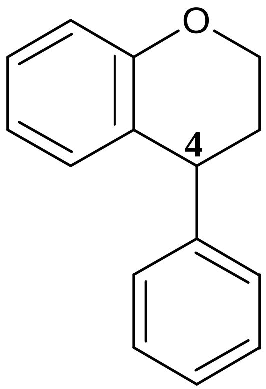


II



III

Fig. 2.2. Basic structure of flavonoids. Structures above are classified according to the linkage of the aromatic ring to the benzopyrano moiety; I: flavonoids, II: isoflavonoids, and III: neoflavonoids (Grotewold, 2006). Phenol rings were classified to A, B, and C ring according to Cook and Samman (1996).

The variations in dietary flavonoids structure result from the hydroxylation pattern (i.e., number and arrangement of hydroxyl groups), the nature and extent of alkylation and/or glycosylation of these groups, glycosidic moieties, methoxy groups, as well as from conjugation between the aromatic rings (Heim et al., 2002; Krämer et al., 2001).

Polymerization of the nuclear structures (Fig 2.2) yields tannins and other complex species occurring in red wine, grapes, and black tea (Cook and Samman, 1996). For instance, during the fermentation of green tea leaves (*Camellia sinensis*) to black tea, there will be a polymerization process of flavanols to tannins and other complex compounds due to the enzymatic oxidation (Balantine et al., 1997).

The antioxidant activity of flavonoids and their metabolites *in vitro* depends on the arrangement of the functional groups around the nuclear structure (Heim et al., 2002). The free radical scavenging activity of flavonoids is mainly attributed to the high reactivity of hydroxyl substituents which is substantially influenced by the configuration and the number of hydroxyl groups (Cao et al., 1997; Burda and Oleszek, 2001).

Plumb et al. (1999) reported that the antioxidant properties of flavonol glycosides from tea decreased as the number of glycosidic moieties increased, therefore, carbohydrate moieties in the flavonoid structure will affect the total antioxidant activity of flavonoids.

The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts, activate antioxidant enzymes, and inhibit oxidases (Elliott et al., 1992; Cos et al., 1998; Ferrali et al., 1997).

The daily human intake of flavonoids varies considerably according to geographic regions and cultures. For instance, it is estimated to be 23 mg daily in the Netherlands (Hertog et al., 1992a); therefore, it can be as low as 25 mg to as high as 1g (Bravo, 1998; Leibovitz and Mueller, 1993). This can be elevated to 2-3 g/day with a high dietary intake of herbs and spices (Hertog et al., 1992b).

Flavonoids are considered as multifunctional bioactive compounds. They have been shown to have a wide range of biological activities, including antiinflamatory, antioxidant, antiviral, antibacterial, antimutagenic, antiallergic, and hepatoprotective effects (Bors and Saran, 1987; Robak and Gryglewski, 1988; Wall, 1992; Middleton and Kandaswami, 1994).

2.3.5. Flavonoids Function in Plants

Flavonoids are secondary metabolites that have been credited with diverse key functions in plant growth and development (Andersen and Markham, 2006).

According to Cadenas and Packer (2002), flavonoids are grouped into anthocyanins and anthoxantins. Anthocyanins are the most important group of water-soluble plant pigments. They are glycosides of anthocyanidin and they are responsible for the red, blue, and purple colours of flowers and fruits. The anthoxantins include flavonols, flavanols, flavones, flavans, and isoflavones. They are colourless or white to yellow.

In plants, phenolics (including flavonoids) may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective against parasites, wounding, air polution and exposure to extreme temperatures (Zobel, 1997).

Anthocyanin biosynthesis has been consistently observed to be induced in plants following exposure to supplementary UV radiation as a protective role of flavonoids against solar UV (Chimphango et al., 2003; Brandt et al., 1995; Lancaster et al., 2000). Gamma irradiation triggered the biosynthesis of anthocyanin in wild-type *Arabidopsis* plants, which were subsequently better scavengers of superoxide radicals than were nonirradiated or anthocyanin-deficient mutants (Nagata et al., 2003).

Isoflavonoids are extremely toxic to fungal pathogens. These flavonoids inhibit fungal spore germination, germ tube elongation, and hyphal growth through causing damage to membrane systems (Skipp and Bailey, 1977; Higgins, 1978).

2.3.6. Effect of processing on vitamin C and polyphenols

Vitamin C is a six-carbon lactone that occurs as L-ascorbic acid and dehydroascorbic acid in fruits and vegetables. Ascorbate is an electron donor and this property explains its function as an antioxidant or reducing agent (Padayatty et al., 2002). Ascorbic acid sequentially donates two electrons from the C2-C3 double bonds forming ascorbate free radical. Although ascorbate free radical is unstable, but it has some properties that makes vitamin C an ideal electron donor (antioxidant) in comparison with other antioxidant possessing molecules. According to Buettner and Moseley (1993), These properties are:

1. Ascorbate free radical is relatively unreactive with other compounds to form potentially harmful free radicals.
2. It can be reversibly reduced to ascorbate and function again as an electron donor molecule.
3. It can undergo further oxidation to form more stable product, dehydroascorbic acid (DHA), which can exist in more than one structural form.

Ascorbic acid also acts as an oxygen scavenger, removing molecular oxygen in polyphenol oxidase reactions. According to Rico et al. (2007) polyphenol oxidase inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed *o*-quinones to their precursor diphenols.

There are many reasons explain the decrease in vitamin C concentration during food processing. Of those, the thermal and/or mineral catalyzed oxidation of this highly reactive compound (Vanderslice et al., 1990; Iwase and Ono, 1997), the leaching of this water soluble compound into the processing water (Iwase and Ono, 1997; De Pascual-Teresa et al., 2000) and the changes in moisture content of the processed food (Vanderslice et al., 1990).

González-Aguilar et al. (2007a) reported a reduction in total ascorbic acid content of UV-C irradiated fresh-cut mango fruits due to the oxidation of ascorbic acid by effect of the increment in UV-C exposure time.

Tzortzakis et al. (2007) reported that there were no significant changes in vitamin C content of tomato fruits that were stored in ozone-enriched atmosphere up to 6 days.

Keutgen and Pawelzik (2008) reported a significant loss in vitamin C content of pre-harvest ozone exposed strawberries. They attributed this loss either to the reduced synthesis of ascorbic acid, the degradation of ascorbic acid, and/or to the free radical scavenging processes that took part during the ozonation process.

Oxidation of phenolic compounds is one of the most important aspects of these compounds. The phenolic compounds can be oxidized through auto-oxidation or through enzyme-catalyzed oxidation.

Oxidation of phenolic compounds can result in the browning of tissue (like fruit browning) and it can also result in formation of metabolites that are toxic to animals and plants and can contribute to food spoilage during processing. However, those metabolites can be useful in the inhibition of pathogens (Vermerris and Nicholson, 2006).