การเปรียบเทียบฤทธิ์ต้านออกซิเดชันและการยับยั้งไทโรสิเนสในหัวไชเท้า กระเทียมและขิงที่สดและแปรรูป

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COMPARISON OF ANTIOXIDANT ACTIVITY AND TYROSINASE INHIBITION IN FRESH AND PROCESSED WHITE RADISH, GARLIC AND GINGER

Mr. Apisit Somman

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
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Thesis Title	COMPARISON OF ANTIOXIDANT ACTIVITY AND TYROSINASE INHIBITION IN FRESH AND PROCESSED WHITE RADISH, GARLIC AND GINGER
By	Mr. Apisit Somman
Field of Study	Biotechnology
Thesis Advisor	Associate Professor Napa Siwarungson, Ph.D.

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อภิสิทธิ์ สมหมั่น : การเปรียบเทียบฤทธิ์ต้านออกซิเดชันและการยับยั้งไทโรสิเนสในหัวไชเท้า กระเทียมและขิงที่สดและแปรรูป (COMPARISON OF ANTIOXIDANT ACTIVITY AND TYROSINASE INHIBITION IN FRESH AND PROCESSED WHITE RADISH, GARLIC AND GINGER) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.นภา ศิวรังสรรค์, 70 หน้า.

ประเทศไทยมีความหลากหลายของพืชผักและผลไม้เนื่องมาจากอยู่ในเขตป่าฝน ทำให้มีความหลากหลายทางชีวภาพของพืชสูง โดยพบว่าพืชผักและผลไม้ที่บริโภคเป็นอาหารมีวิตามิน แร่ธาตุและสารไฟโตเคมิคัลหลากหลายชนิด เช่น ฟลาโวนอยด์ ฟีนอลลิค แอนโธไซยานิน แคโรทินอยด์ เป็นต้น จากการศึกษาพบว่าสารดังกล่าวสามารถยับยั้งปฏิกิริยาของอนุมูลอิสระ ป้องกันโรคและทำให้มีสุขภาพดี เช่น หัวไชเท้า *Raphanus sativus* กระเทียม *Allium sativum* และขิง *Zingiber officinale* ซึ่งนิยมบริโภคกันเป็นประจำทุกวันทั้งสดและแปรรูป เช่น การดอง เป็นต้น จึงได้นำมาศึกษาเปรียบเทียบแอคติวิตีของพืชสดและผ่านการแปรรูปโดยเลือกเก็บตัวอย่างจากตลาดในเขตกรุงเทพมหานคร ผลการทดลองพบว่าค่าการต้านอนุมูลอิสระโดยวิธีทดสอบ 1,1-diphenyl-2-picryl-hydrazyl (DPPH) พบสูงที่สุดในหัวไชเท้าแปรรูป 84.99 ± 11.39% และขิงสด 80.90 ± 5.70% ค่าการต้านอนุมูลอิสระโดยวิธีทดสอบ 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) พบสูงที่สุดในขิงแปรรูปและกระเทียมสด 109.39 ± 4.34% และ 48.30 ± 8.38% ปริมาณสารฟลาโวนอยด์พบในกระเทียมมากที่สุดทั้งสดและแปรรูป 8.85 ± 1.08 และ 2.26 ± 0.88 มิลลิกรัมต่อ 100 กรัม ตามลำดับ อีกทั้งยังพบปริมาณสารฟีนอลิคสูงที่สุดในกระเทียมแปรรูปและสด 19.48 ± 5.51 และ 1.76 ± 0.33 มิลลิกรัมต่อ 100 กรัม ตามลำดับ ค่าการยับยั้งเอนไซม์ไทโรสิเนสพบว่า ขิงสดมีความสามารถในการยับยั้งสูง 363.71 ± 58.75% และกระเทียมแปรรูป 151.42 ± 15.12% การศึกษาดังกล่าวเพื่อเป็นแนวทางในการเลือกบริโภคอาหารที่เป็นแหล่งของสารไฟโตเคมิคัลซึ่งสามารถต้านอนุมูลอิสระและยับยั้งเอนไซม์ไทโรสิเนสและเป็นประโยชน์ต่อสุขภาพ

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORDS: ANTIOXIDANT / TYROSINASE / RADISH / GARLIC / GINGER

APISIT SOMMAN: COMPARISON OF ANTIOXIDANT ACTIVITY AND TYROSINASE INHIBITION IN FRESH AND PROCESSED WHITE RADISH, GARLIC AND GINGER. ADVISOR: ASSOC. PROF. NAPA SIWARUNGSON, Ph.D., 70 pp.

In Thailand, there is a diversity of vegetables and fruits due to being a tropical rain forest as well as these are good source of loaded with vitamins, minerals and phytochemicals. Examples of phytochemicals are flavonoid, phenolic, anthocyanin and carotenoid which have been proved to be antioxidant, prevented some diseases and lead to better health. The fresh and processed white radish *Raphanus sativus,* garlic *Allium sativum* and ginger *Zingiber officinale* are consumed on daily basis. They are popular for cooking and preservation e.g. pickle. The objective of this work is to compare antioxidant activity and tyrosinase inhibition between fresh and processed vegetables. This study determined the antioxidant activity such as 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), total flavonoid and phenolic contents as well as tyrosinase inhibition in fresh and processed vegetables. They were collected from three representative markets in Bangkok. The results showed that DPPH in processed white radish was 84.99 ± 11.39% and fresh ginger was 80.90 ± 5.70%. The ABTS test on processed ginger and fresh garlic were 109.39 ± 4.34% and 48.30 ± 8.38% respectively. The highest total flavonoid contents of garlic were 8.85 ± 1.08, 2.26 ± 0.88 mg/100g in fresh and processed respectively. Total phenolic contents of garlic were 1.76 ± 0.33, 19.48 ± 5.51 mg/100g in fresh and processed respectively. The tyrosinase inhibitory activity was highest in fresh ginger 363.71 ± 58.75% and processed garlic 151.42 ± 15.12%. These studied vegetables could be an alternative choices for the advantage consumption diet. They have been shown to be a novel rich source of phytochemicals. These can be antioxidant, tyrosinase inhibition and healthiness.

Field of Study: Biotechnology Student's Signature .....

Academic Year: 2014 Advisor's Signature .....

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LIST OF ABBREVIATIONS

ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
$AlCl_3.6H_2O$	aluminum chloride
°C	celsius
CAT	Catalase
DPPH	1,1-diphenyl-2-picrylhydrazyl
g	gram
GPX	Glutathione peroxidase
GR	Glutathione ruductase
GST	Glutathione S-transferase
L	liter
L-DOPA	L-3,4-dihydroxyphenylalanine
M	molar
mg	milligram
ml	milliliter
mM	millimolar
$NaNO_2$	sodium nitrite
NaOH	sodium hydroxide
nm	nanometer

OD	optical density
ORAC	oxygen radical absorbance capacity
pH	positive potential of the hydrogen ions
RCS	reactive chlorine species
RNS	reactive nitrogen species
ROS	reactive oxygen species
SOD	superoxide dismutase
TEAC	trolox equivalent antioxidant capacity
U	unit
UVA	ultraviolet A
UVB	ultraviolet B
µl	microliter
%RSA	% radical scavenging activities

CHAPTER I

Introduction

The tropical rainforests of Thailand are marked by a great diversity of vegetable and fruit plants. Many of these products (e.g. herbs, vegetables and fruits) possess nutritionally beneficial properties that have been the subject of many studies. At present, consumers are becoming more interested in the benefits of edible plants due to their value as sources of vitamins, minerals, and other phytochemicals. Many studies show the benefits of both vegetables and fruits known to possess antioxidant and anti-melanogenic properties and the ability to prevent other diseases [1-3]. White radish *Raphanus sativus*, garlic *Allium sativum* and ginger *Zingiber officinale* are three such root vegetables consumed on a daily basis which are popular both in cooking and for food preservation e.g. pickling [4]. They possess excellent health benefits as they are a good source of antioxidants and tyrosinase inhibition from phytochemicals such as flavonoids and phenolic compounds which are of value for the prevention of certain non-communicable diseases (NCDs), for example those caused by free radicals [5]. Tyrosinase inhibitors have been of interest due to the key role played by tyrosinase in both mammalian melanogenesis and fruit or fungal enzymatic browning. Melanogenesis has been defined as the entire process leading to the formation of dark macro-molecular pigments, i.e. melanin [6]. Previous reports confirm that antioxidants and tyrosinase are among the components providing the nutritional quality of vegetables and fruits in addition to their beneficial health effects and disease prevention potential [7]. The antioxidant activity and tyrosinase inhibition qualities of many

vegetables and fruits have been widely studied. This study indicates that the antioxidant and antityrosinase capacity of the three raw vegetable materials i.e. white radish, garlic and ginger in Thailand, are due to the presence of radical scavenging phenolics, flavonoids and antityrosinase [8].

1.1 Antioxidant

Antioxidants are present in foods and our bodies at low concentrations compared to the oxidizable substrate. They are of interest to health professionals because they help to protect the body against damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as reactive chlorine species (RCS) associated with degenerative diseases. Antioxidants act at a different level in the oxidative sequence in lipid molecules. They may decrease oxygen concentration by preventing first-chain initiation by scavenging initial radicals including hydroxyl or metal catalysts (Figure 1) [9]. Protection against ROS and RNS-induced damage is provided by complex antioxidant defense systems, comprising endogenous enzymatic and non-enzymatic antioxidants (e.g. superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and exogenous antioxidants (i.e. vitamin C, vitamin E, carotenoids and polyphenols), the latter provided mainly through dietary sources [3].

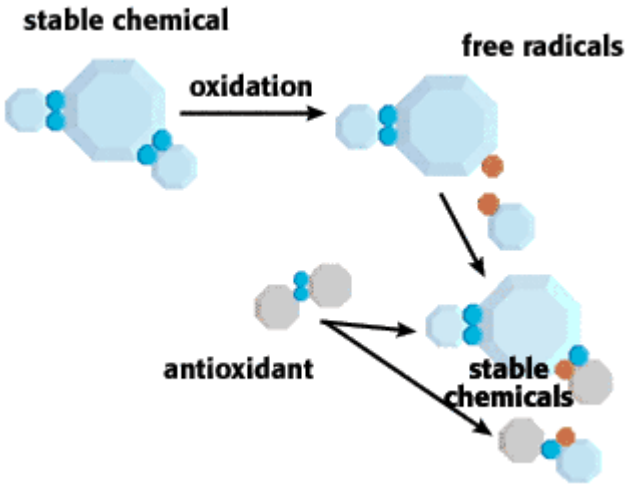

Figure 1 Antioxidant reaction
(http://www.thehorse.com/images/content/0104/antioxidants.gif)

Natural antioxidants come from a variety of dietary sources, for example, phenolic and polyphenolic compounds among others. The mechanism by which these antioxidants exert their effects may vary depending on the compositional characteristics of the food in question. Moreover, the beneficial health effects of plant food consumption have been described. Phenolic containing compounds in foods are associated with reduction of the risk of cardiovascular diseases, cancer and the other diseases. This is believed to be achieved through the prevention of lipid oxidation, protein cross-linking, DNA mutation and tissue damage. Additionally, phenolic compounds and some of their derivatives are very efficient in preventing auto-oxidation; however, only a few phenolic compounds are currently allowed as supplementary food antioxidants. The list of approved phenolic antioxidants has been extensively studied but the toxicology of their degeneration products is still not clearly documented [9].

The activity of an antioxidant can be estimated by quantitatively determining primary or secondary products of anti-oxidation of a lipid, or by monitoring other variables. It is also possible to use a luminescence apparatus which is a photochemistry device which measures antioxidant activity of hydrophilic and lipophilic compounds. There are numerous methods of antioxidant activity including ORAC (oxygen radical absorbance capacity) and TEAC (Trolox equivalent antioxidant capacity). Recent literature has described tests that use artificial radicals such as DPPH (1,1-diphenyl-2-picrylhydrazyl). The antioxidant mixtures present, for example, an extract to scavenge ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and the disappearance of the blue/green color of this radical upon the action of antioxidants, have also been described. Moreover, all of the tests cited offer means of evaluating antioxidant activity of food phenolics and other constituents [9].

Types of antioxidants

Antioxidants may be classified as free radical terminators, chelators of metal ions and oxygen scavengers that react with oxygen in a closed system. First, primary antioxidants react with high-energy lipid radicals to convert them to thermodynamically more stable products. Secondary antioxidants, known as preventive antioxidants, function by retarding the rate of chain initiation by breaking down hydroperoxide [9].

Sources of antioxidants

Edible plants contain various phytochemicals with different bioactive properties including antioxidant activity [10]. The compounds that appear to be responsible for the activity of antioxidants have been reported present in a number of fruits and vegetables. Antioxidant content levels differ according to species, area of cultivation,

post-harvest conditions and other factors. Fruits and berries are a good source of antioxidants containing for instance, carotenoid, ascorbic acid, α-tocopherol, flavonoids and phenolic acids [3, 10]. It has been known for a long time that phenolic and other antioxidant components are closely associated with the sensory attributes of fresh and processed plant foods [11].

Benefits of antioxidants

Antioxidants are popularly known to promote good health and prevent disease. It is essential to consume antioxidants because the body cannot produce them endogenously. However, vitamins, minerals, phytochemicals, and enzymes must come from daily dietary intake to help provide added protection for the body [3, 12]. For example, they protect the skin from ROS, provide immune system support and prevent some diseases and may even have anti-aging potential [3, 10].

Free radicals

Free radicals are any atoms, molecules or groups of atoms with a single unpaired electron, which are highly reactive substances that can result in chain reactions (Figure 2). These chain reactions involve a number of steps each of which forms a free radical that triggers the next step. The free radical species include reactive oxygen species (ROS), reactive nitrogen species (RNS), carbon-centered radicals, and sulfur-centered radicals. RNS in living systems mainly include nitric oxide (NO) and nitrogen dioxide. Nitric oxide is a free radical which can also produce hydroxyl radicals and nitrogen dioxide radicals [13]. Free radicals play a role in the activation or inhibition of signaling pathways include damaging agents in living organisms. They are involved in diverse pathological processes, such as aging, some diseases, and the other effects [14, 15].

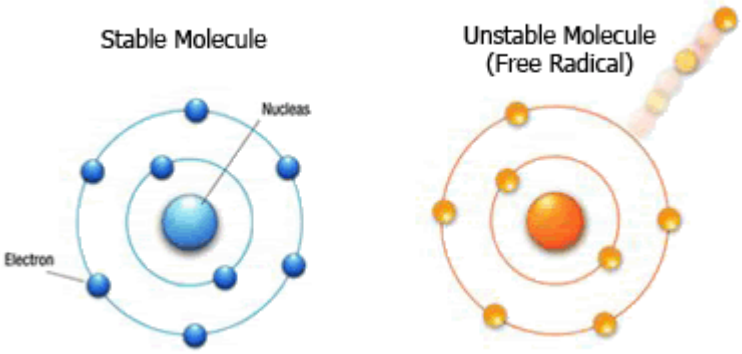

Figure 2 Free radicals
(http://coconutcreamcare.files.wordpress.com/2012/08/freeradical1.gif)

1.2 Phenolic compounds

Phenolic phytochemicals are a large class of natural substances that can be found in many edible plant products which have strong antioxidant activity. In this capacity they are able to scavenge reactive oxygen species (ROS) generated endogenously and by chemical carcinogens. Phenolic compounds are categorized into different groups depending on their structure and are subcategorized within each group according to the number and position of the hydroxyl group and the presence of other constituents (Figure 3). The most widespread and diverse group of the polyphenols in fruits and vegetables are the flavonoids which are built upon C6-C3-C6. In addition, other phenolic compounds such as benzoic acid or cinnamic acid have been identified in fruits and vegetables (Figure 4) [16]. Natural phenolic compounds from plants include many secondary metabolites such as alkaloids and terpenoids. They are formed by several different biosynthesis pathways in plants. Phenolic bioactive compounds in foods and nutraceuticals originate from one of the main classes of secondary metabolites in plants and are of interest for their antioxidant properties [17]. Plants and

foods derived from them contain a large variety of phenolic derivatives including phenol, phenylpropanoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, lignans and lignins. Many properties of plant products are associated with the presence, type and content of their phenolic compounds. They lend a property of astringency to foods, have beneficial health effects or potential anti-nutritional properties when present in large quantities, all of which are significant in consumer foods [9].

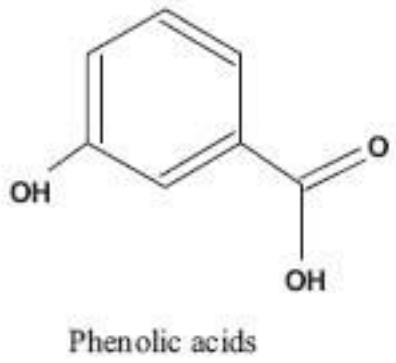

Figure 3 Phenolic structure
(http://www.foodnetworksolution.com/uploaded/phenolic%20compond.bmp)

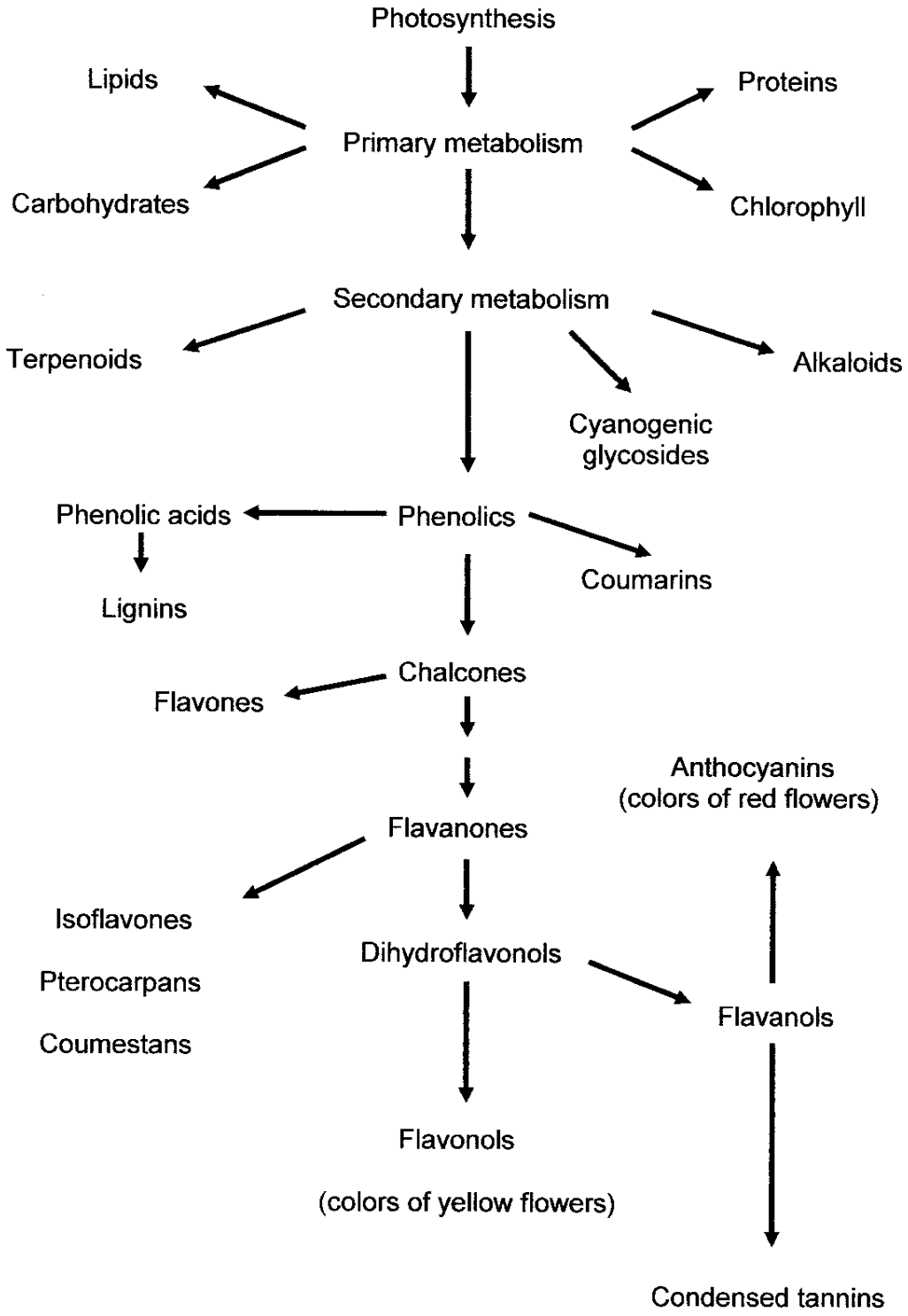

Figure 4 Overview phenolic compounds

(http://www.intechopen.com/source/html/38573/media/image1.png)

1.3 Flavonoids

Flavonoids or bioflavonoids are phenolic compounds which form a class of secondary metabolites in plants and are widely distributed in leaves, seeds, barks and flowers of plants. Flavonoids, which are water soluble, are the most important plant pigments. The flavonoids comprise 5 major subgroups: chalcone, flavone, flavonol, flavanone and anthocyanins (Figure 4, 5 and 6) [18, 19]. Common storage and processing methods such as peeling, chopping, boiling, microwaving and frying reduce the total content of flavonoids [9, 20, 21]. Flavonoids are found in many plants such as vegetables and fruits. In addition, beverages, herbs, barks, garlic, grapes, beans, wine, green tea, and others are also found [18, 19].

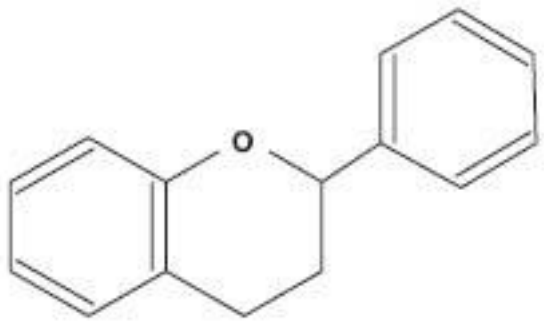

Figure 5 Structure of flavonoids
(http://www.foodnetworksolution.com/uploaded/phenolic%20compond.bmp)

Benefits of flavonoids

Flavonoid is a secondary metabolite in plants mostly found in vegetables, fruits, wines, teas and cocoa [18]. The flavonoids appear to play a healthful role in the human diet. They contribute to lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men, and have anti-allergic and anti-

inflammatory anti-tumor properties and may even protect against oxidative stress [18, 21, 22].

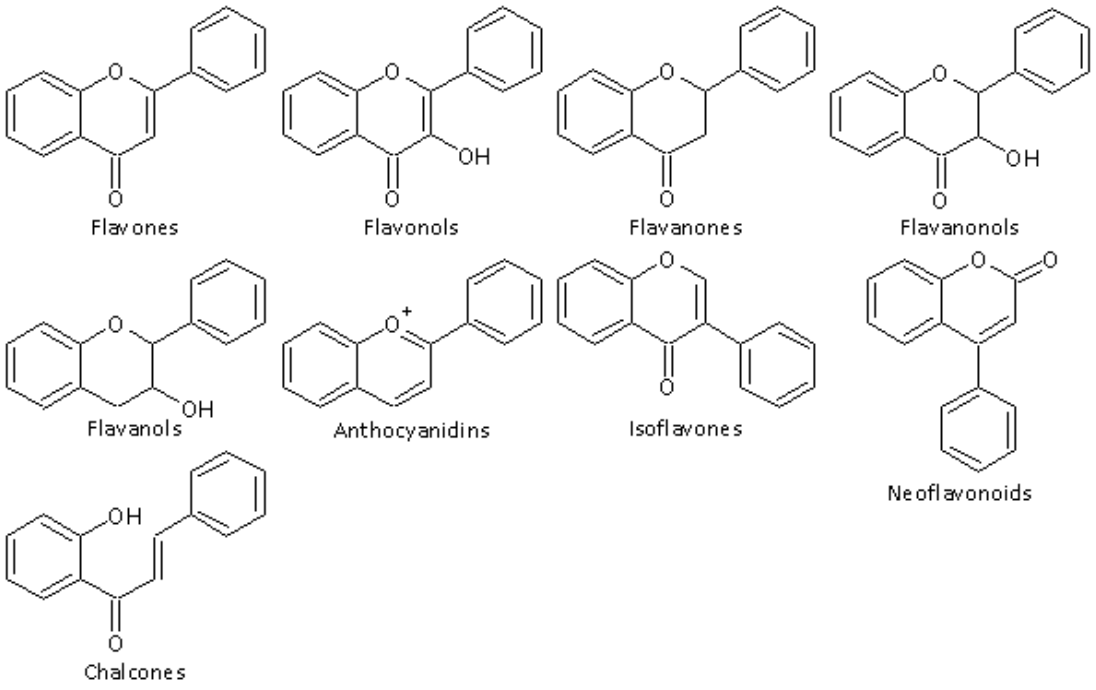

Figure 6 Chemical structures of flavonoids
(http://www.tuscany-diet.net/wp-content/uploads/2014/01/Flavonoid-subgroups.gif)

1.4 Tyrosinase

Tyrosinase (monophenolmonooxygenase, EC:1.14.18.1) is a copper containing enzyme responsible for enzymatic browning in plants, and for producing undesirable changes in the color, flavor and the nutritive value of plant-derived foods and beverages [23-25]. Tyrosinase has a unique ability to catalyze two distinct reactions in the course of melanin synthesis: the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone after a further series of conversions to melanin [24, 25]. Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions,

such as kojic acid [26]. Tyrosinase is also widely distributed in microorganisms, plants and animals. It can be extracted from the champignon (white button) mushroom (*Agaricus bisporus*). Mushroom tyrosinase is a popular model for studies on melanogenesis and has been extensively used as a target enzyme for screening and characterizing potential tyrosinase inhibitors. L-tyrosine was used as the substrate in this experiment because the mode of inhibition depends on the structures of both the substrate and inhibitors [27]. Moreover, studies on tyrosinase inhibition have used mushroom tyrosinase since it is commercially widely available. Tyrosinase has been ascribed other functions apart from melanin production. Several biotechnological applications include numerous uses as electrochemical biosensors in a number of phenolic compounds. In addition, tyrosinase has been applied to activate tyrosine residues in polypeptides for protein cross-linking on chitosan films, and in direct protein-protein cross-linking. Moreover, tyrosinase can be applied to remove phenol from wastewater and for the bioconversion of L-tyrosine to L-DOPA [28].

1.5 Principle of assays

Principle of antioxidant assays

The principle of assays was to reduce redox-active compounds as monitored by spectrophotometry. The most popular assays in use adopted for the current study are 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Figure 7) and 2,2-azino-bis-3-ethylbenzthiazo-line-6-sulphonic acid (ABTS) assay (Figure 8), ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity ORAC). Ascorbic acid (vitamin C) was used to quantify antioxidant capacity as positive control. The DPPH assay is based

on the reduction of purple DPPH to 1,1-diphenyl-2-picryl hydrazine whereas the ABTS assay is based on the generation of a blue/green ABTS that can be reduced by antioxidants. Both assays are simple to apply and thus often used, nevertheless, they are limited as they use nonphysiological radicals [29].

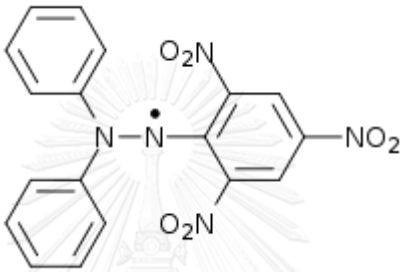

Figure 7 Structure of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (http://upload.wikimedia.org/wikipedia/commons/thumb/2/2a/DPPH.svg/219px-DPPH.svg.png)

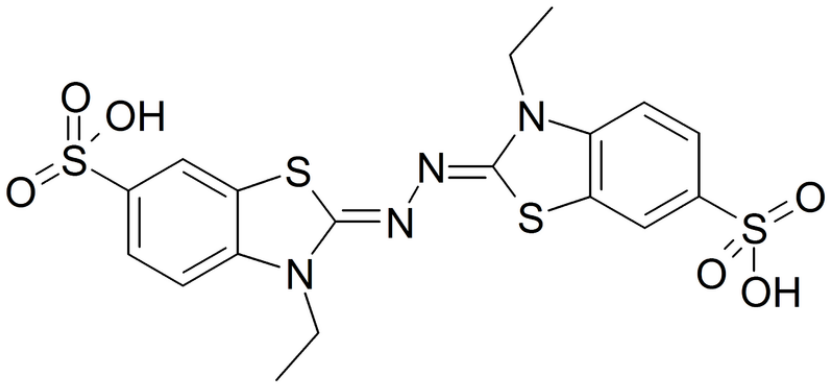

Figure 8 Structure of 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) (http://upload.wikimedia.org/wikipedia/commons/thumb/1/1f/ABTS.png/800px-ABTS.png)

Principle of phenolic content

Reduction of Folin–Ciocalteu reagent to a blue-colored complex in an alkaline solution occurs in the presence of phenolic compounds. This reagent was sensitive to reduce compounds which can be quantified by spectrophotometry. The phenolic content of our sample food substances were calculated as gallic acid for the standard curve [30].

Principle of flavonoid content

Acid stable complexes were composed of the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols with aluminum chloride. Aluminum chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. The flavonoid contents were calculated as rutin for the standard curve [31].

Principle of tyrosinase assay

Tyrosinase inhibition assay was determined based on the inhibition of the conversion of a specific substrate of tyrosinase and L-DOPA into a colored product, DOPACHROME, which has maximum absorption at 492 nm [32]. Tyrosinase extracted from the champignon mushroom (*Agaricus bisporus*) is a popular model for studies on melanogenesis [28].

1.6 White radish

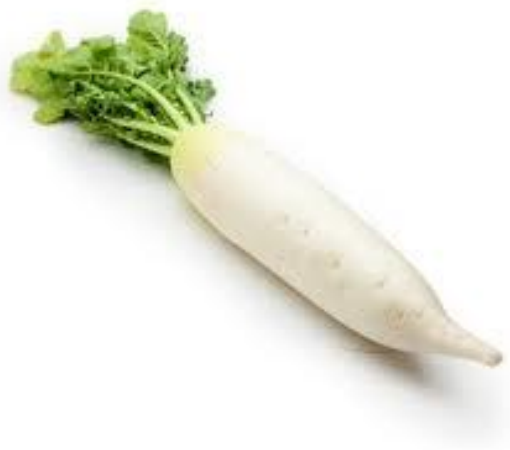

Figure 9 White radish (*Raphanus sativus*)
(http://jundishes.com/wp-content/uploads/2013/03/DaikonRadish.jpeg)

Scientific classification

Kingdom: Plantae

Order: Brassicales

Family: Brassicaceae

Genus: *Raphanus*

Species: *R. sativus*

Radish is a rhizome plant commonly used for consumption. The root can be eaten raw, cooked or preserved in salt. It is well-known as a root vegetable and is used as an ingredient in a variety of foods in East Asia including soups such as a food preservative in Japanese foods. In addition, radish extract is used as a popular whitening agent in skincare cosmetics. Furthermore, people in Thailand commonly use it to cook noodle soups, for pickling and preserved food. The white radish found in Thailand

contains polysaccharides, proteins and vitamin C, while fresh radish roots contain many phenolic compounds such as kempferol, cyanidin, gentisic acid, hydrocinnamic acid, vanillic acid, pelargonidin, luteolin, myricetin and quercetin. The aqueous extract of radish roots and leaves are a common element in traditional medicine for their antimicrobial, antimelanogenic and antioxidant properties. However, many other beneficial properties, especially for cosmetic and dermatological applications, are not widely known or studied although traditionally, Thai women have used slices of fresh white radish root for skin treatments due to its antityrosinase properties [1, 33, 34].

1.7 Garlic

An important medicine as well as a spice widely used in Western and Middle Eastern cooking, different parts of garlic (*Allium sativum*), including the flower, bud, leaf, fruit and rhizome, have been cultivated for over 3,000 years in India and China. It has been used for treatment of a number of ailments and discomforts including stomachache, cough, anorexia, eczema, diarrhea, rheumatism, jaundice, anemia, constipation, asthma, gastritis and can also function as an anticonvulsant and antiemetic agent. The fresh rhizomes of garlic are dried and used as digestive stimulants and anti-inflammatory agents in China and tropical Asia [9, 35].

Figure 10 Garlic (*Allium sativum*)

(http://po-zdravidnes.com/language/bg/uploads/img_original/top10__1/top10__21875aaaf795b7f1d54c9c335fd7a9a0.jpg)

Scientific classification

Kingdom: Plantae

Order: Asparagales

Family: Amaryllidaceae

Genus: *Allium*

Species: *A. sativum*

The bioactive components of garlic are flavonoids, the main pungent component of the rhizome. The antioxidant activity of the phenolic constituents of garlic has been documented. The relevant compounds also serve as good antimicrobial agents [36]. One of the most widely consumed root vegetables, garlic is classified on

the basis of color into red and white garlic. Garlic are rich of flavonoids and serve as a major source of flavonols in the diet [9, 37].

1.8 Ginger

Scientific classification

Kingdom: Plantae

Order: Zingiberales

Family: Zingiberaceae

Genus: *Zingiber*

Species: *Z. officinale*

Figure 11 Ginger (*Zingiber officinale*)
(http://202.143.138.115/e-doc/img/zingiber3.jpg)

Ginger is an important herb which is derived from the rhizomes of the plant. Rhizomes are eaten raw or cooked as vegetables and used for flavoring food as well as treatment of a variety of diseases. As traditional medicine, rhizomes of ginger plants have been consumed by women during labor. Rhizomes are also taken as carminatives to relieve flatulence [26]. Moreover, they are considered effective as a natural alternative to promote health due to their antioxidant properties. The natural antioxidant properties of ginger species have been studied in terms of their functional benefits for health deriving from phenols, vitamins, as well as secondary metabolites [26, 38, 39]. In Thailand, people have traditionally used rhizome of ginger for traditional medicine, as an essential oil to aid relaxation and for use in massage, and as a beverage in ginger tea. Previous studies on the medical use and on supplements including antioxidant properties, phytochemicals and tyrosinase inhibitors of ginger species are confined to the rhizomes of the plant. Rhizomes of gingers have been reported to have tyrosinase inhibition properties. Skin-lightening cosmetic products were recently developed from ginger rhizomes. Despite the rhizome of ginger having been used for food flavoring and in traditional medicine, little research has been done on their antioxidant and tyrosinase inhibition properties [26].

1.9 Literature review

Vegetables and fruits have a wide variety of antioxidant properties, such as, radish, garlic and ginger, are consumed daily in Thailand. They are a source of phytochemicals with antioxidant tyrosinase inhibition properties. Phytochemicals are a variety of compounds in plants with active ingredients, for example, phenolic acids,

carotenoids, polyphenols, phytoestrogens, saponins and others. They are found in vegetables, fruits, beans, and grains. Though they are popular food plants both fresh and preservation, previous studies have not looked at the nutritional compounds in preserved food plants in Thailand. In general, food preservation uses salt or sugar, for example, pickling. Some studies have examined local species such as red radish, onion, and ginger species. However, these studies focused on properties for antioxidants, health promotion, disease prevention, tyrosinase inhibitors, anti-inflammatories, antimicrobials and anti-cancers. The current study provides a comparison of the antioxidant properties and tyrosinase inhibition in white radish, garlic and ginger. It examines their antioxidant contents in both fresh and processed forms. The food samples were selected from three representative markets in Ratchathewi, Prathumwan and Bangrak in Bangkok, Thailand.

The study of radish (*Raphanus sativus*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) is interesting. At first, radish is an important tyrosinase inhibitor which has been studied in fresh form, but not in the pickled form. Ghasemzadeh et al. (2012) studied white radish and found they contained total flavonoids of 0.016 mg/g (1.6 mg/100g) with antioxidant activity of 47.33% [40]. Jakmatakul et al. (2009) reported the contents of total phenols, total flavonoids and L-ascorbic acid (per 1 mg of dried extract) were found to be 6.59, 0.33 and 8.28 µg, with 9.62 mg/ml of tyrosinase inhibition and 0.64 mg/ml of DPPH for the methanolic extract [33]. while Kamkaen et al. (2007) studied Chinese radish. Maheswari et al. (2012) reported the antioxidant activity and total phenolic content of white radish extracted by ethanol was 154.72 µM of mg gallic acid equivalents (GAE)/ g of fresh material (FM) [41]. De Martino et al. (2012) studied *in vitro* phytotoxicity and antioxidant activity of selected flavonoids.

They reported the high antioxidant value of *Raphanus sativus* from flavonoids [42]. Rosa and Lule Perez (2004) studied phytochemicals in radish and found many phytochemicals such as phenolic compounds [34]. Weerapreeyakul et al. (2012) studied radish and found 2,425.8 µg/ml of radical scavenging activity and 102.5 µg/ml tyrosinase inhibitory activity [32]

Garlic is an important traditional medicine that has been used to treat and prevent diseases because of its phytochemical content. Benkeblia (2005) reported the highest DPPH radical scavenging activity in garlic extract was 196% [43]. while Bozin et al. (2008) reported extract from garlic can reduce DPPH radical formation [35]. Jae-hee et al. (2009) reported garlic's significant antioxidant activity and protective effects against oxidative DNA damage regardless of processing method [44]. Kim et al. (2013) did a comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. They reported total phenolic and flavonoid content of garlic subjected to different thermal processing steps were higher than those of fresh garlic [45]. Marta et al. (2007) reported garlic to be effective in prevention of cardiovascular disease because of its hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects. They also found garlic to have many other biological activities including antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities [46]. Mei-chin and Wen-shen (1998) studied the antioxidant activity of several members of the *Allium* family finding it possessed antioxidant properties [8]. Queiroz et al. (2009) reported garlic *Allium sativum* L. and ready-to-eat garlic products had 21.69% DPPH fresh [36]. Rahman et al. (2012) reported extract of garlic has been presumed to be a very strong antioxidant content [47]. while Stajner et al. (1998)

investigated the antioxidant abilities of cultivated and wild species of garlic reporting that the highest quantities of $o_2^{\cdot-}$ and $OH^{\cdot}$ were observed in the aboveground parts of wild *Allium vienale* [48]. Victor et al. (2011) reported the contents of total phenolic compounds was 3.4 mg gallic acid equivalents (GAE)/g (340 mg/100g) while flavonoids were not detected in any of the samples [49].

Ginger is an important traditional medicine with several uses including for relaxation, disease treatment, ginger tea and in the improvement of nutrient absorption. Chan et al. (2008) studied antioxidant and tyrosinase inhibition properties of 26 species' leaves and rhizomes of ginger and found leaves of the *Etlingera* species had the highest total phenolic content and ascorbic acid equivalent antioxidant capacity (AEAC) [26]. Charanjit and Kapoor (2002) reported that antioxidant and total phenolic contents of ginger was 71.8% antioxidant and high phenolic content [2]. Ghasemzadeh et al. (2010) studied antioxidant activities, total phenolic and flavonoid content in two varieties of Malaysian young ginger. Their results showed that extracts from the leaves, stems and rhizomes of two *Zingiber officinale* varieties had antioxidant activity in DPPH assay and phenolic content of the leaves as well as higher total amounts of phenolic and flavonoid contents than those of the rhizomes and stems [50]. Masuda et al. (2004) studied antioxidant properties of gingerol related compounds from ginger showing that gingerol extract can be shown to display inhibitory effects of autoxidation [51]. Shirin et al. (2010) reported ginger extract reduced the power and free radical scavenging activity of DPPH [52]. Stoilova et al. (2007) reported the antioxidant effect by DPPH of ginger extract was 91.1% for free radical scavenging [39]. Weerapreeyakul et al. (2012) studied ginger's antioxidative and tyrosinase inhibitory activity. They reported

radical scavenging activity of 349.5 µg/ml and tyrosinase inhibitory activity of 624.5 µg/ml [32].

Finally, the study was focus on fresh and processed plant foods (garlic, ginger and white radish) to compare their antioxidant activity and tyrosinase inhibition properties. It was test antioxidants using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay as well as measuring total phenolic and flavonoid content using the tyrosinase inhibition method L-3,4-dihydroxyphenylalanine (L-DOPA). The L-DOPA was intermediate to react with tyrosinase taken from mushroom and other sample extracts. Both antioxidant and tyrosinase will be reported as percentages of activity while phenolic and flavonoid compounds will be reported as total content. In this study, fresh and preserved local species in Thailand, such as white radish, Thai garlic and ginger will be used for comparison. Additionally, the study will compare antioxidant and tyrosinase activity in fresh and processed samples at 10, 20 and 30 days. It is believed the information of this study will be useful for the selection of fresh vegetables for consumption to promote health or prevent non-communicable diseases.

1.10 The objective of this study

The objective of this study is to compare fresh and processed white radish *Raphanus sativus,* garlic *Allium sativum* and ginger *Zingiber officinale* for antioxidant activity using DPPH and ABTS radical scavenging, total phenolic and flavonoid content as well as tyrosinase inhibition activity. Samples were selected in three representative markets in Bangkok, Thailand at Rachathewi, Prathumwan and Bangrak.

There were 27 samples consisting of nine white radishes, nine garlic cloves and nine pieces of ginger for analysis.

CHAPTER II

Materials and Methods

2.1 Materials

After a survey of representative markets in Bangkok Thailand fresh samples of about 1 kg. of white radish *Raphanus sativus*, garlic *Allium sativum* and ginger *Zingiber officinale* were collected from three green groceries in three markets in Ratchathewi, Prathumwan and Bangrak. The bottles used for tests were cleaned and sterilized by autoclave at 121°C, pressure 150 pounds per square inch for 15 minutes.

2.2 Equipments

50 mL centrifuge tubes	Sigma-Aldrich Co. (St. Louis, MO, USA)
Balances	PB 303-S METTLER TOLEDO
Blender	MX- J210GN National
Centrifuge	J-30I Beckman Coulter
pH-Meter	G 76 D METTLER TOLEDO
Magnetic stirrer	MS 115 Ceremag midi
Micropipette	PIPET MAN
Microtube size 1.5 ml	E1004 Hycon
Spectrophotometer	Genesys 10 Biomate 3
Water bath	WB 29 Memmert

Vortex mixer	Type 232 Fisher scientific

2.3 Chemicals and reagents

Acetic acid	Lab Scan, Ireland
1,1-diphenyl-2-picrylhydrazyl	Sigma-Aldrich Co. (St. Louis, MO, USA)
2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)	Sigma-Aldrich Co. (St. Louis, MO, USA)
Aluminum chloride	Sigma-Aldrich Co. (St. Louis, MO, USA)
Dimethyl sulfoxide	Sigma-Aldrich Co. (St. Louis, MO, USA)
Ethyl alcohol absolute	Merck, Germany
Folin–Ciocalteu's reagent	Sigma-Aldrich Co.(St. Louis, MO, USA)
Gallic acid	Sigma-Aldrich Co. (St. Louis, MO, USA)
Hydrogen peroxide	Witthayasom Co., Ltd
L-3,4-dihydroxyphenylalanine (L-DOPA)	Sigma-Aldrich Co. (St. Louis, MO, USA)
L-ascorbic acid	Witthayasom Co., Ltd
Methanol	Burdick Jackson, USA
Peroxidase from horseradish	Sigma-Aldrich Co. (St. Louis, MO, USA)
Rutin	Sigma-Aldrich Co. (St. Louis, MO, USA)
Sodium carbonate	Sigma-Aldrich Co. (St. Louis, MO, USA)
Sodium hydroxide	Ajax Finechem, Australia

Sodium nitrite	Sigma-Aldrich Co. (St. Louis, MO, USA)
Sodium phosphate	Sigma-Aldrich Co. (St. Louis, MO, USA)
Tyrosinase from mushroom	Sigma-Aldrich Co. (St. Louis, MO, USA)

2.4 Ingredients for Processes

Salt	Thai Refined Salt Co., Ltd.
Sugar	Mitr Phol Co., Ltd.
Vinegar	Aorsorror Co., Ltd.

2.5 Preparation of fresh sample

About 1 kg of each sample was selected from three markets. The single composite samples were prepared by churning into a homogenized single sample and then mixed with more homogenized sample once again. Following this, supernatants of the solutions were collected into 50 ml by 6,000×g and centrifuged for 15 minutes. The samples were stored in a freezer (−20 °C) for later analysis.

2.6 Preparation of processed sample

2.6.1 White radish

Approximately 1 kg of samples were selected from three markets. The cut samples were washed in distilled water and dried at room temperature. Then, they were chopped by knife and pickled with salt for 30 minutes after which they were cleaned

again and dried and placed into three bottles per outlet for the three representative markets. The preparation syrup for preservation was boiled at 100 °C with 1L of distilled water, 500 g of sugar and 100 g of salt. Next, these were dissolved to create a homogeneous solution which was cooled at room temperature. After that, the samples were mixed with syrup and left to stand at room temperature for 30 days and samples taken every 10 days, so at 10 days, 20 days and 30 days, for analysis.

2.6.2 Garlic

A kilogram of garlic was collected from three markets. Samples were soaked in distilled water overnight then cut and cleaned. Next, they were put into three bottles and the combined syrup was prepared. The bottles were left at room temperature for 30 days and samples taken every 10 days at 10 days, 20 days and 30 days for analysis.

2.6.3 Ginger

A kilogram of ginger selected from three markets was cut, peeled, washed with distilled water and dried at room temperature. After mixing with syrup and being placed into three bottles they were left to stand at room temperature for 30 days and samples taken every 10 days at 10 days, 20 days and 30 days for analysis.

All samples used the same supernatants.

2.7 Preparation of syrup for processes

2.7.1 White radish

1L of distilled water poured into a container was boiled while mixing in 500 g of sugar and 100 g of salt. After the solutes were dissolved, the solution was left to stand at room temperature to pickle samples.

2.7.2 Garlic

100 g of salt, 1L of vinegar and a kilogram of sugar were mixed in 1L boiled distilled water then cooled at room temperature for preserved samples.

2.7.3 Ginger

A kilogram of sugar and 50g of salt were combined in 1L boiled distilled water with 100 ml vinegar. After the ingredients became a homogeneous solution it was left to stand at room temperature for later use.

2.8 Extraction

The extraction method used in this study is the same as that described by Kubola et al. (2011) [53]. A 1g sample was taken and mixed with 10 ml of 80% methanol in a shaker at a speed of 180×g for 2 hours and centrifuged at 1,400g for 20 minutes. The supernatant was decanted for analysis. The pellet was re-extracted under identical conditions and combined supernatants were used to analyze DPPH and ABTS radical scavenging activity, total flavonoid and total phenolic content as well as tyrosinase inhibition activity.

2.9 DPPH radical scavenging activity

The samples were analyzed using the method described by Jo et al. (2012) and Lee et al. (2003) [54, 55]. Each sample of 0.1 ml volume was mixed with 0.9 ml of 0.5 mM DPPH and made into ethanol. Then, the mixture was stirred by vortex and allowed to stand at room temperature for 10 minutes in the dark. The absorbance of the samples was measured at 517 nm against a blank. L-ascorbic acid (1mg/ml) was used as positive control and all tests were carried out in triplicate, and the percentage inhibition of DPPH radical scavenging activity was calculated with the following formula:

% RSA = [1-(absorbance of sample/ absorbance of control] × 100

2.10 ABTS radical scavenging activity

The determination of ABTS was performed as described by Jo et al. (2012) and Muller et al. (1985) [54, 56]. A sample of 0.1 ml was mixed with 0.1 ml of 0.1M potassium phosphate buffer pH 5.0, and 20 µl of 10 mM hydrogen peroxide were mixed and pre-incubated at 37 °C for 5 minutes. After pre-incubation, 30 µl of 1.25 mM ABTS and 30 µl of 1 unit/ml peroxidase were added to the mixture and then it was incubated at 37°C again for 10 minutes. Then, the mixture was measured at 405 nm against a blank. L-ascorbic acid (1mg/ml) was used as positive control and all tests were carried out in triplicate, and the ABTS was calculated with the following formula:

%RSA = [1–(OD of sample/ OD of control)]×100

2.11 Total flavonoid content

Total flavonoid content was determined using the method of Kubola et al. (2011) and Dawanto et al. [57, 58]. 0.5 ml of extract was mixed with 2.25 ml of distilled water with the addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes, 0.3 ml of 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for 5 minutes before 1 ml of 1 M NaOH was added and well mixed. The absorbance was measured at 510 nm. The total flavonoid was expressed as mg rutin for the standard curve.

2.12 Total phenolic content

Total phenolic content was determined using Folin–Ciocalteu reagent following Kubola et al.(2011) and Singleton et al. (1965) [57, 59]. Briefly, 0.3 ml of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent and allowed to stand at room temperature for 5 minutes, after that 2.25 ml of sodium carbonate (60 g/l) solution was added to the mixture and stood at room temperature for 90 minutes, the amount of absorbance was measured at 725 nm. The total phenolic content was expressed as mg gallic acid for the standard curve.

2.13 Tyrosinase inhibition activity

Tyrosinase inhibition activity was measured according to the procedures described by Liu et al. (2012) [60], 100U/ml tyrosinase was prepared in 0.3 ml of 0.1M sodium phosphate buffer (pH 6.8), 0.3 ml of 0.1M sodium phosphate buffer (pH 6.8) and 0.3 ml samples were mixed by vortex and incubated at 37^{o}C for 10 minutes. Then, 0.3 ml. of 15% L-DOPA was added, mixed well and incubated at 37^{o}C for 20 minutes. The OD value was measured at 475 nm against a blank. L-ascorbic acid (1mg/ml) was

used as positive control and all tests were carried out in triplicate, and the percentage of inhibition of tyrosinase activity was calculated with the following formula:

% inhibition activity = [(OD of control - OD of (sample-sample blank))/ OD of control] ×100

2.14 Statistical analysis

Data is presented as mean ± standard deviation (SD) of triplicate examination. Analysis of variance using SPSS version 17 software was used for comparisons with $P < 0.05$. Individual differences were analyzed using LSD test.

CHAPTER III

RESULTS

3.1 DPPH radical scavenging activity

Table 1 The DPPH radical scavenging activity of fresh and processed white radish, garlic and ginger

Sample	% of DPPH radical scavenging activity per 100 g			
	Fresh	Processed vegetables		
		10 days	20 days	30 days
White radish	25.72 ± 7.73[a]	80.18 ± 9.15[a]	84.99 ± 11.39[a]	54.13 ± 9.31[a]
Garlic	25.53 ±4.99[b]	31.78 ± 3.16[b]	56.67 ± 5.03[b]	55.62 ± 7.16[b]
Ginger	80.90 ± 5.70[c]	59.68 ± 10.68[c]	72.83 ± 10.67[c]	63.85 ± 6.94[c]

[a,b] The number is significantly different when compared with the fresh samples ($P < 0.05$).

[c] The number is not significantly different when compared with the fresh samples ($P > 0.05$).

Firstly, the samples were preserved before the test activity. White radish, garlic and ginger were mixed with syrup made from sugar, salt and water. After that, they were left to stand at room temperature for 30 days and samples taken every 10 days. The samples were extracted by methanol. Two parts were analyzed including fresh and processed samples. DPPH was used to analyze activity. The results show DPPH of white radish, garlic, and ginger were 25.72%, 25.53%, and 80.90% respectively in fresh compared with the processed samples at 10, 20 and 30 days. In processed samples, activity at 10 days were 80.18%, 31.78%, and 59.68% while at 20 days they were 84.99%, 56.67%, and 72.83% and at 30 days, 54.13%, 55.62% and 63.85%, respectively (Table 1), (Figure 12). The fresh and processed plants showed significant differences ($P < 0.05$) but fresh and processed ginger did not differ significantly ($P > 0.05$). As a result, comparison of fresh and processed plants showed statistical

differences. While the fresh white radish and garlic had lower antioxidant efficiency than processed, fresh ginger had more antioxidant activity than the processed ginger. In contrast, fresh ginger had higher antioxidant efficiency than processed due to degeneration of activity. However, both fresh and processed samples showed decreased activity whereas they can be consumed with benefit from the phytochemical content in the plants. In the end, the preserved food still has the best activity for consumption.

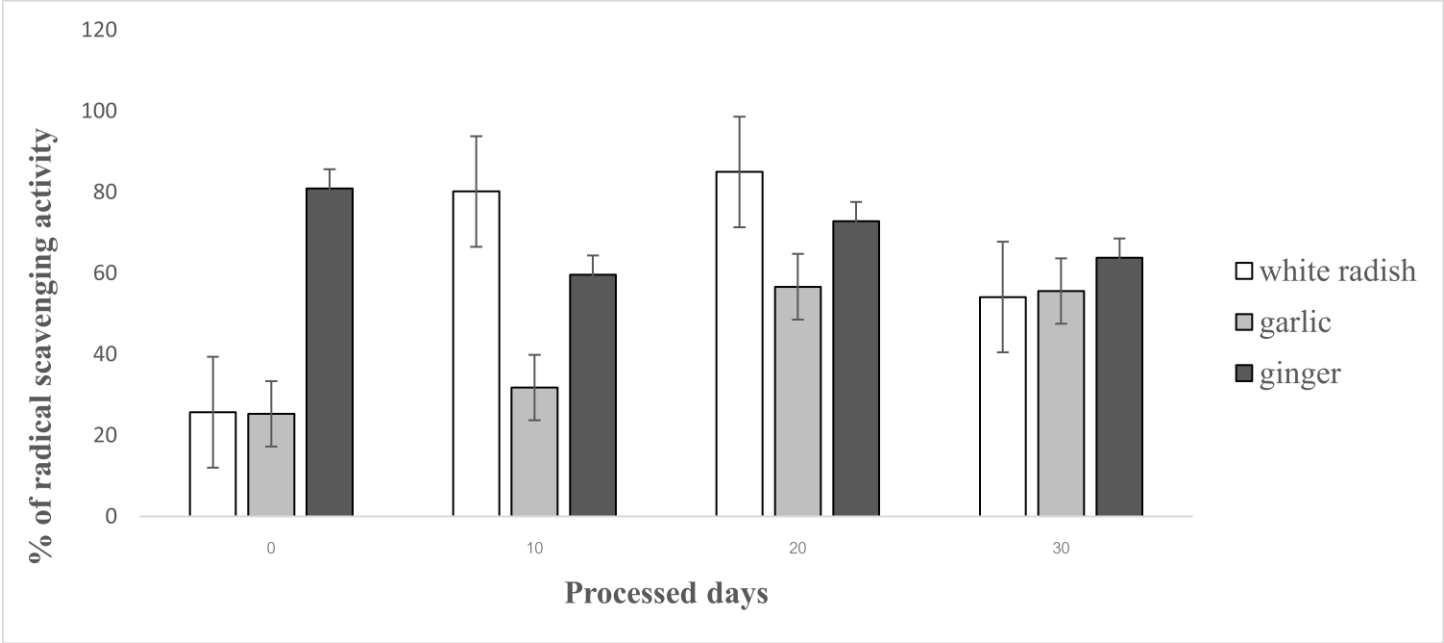

Figure 12 % of DPPH radical scavenging activity per 100 g of white radish, garlic and ginger at 0, 10, 20 and 30 days respectively.

3.2 ABTS radical scavenging activity

Table 2 The ABTS radical scavenging activity of fresh and processed white radish, garlic and ginger

Sample	% of ABTS radical scavenging activity per 100 g			
	Fresh	Processed vegetables		
		10 days	20 days	30 days
White radish	42.20 ± 12.73[a]	38.49 ± 6.62[a]	84.28 ± 6.26[a]	48.33 ± 3.22[a]
Garlic	48.30 ± 8.48[b]	96.63 ± 4.00[b]	52.62 ± 3.73[b]	19.41 ± 5.31[b]
Ginger	38.09 ± 5.95[c]	109.39 ± 4.34[c]	82.73 ± 8.10[c]	42.14 ± 3.89[c]

[a,b,c] The number is significantly different when compared with the fresh samples ($P < 0.05$).

The experiment used 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay. This assay used hydrogen peroxide with peroxidase enzyme as substrate. The reaction expresses a blue/green color and it was measured at 405 nm. First, fresh white radish, garlic, and ginger were 42.20%, 48.30%, 38.09% respectively. At 10 days, they were 38.49%, 96.63%, and 109.39% respectively and at 20 days, they were 84.28%, 52.62%, and 82.73% respectively. The last day of pickling, they were 48.33%, 19.41%, and 42.14% respectively. The highest level of ABTS of the processed white radish, garlic and ginger were 84.28%, 96.63% and 109.39%, respectively, compared with the fresh forms. Both of them had significant differences ($P < 0.05$) (Table 2), (Figure 13). Secondly, the trend of ABTS was similar to the DPPH indicating that good antioxidant properties presented in this study might be due to the amount of total phenolic and flavonoid content (Figure 14). Thus, these results suggest that the methanolic extracts have a scavenging effect on radical generation helping prevention or improvement in oxidative damage. In the same way, the fresh and processed samples were similar. Lastly, the ABTS test indicates the samples' radical scavenging activity

demonstrating that these foods can be selected for consumption either fresh or preserved.

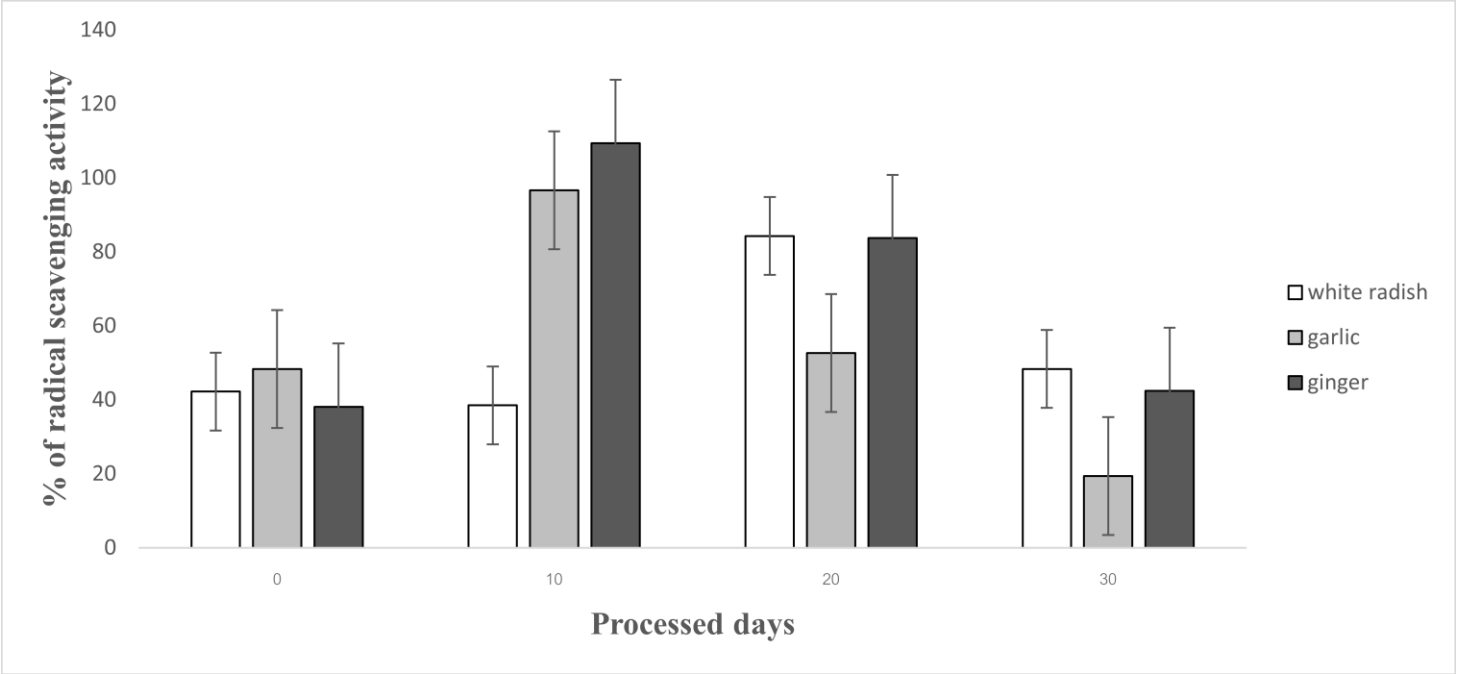

Figure 13 % of ABTS radical scavenging activity per 100 g of white radish, garlic and ginger at 0, 10, 20 and 30 days respectively.

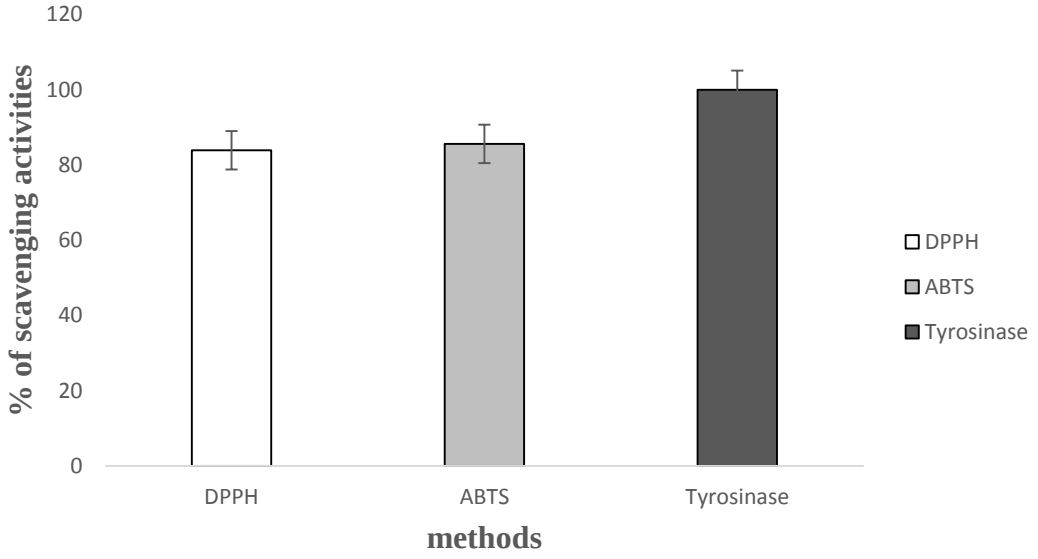

Figure 14 Percentage of scavenging activities of vitamin C (1mg/ml) as positive control

3.3 Total flavonoid content

Table 3 The total flavonoid content of fresh and processed white radish, garlic and ginger

Sample	Total flavonoid contents per 100 g			
	Fresh	Processed vegetables		
		10 days	20 days	30 days
White radish	1.84 ± 0.48^{a}	0.50 ± 0.25^{a}	0.33 ± 0.11^{a}	0.23 ± 0.07^{a}
Garlic	8.85 ± 1.08^{b}	2.26 ± 0.88^{b}	0.70 ± 0.34^{b}	0.10 ± 0.02^{b}
Ginger	6.47 ± 0.88^{c}	2.03 ± 0.58^{c}	0.16 ± 0.02^{c}	0.04 ± 0.01^{c}

^{a,b,c} The number is significantly different when compared with the fresh samples ($P < 0.05$).

Flavonoids are complex structures belonging to the polyphenol distribution. It has many functions in plants. These are defensive compounds used against insects, several pathogens and function as natural antioxidants [45]. First, the total flavonoid content (TFC) in processed vegetables compared with fresh samples is shown in Table 3. These were greater in the fresh than in processed samples at 10, 20, and 30 days.

There were significant differences ($P < 0.05$) between the fresh and processed samples. The highest total flavonoid content was found in fresh and processed garlic. As time passed, activity declined in value continuously. Fresh white radish, garlic and ginger have higher activity than processed samples due to activity of phytochemicals. Processed samples showed a decrease at 10, 20 and 30 days respectively because they were preserved in salt, sugar and vinegar (Figure 15). The activity of flavonoid decreased due to inactive or degenerate flavonoids. The flavonoid and total phenolic content decreased in processed samples. This result is similar to that of total phenolic content. However, the processed vegetables are decreased both content and they still showed antioxidant properties.

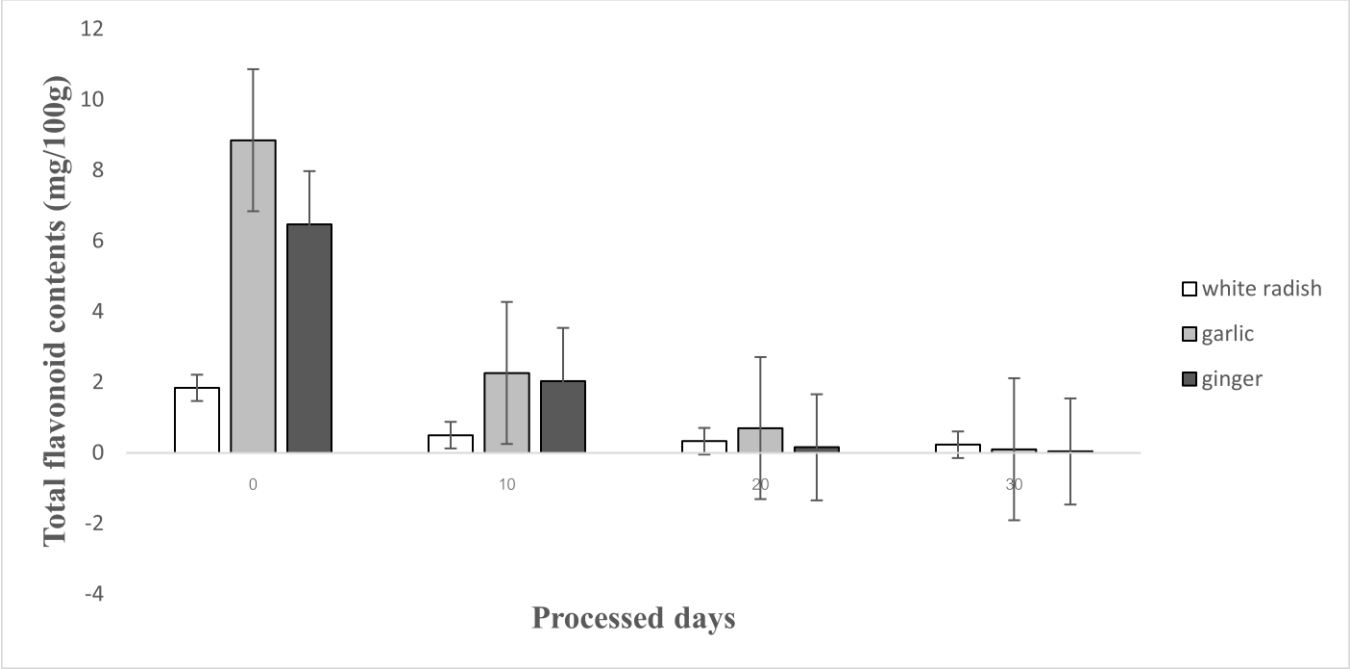

Figure 15 Total flavonoid contents per 100 g of white radish, garlic and ginger at 0, 10, 20 and 30 days respectively.

3.4 Total phenolic content

Table 4 The total phenolic content of fresh and processed white radish, garlic and ginger

Sample	Total phenolic contents per 100 g			
	Fresh	Processed vegetables		
		10 days	20 days	30 days
White radish	0.53 ± 0.07^{a}	0.16 ± 0.13^{a}	0.13 ± 0.02^{a}	0.09 ± 0.02^{a}
Garlic	1.76 ± 0.33^{b}	19.48 ± 5.51^{b}	0.07 ± 0.01^{b}	0.05 ± 0.00^{b}
Ginger	1.63 ± 0.21^{c}	3.23 ± 1.91^{c}	0.03 ± 0.00^{c}	0.03 ± 0.00^{c}

^{a,b,c} The number is significantly different when compared with the fresh samples ($P < 0.05$).

Firstly, total phenolic content (TPC) is shown in Table 4. The result of TPC analysis of three fresh and processed vegetable samples decreased from 0.53 to 0.09 in white radish, while both garlic and ginger increased at 10 days and decreased at 20 and 30 days respectively (Figure 16). The TPC in fresh and processed samples showed statistically significant differences ($P < 0.05$). Even though the TPC of white radish was lower in processed samples it still supported antioxidant activity and tyrosinase inhibition. Similarly, the highest levels of TPC of garlic and ginger were at 10 days, but lower at 20 and 30 days compared to fresh samples showing that ingredients preserved in syrup may conserve total phenolic content at 10 days. This result is similar to flavonoids. Phenolic content decreased as flavonoid in processed samples. However, this experiment used Folin–Ciocalteu reagent and calculated phenolic content with a Gallic acid standard curve. Finally, it can be concluded that total phenolic content of preserved samples supported antioxidants.

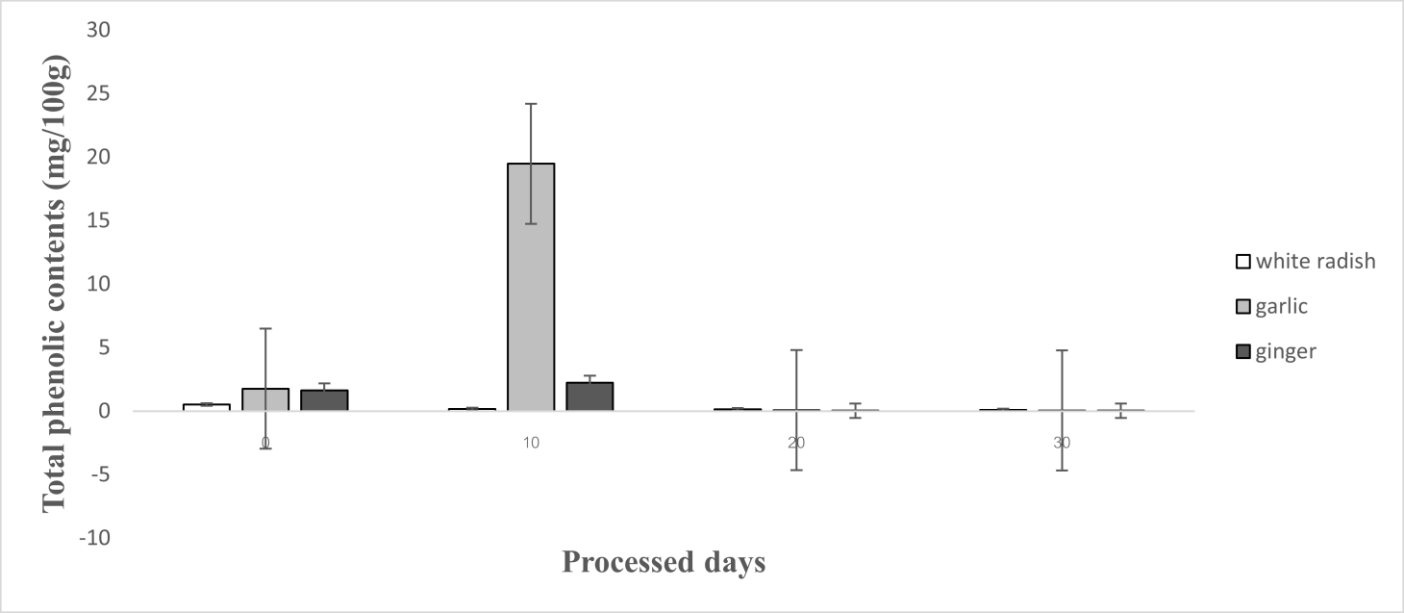

Figure 16 Total phenolic contents per 100 g of white radish, garlic and ginger at 0, 10, 20 and 30 days respectively.

3.5 Tyrosinase inhibition activity

Table 5 Tyrosinase inhibition activity of fresh and processed white radish, garlic and ginger

Sample	% of Tyrosinase inhibition activity per 100 g			
	Fresh	Processed vegetables		
		10 days	20 days	30 days
White radish	129.55 ± 15.09[a]	75.86 ± 11.77[a]	76.49 ± 14.17[a]	30.53 ± 7.37[a]
Garlic	117.74 ± 17.02[b]	90.88 ± 16.58[b]	151.42 ± 15.12[b]	93.67 ± 17.42[b]
Ginger	363.71 ± 58.73[c]	128.10 ± 14.53[c]	73.90± 14.93[c]	129.37 ± 37.86[c]

[a,b,c] This number is significantly different compared with the fresh samples ($P < 0.05$).

The highest level of tyrosinase inhibition in fresh samples of ginger, white radish and garlic were 129.55%, 117.74%, and 363.71% respectively. At 10 days, the processed ginger, garlic, and white radish had 128.10%, 90.88%, and 75.86%, tyrosinase inhibition respectively while at 20 days, they had 151.42%, 76.49%, and 73.90%, and finally at 30 days, 129.37%, 93.67%, and 30.53%, respectively. Ginger had the highest percentage tyrosinase inhibition in fresh samples, greater than both white radish and garlic because both phenolic and flavonoid content was effectively preserved. In processed samples, garlic had higher tyrosinase inhibition than white radish and ginger (Table 5), (Figure 17). This study found that the best level of tyrosinase inhibition was in fresh samples but there were statistically significant differences ($P < 0.05$) in fresh as well as processed samples showing that although they were preserved for 10, 20, and 30 days and they still possessed tyrosinase inhibition.

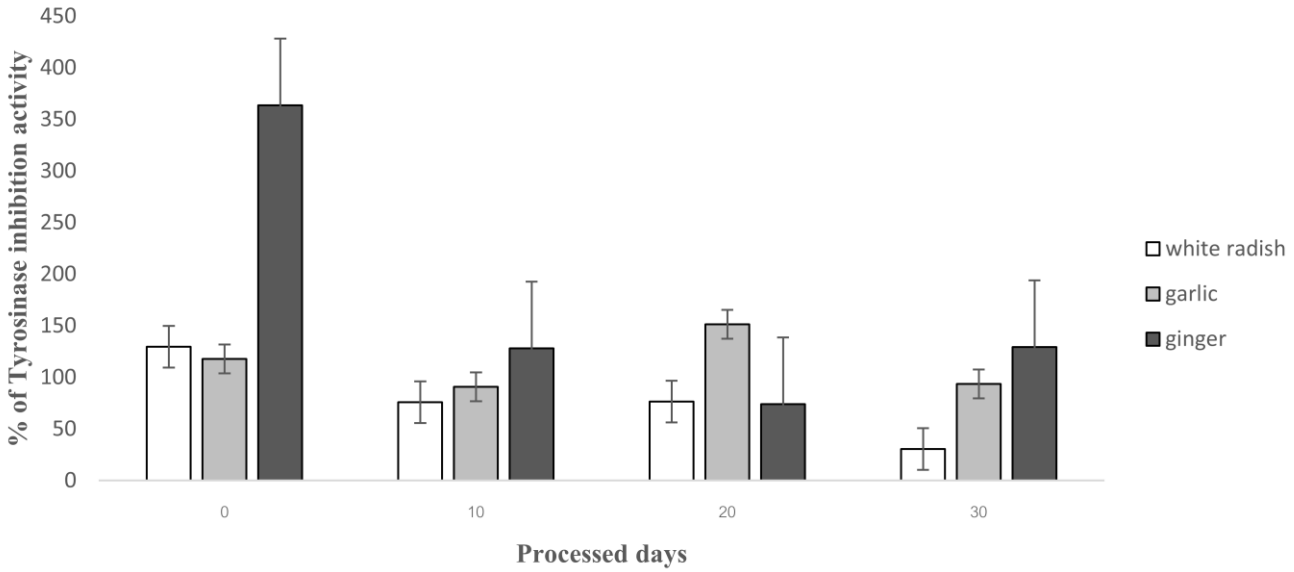

Figure 17 Percentage of tyrosinase inhibition activity per 100 g of white radish, garlic and ginger at 0, 10, 20 and 30 days respectively.

CHAPTER IV

DISCUSSIONS

4.1 DPPH radical scavenging activity

This experiment used the DPPH method to test antioxidant activity in fresh and processed samples of white radish, ginger and garlic using vitamin C as a positive control. The results show differences between fresh and processed vegetable samples. White radish, garlic and ginger showed differences ($P < 0.05$) at 10, 20 and 30 days due to short-term processes. Analysis found statistical differences with all tests (fresh, 10, 20, 30 days) which were compared with 1mg/ml vitamin C concentration as a positive control and found 83.87% more activity than in fresh white radish samples at 10 and 30 days and likewise for both fresh and processed garlic and ginger. The white radish and garlic showed increased activity because phytochemicals may dissolve in solution. Thus, this method of preservation can reduce free radicals. The greatest antioxidant activity found was in fresh ginger as well as processed white radish. However, either fresh or pickling and both still preserved antioxidant properties. This study compared processed with fresh samples of white radish, garlic and ginger to determine the free radical scavenging activity ability in these favorite additives. Although red radish pigment had almost the same antioxidative activity as BHT at the same concentration the inhibition percentage could reach more than 93% by the addition of 0.01% pigment but it was found that white radish could have the same level of antioxidative activity [34]. Rahmam et al. [47] studied fresh garlic extract ,presumed to be a very strong antioxidant, by DPPH scavenging methods and found a high level of antioxidant

activity. Thus, fresh or processed vegetables can be a rich source of antioxidants. Ghasemzadeh et al. [50] studied ginger's bioactive constituents using DPPH assay and measured the total amount of phenolic and flavonoid compounds in rhizomes and stems. The relationship between total phenolic and flavonoid content and antioxidant activity in ginger is believed to account for their scavenger properties. Consequently, this study showed the percentage of activity of antioxidants that could reduce free radicals as supported by Ghasemzadeh et al. [50].

Antioxidants, as protectors of our cells, can prevent chronic diseases from free radicals, and promote good health. Antioxidants can be consumed from healthy foods or supplements like vitamin A, vitamin C, vitamin E, anthocyanin, beta-carotene, lycopene, phenolic compounds and more. Finally, both natural and synthetic antioxidants have been shown to reduce cell damage caused by free radicals and promote normal function of cells in our body.

4.2 ABTS radical scavenging activity

The effect of the ABTS activity test was similar to the DPPH activity test in that they were highly antioxidant due to bioactive compounds in our plant samples (e.g. phenolic compounds). There are several general methods to test for antioxidants as DPPH, ABTS, ORAC among others. The present study explains the association between ABTS and DPPH activity tests and their differences. The percentage of ABTS activity in fresh samples was significantly higher than by DPPH for white radish and garlic. There were many differences between 10, 20, and 30 days of exposure to solution in processed samples due to the solubility and degradation of bioactive

compounds. However, the study of Maheswari et al. [41] found that white radish had similar levels of activity by the ABTS test compared to DPPH. Gorinstein et al. [37] showed that the amount of ABTS in garlic was 27.16 - 43.73 µM Trolox Equivalent/g which was comparable to Leelarungrayub et al. [61].

4.3 Total flavonoid content

Flavonoid, a phenolic compound, was found in higher quantities in garlic and ginger than in white radish. On the other hand, Jakmatakul et al. [33] reported *Raphanus sativus* was extracted in 0.33µg (0.00033 mg) of methanol to get quercetin equivalent. Bozin et al. [35] reported that decreased flavonoid content was most probably caused by increased sulphur compounds and terpenoid substances present in the essential oil of mature garlic bulbs. Ghasemzadeh et al. [50] studied the level of flavonoid in different solvent extracts (methanol, acetone and chloroform) of the leaves, rhizomes and stems of two varieties of ginger as shown by TFC and found that the TFC increased in methanolic extract. Moreover, the TFC of the leaves was greater than in the rhizomes. In the present study, a positive relationship was observed between antioxidant activity and flavonoid content. Many researchers have shown high total flavonoid content and increased antioxidant activity and there was a linear correlation between total flavonoid content and antioxidant activity [40].

4.4 Total phenolic content

The highest total phenolic content of both fresh and processed samples was found in garlic with white radish and ginger having less. The quantity in

processed samples decreased at 10, 20 and 30 days. Phenolic content was highest in garlic samples at 10 days which is consistent with the previous research. Results of this present study indicate that phenolic content decreased in processed garlic samples at 10, 20, and 30 days respectively, because phenolic compounds may be soluble or their activity degraded over time. In contrast, phenolic compounds in garlic increased at 10 days due to solubility in solution. The other previous research has shown that phenolic compounds were important components of the garlic. Chan et al. [26] studied total phenolic content in leaves of 26 species of ginger, 14 of which had significantly higher TPC in leaves than in rhizomes. Stoilova et al. [39] studied the total phenols of the alcohol extract and found they reached 870.1 mg/g dry extract but the differences in the present study may be due to the use of local species from representative markets in Bangkok [26],[39]. The content of phenolic acids in the roots of the radish was much less than in the leaves due to the lesser phenolic content of the root [34]. Yara et al. [36] studied ready-to-eat garlic products and found that there was a significant decrease in the total phenolic content due to the length of storage, being almost halfway to expiration date. This relationship was confirmed with the processed vegetables in this study. Charanjit et al. [2] reported the highest TPC of ginger was found in fresh samples. Therefore, the result of this present study was similar to the other articles.

4.5 Tyrosinase inhibition activity

The highest level of tyrosinase inhibition in fresh samples were found in ginger, white radish and garlic respectively. In the processed samples it was garlic, ginger and

white radish respectively. Kamkaen et al. [1] reported *in vitro* tyrosinase inhibition (78.98% and 88%, respectively) compared to kojic acid (65% inhibition in methanol and 82% inhibition in 50% propylene glycol) which were different because of procedure on extraction. The difference between fresh and processed samples related to quality such as freshness, storage at room temperature, humidity, moisture, etc. The highest of all inhibition activity was significantly higher than the vitamin C as positive control. Both fresh and processed white radish showed decreased activity respectively. Garlic had decreased activity at 10 days while activity increased at 20 and 30 days. Ginger had decreased activity at 10 and 20 days whereas it was increased at 30 days. The garlic showed increased activity at 20 days due to phytochemicals increased solubility and degeneration at 30 days. In the same way, ginger may be degraded at 20 days and have increased activity at 30 days because phytochemicals may have increased in solution. Other research has studied tyrosinase inhibition in the target plants for instance, Jakmatakul et al. [33] reported that white radish appeared to be a good candidate for application due to its ability to inhibit tyrosinase and to scavenge several types of reactive oxygen species. Kamkaen et al. [1] also studied Chinese radish and found that it contained antityrosinase activity. Garlic was examined for its antioxidant and anti-tyrosinase activity and found to be a tyrosinase inhibitor. Chan et al. [26], studied both leaves and rhizomes of ginger and reported very strong tyrosinase inhibition activity. Besides showing promising tyrosinase inhibition ability, leaves of these species also had high antioxidant activity. Lastly, tyrosinase inhibition is related to antioxidant activity due to phytochemical content which promotes good health through anti-inflammatory effects, reduction of free radicals, and prevention of NCDs.

CHAPTER V

CONCLUSION

White radish, garlic, and ginger are commonly consumed vegetables in Thailand known to contain varying degrees of phytochemicals (e.g. flavonoid and phenolic compounds). In this study, the antioxidant, flavonoid, phenolic content and tyrosinase inhibition ability of the fresh and processed forms of the three plant foods were compared. Plants high in phytochemicals are a source of natural antioxidants in the form of bioactive compounds possessing antioxidant properties. The present study obtained samples of the three fresh vegetables for analysis and found they had high levels of phenolic and flavonoid content, antioxidant activity and tyrosinase inhibition ability. Therefore, they are good sources of phytochemicals which could offer enormous opportunities for the food industry. The data of samples from three representative markets in Bangkok demonstrate some differences in antioxidant content between fresh and processed forms. The fresh samples had greater activity than the processed forms while the latter still contained significant antioxidant activity thus still possessing health benefits. The best antioxidant and tyrosinase content levels were found in the fresh plants while the best total flavonoid and phenolic content were found in samples preserved for 10 days in solution. Finally the information gained from this study will be used for the selection of raw vegetables for consumption to promote health or prevent non-communicable diseases.

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APPENDIX

APPENDIX A

Figures of processed white radish

1. 0 Day of processed white radishes

2. 10 Days of processed white radishes

3. 20 Days of processed white radishes

4. 30 Days of processed white radishes

APPENDIX B

Figures of processed garlic

1. 0 Day of processed garlics

2. 10 Days of processed garlics

3. 20 Days of processed garlics

4. 30 Days of processed garlics

APPENDIX C

Figures of processed ginger

1. 0 Day of processed gingers

2. 10 Days of processed gingers

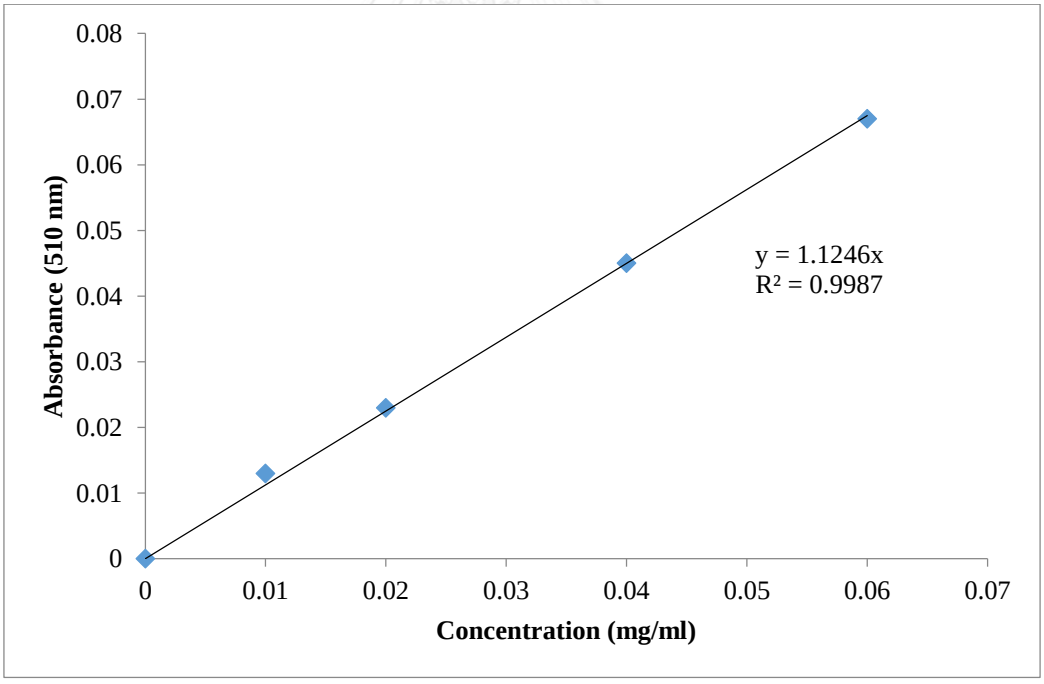
3. 20 Days of processed gingers

4. 30 Days of processed gingers

APPENDIX D

Standard curve for rutin

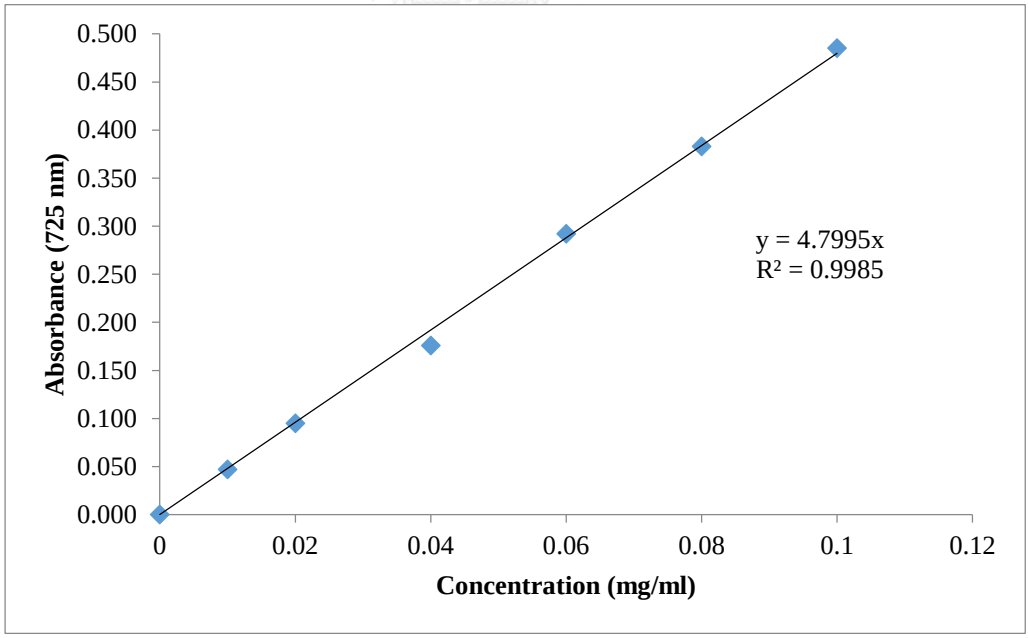
Concentration (mg/ml)	Absorbance
0.08	0.043
0.06	0.067
0.04	0.045
0.02	0.023
0.01	0.013



APPENDIX D

Standard curve for gallic acid

Concentration (mg/ml)	Absorbance
0	0
0.1	0.485
0.08	0.383
0.06	0.292
0.04	0.176
0.02	0.095
0.01	0.047



APPENDIX E

Preparation of syrup for processes

1. White radish

Water	1000	ml
Sugar	500	g
Salt	100	g

2. Garlic

Water	1000	ml
Sugar	1	kg
Salt	100	g
Vinegar	1000	ml

3. Ginger

Water	1000	ml
Sugar	1	kg
Salt	50	g
Vinegar	100	ml

APPENDIX F

Preparation of chemicals and reagents

1.	0.5 mM DPPH		
	1,1-diphenyl-2-picrylhydrazyl	0.0394	g
	Ethyl alcohol absolute	200	ml
2.	Acetic acid buffer pH 5.5		
	Sodium acetate	0.41	g
	Distilled water	500	ml
	Glacial acetic acid for pH adjustment		
3.	10 mM H_2O_2		
	H_2O_2	0.56	ml
	Distilled water	99.44	ml
4.	0.05 M phosphate-citrate buffer pH 5.0		
	0.2 M Na_2HPO_4	25.7	ml
	0.1 M $C_6H_8O_7$. H_2O	24.3	ml
	Distilled water	50	ml
5.	0.1 M Potassium Phosphate buffer pH 5.0		
	Distilled water	1000	ml
	K_2HPO_4	3.657	g
	KH_2PO_4	10.751	g

6. 0.1 M Sodium phosphate buffer pH 6.8

NaH_2PO_4	13.46	g
Na_2HPO_4	1.95	g
Distilled water	1000	ml

7. 1 M NaOH

NaOH	4	g
Distilled water	100	ml

8. 0.15% L-DOPA in 0.1 M Sodium phosphate buffer pH 6.8

L-3,4-dihydroxyphenylalanine	0.15	g
0.1 M Sodium phosphate buffer pH 6.8	100	ml

9. 5% (w/v) $NaNO_2$

$NaNO_2$	5	g
Distilled water	100	ml

10. 10% $AlCl_3$ (w/v)

$AlCl_3$	10	g
Distilled water	100	ml

11. 1U/ml Peroxidase

Peroxidase	1000	U
0.05 M phosphate-citrate buffer pH 5.0	1000	ml

12. 100U/ml Tyrosinase in 0.1 M Sodium phosphate buffer pH 6.8

Tyrosinase from mushroom	25000	U
0.1 M Sodium phosphate buffer pH 6.8	250	ml

13. 1 mg/ml Ascorbic acid

Ascorbic acid	10	mg
Distilled water	10	ml

14. 60g/L Sodium carbonate

Na_2CO_3	30	g
Distilled water	500	ml

15. ABTS

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)	0.342	g
0.05 M phosphate-citrate buffer pH 5.0	500	ml

16. Folin–Ciocalteu’s reagent

Folin–Ciocalteu’s reagent	10	ml
Distilled water	90	ml

VITA

My name is Apisit Somman. I was born in 1989 in Lampang province and graduated Bachelor of Science (Agriculture) from King Mongkut's Institute of Technology Ladkrabang in 2011. Now, I have studied Master of Science (Biotechnology), Faculty of Science, Chulalongkorn University since 2011. I joined oral presentation subject : Comparison of antioxidant activity and tyrosinase inhibition in fresh white radish, garlic and ginger in the International Conference on Global Trends in Academic Research (ICMRP-December 17-18, 2014) at Kuala Lumpur, Malaysia. Finally, some section of a thesis has been accepted for publication in Journal of Food Measurement & Characterization.