OVERVIEW OF PLANT-BASED NATURAL ANTIOXIDANTS AND EFFECT OF THERMAL DECOMPOSITION

by

NEHA MAHESHWARI

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Major Professor
J. Scott Smith

Abstract

The popularity of convenience foods and consumer awareness have indirectly increased the demand for novel and naturally occurring compounds that can delay oxidative deterioration and maintain nutritional quality of foods. Natural antioxidants from certain herbs and spices such as rosmarinic acid from rosemary, thymol from oregano, eugenol from clove, curcumin from turmeric are rich in polyphenolic compounds that provide long term oxidative stability as well as offer additional health benefits. High antioxidative capacity of herbs and spices phenolics could potentially substitute synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), octyl gallate, and *tert*-butylated hydroquinone (TBHQ) in the food system. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are thermally unstable and decompose at higher temperatures. However, widely used cooking methods such as baking, frying, boiling, and roasting use high thermal temperature that can chemically degrade herbs and spices and diminish their antioxidative capacity, but they have been little studied. In this context, this review deals with the need of natural antioxidants, spices and herbs as natural antioxidants, their origin, chemical composition, pharmacological, and antioxidant properties. Moreover, the impact of temperature on total antioxidant capacity (TAC) of various herbs and spices such as cinnamon, clove, nutmeg, mace, oregano, rosemary, sage, and turmeric is highlighted. Different antioxidant assays are also studied and this approach revealed that there is a clear correlation between total phenolic content (TPC) and TAC of herbs and spices and specific phenolic compounds are responsible for the antioxidative capacity of particular herb and spice. These findings identified the optimum cooking temperature-time combination which results in the highest retention of antioxidative capacity and assures higher quality of food for the maintenance of human health.

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Acknowledgements

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K-State has given me friendships that I will cherish all of my life. I would like to thank Vijaya Iyer, Ranjni Chand, Rochni Chand, Phil Schumm, Trish and Gill Stevens, David Stuckenschmidt, and K-State alumni Michelle and Nicholle Haupt for their friendship and making the experience at K-State a memorable one. The unwavering friendship of my friends back in India and their confidence in me has helped me through thick and thin. I owe a big thank you to my best friend, Capt. Shashi Kanth, for his love, understanding, and continuous support. Last but not the least, I especially thank my mom. She sacrificed her life for me and provided unconditional love and care. I would like to wholeheartedly thank my family for their faith in me and their constant source of encouragement and support despite the physical distance between the continents. I love them so much, and I would not have made it this far without them.

Dedication

I dedicate this report to
my mother Sneha Maheshwari,
my family, and
my best friend Capt. Shashi Kanth
for their constant support and unconditional love.
I love you all dearly.

Chapter 1 - Literature Review

Introduction

Food lipids, a diverse group of organic compounds that includes fats, oils, waxes, steroids, and phospholipids are a fundamental part of our diet. As nutrients, food lipids, serve as an important source of energy, provide essential fatty acids such as omega-3-fatty acid, and act as solvent and absorption vehicle for fat-soluble vitamins. In addition, lipids contribute desirable qualities such as flavor, texture, structure, smoothness, juiciness, heat transfer, aroma, and lubricity to food (Kinsella 1988; O'Keefe 2002).

However, lipids are one of the most chemically unstable food components. When foods are exposed to air, light, or metal ions, lipids readily undergo auto-oxidation and produce primary and secondary volatile by-products that cause quality deterioration including off-flavor, rancid odor, discoloration, as well as produce some toxic compounds (hydroperoxides, epoxides, oxycholestrols, dimes, etc.), and ultimately make that food unacceptable to consumers. Lipid oxidation also influences the nutritional quality by damaging proteins, blocking essential amino acids, oxidizing amino acids, and forming protein radicals (Labuja and Dugan 1971; Zheng and Wang 2001; Bandoniene and others 2002; Kołakowska 2003; Suhaj 2006; Hossain and others 2008; Tavassoli and Djomeh 2011).

Mechanism of Auto-oxidation

The auto-oxidation process consists of three phases: initiation, the formation of free radicals; propagation, the free-radical chain reaction; and termination, the formation of non-radical products (Figure 1:1; Shahidi 1997). The initiation process involves the abstraction of a hydrogen atom from a lipid to form a free radical (R•, H•, and ROO•) in the presence of reactive oxygen species, and these radicals propagate and start a chain reaction because the unstability and try to capture the required electron to gain stability. ROOH is a hydro peroxide, one of the major initial oxidation products that quickly decompose to form secondary compounds in termination process such as hexanal, pentanal, and malonaldehyde, which are responsible for off-flavors and odors. These acids, alcohols, aldehydes, carbonyls, and ketones further decompose and polymerize and form toxic compounds (Cuppett and others 1997; Young and Woodside 2001).

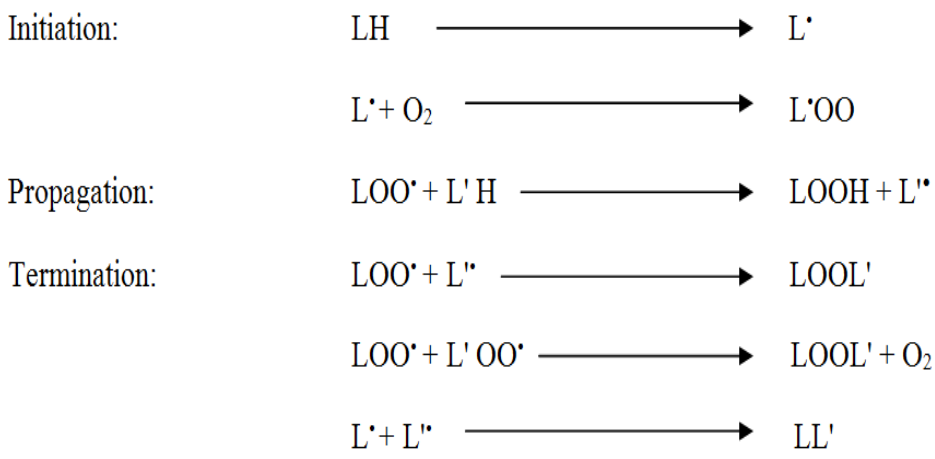


Figure 1:1-Process of lipid auto-oxidation (Cuppett and others 1997)

Health Implications of Oxidative Stress

Oxidative stress, which is caused by free radicals, is also considered to cause various diseases such as diabetes, cardiovascular diseases, cancer, and aging issues (Cardiodrops 2011; Figure 1:2). Metabolism by-products of lipid auto-oxidation such as superoxide, hydrogen peroxide, and hydroxyl radicals also attack biological molecules and leads to cell or tissue damage (Young and Woodside 2001; Kritchevsky 2002).

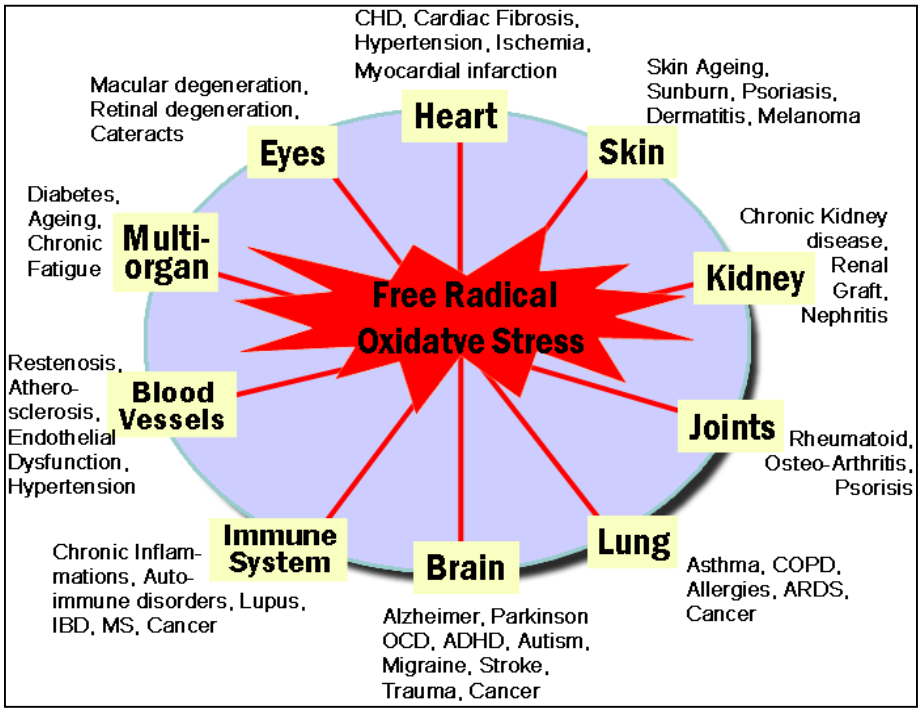


Figure 1:2-Free radical oxidative stress diseases (Cardiodrops 2011)

Antioxidants

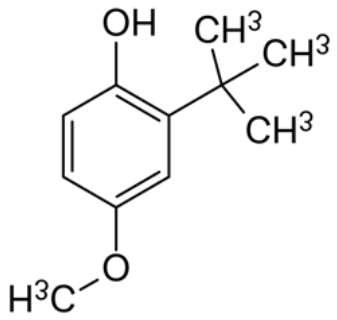
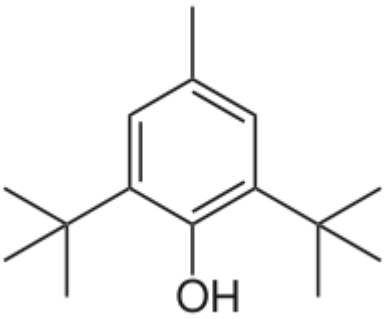
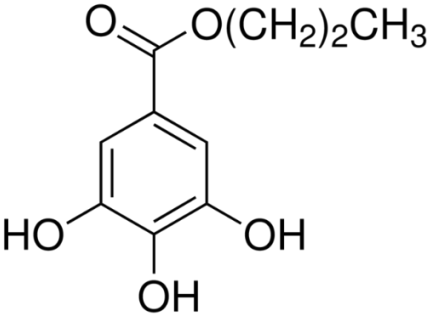
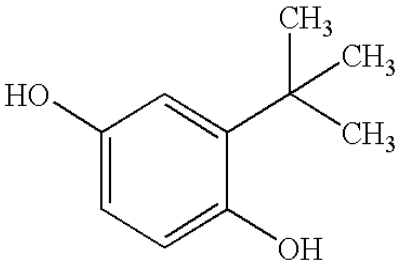
An antioxidant is a substance that helps to inhibit or delay the oxidation process in the body or in the food (Halliwell 1990). Antioxidants can be classified into two major groups on the basis of mechanism: 1. Primary (type 1 or chain-breaking) antioxidants which interfere with one or more of the propagation steps and generate more stable non radical products by reacting with lipid and peroxy radicals. Primary antioxidants terminate the destructive chain reaction by neutralizing the free radicals by four mechanisms: hydrogen donation; electron donation; addition of the component within the aromatic ring of the antioxidant; or formation of a complex between the oxidizing compound with the antioxidant; and 2. Preventive (type 2 or secondary) antioxidants which retard the oxidation process by reducing the rate of chain initiation but they do not convert free radicals to stable products. It includes superoxide dismutase, catalase, and glutathione peroxidase. These antioxidants generally act as chelator, oxygen scavengers, and reducing agents (Kołakowska 2003). Antioxidants have been widely used in dietary supplement is the most efficient and practical way to inhibit or decrease lipid oxidation and to prevent degenerative diseases. Antioxidants not only increase the shelf-life of food products but also reduce the nutritional loss, and raw material waste (Zheng and Wang 2001). The most commonly used primary antioxidants in foods are synthetic antioxidants. However, some natural antioxidants also act as primary antioxidants such as tocopherols and carotenoids.

Synthetic Antioxidants

Synthetic antioxidants are intentionally added to prevent the oxidation of lipids in food. The most commonly used synthetic antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), octyl gallate, and *tert*-butylated hydroquinone (TBHQ). Among these, BHA and BHT are unstable, volatile and decompose at high temperature, while PG is relatively stable because of its low volatility (Chang and others 1977; Hamama and Nawar 1991; Augustyniak and others 2010). TBHQ is considered as the best antioxidant for fried food because of high-temperature stability and effectiveness. Table 1:1 summarizes the functionality and structure of synthetic antioxidants (Chang and others 1977). However, the metabolites of BHA and BHT contribute to possibly toxic effects on living organisms (Chang and others 1977; Kahl and Kappus 1993; Zheng and Wang 2001). Therefore,

potential toxicity and general awareness led to a decreased use of synthetic antioxidants, and increased attention towards natural antioxidants.

Table 1:1-Functionality and structure of synthetic antioxidants (Reische and others 2002)

Antioxidant	**Functionality**	**Structure**
Butylated hydroxyanisol (BHA)	Volatile, distillable. Most effective in animal fats. Used in packaging material. Synergistic with other antioxidants. Good carry-through in baked and fried products.	
Butylated hydroxytoluene (BHT)	Volatile, distillable. Most effective in animal fats. Slight phenol odor. Synergistic with other antioxidants. Decomposes at frying temperatures. Less carry-through in baked and fried products than BHA.	
Propyl gallate (PG)	More effective in vegetable oils than BHA and BHT. Always used in combination with a chelator. Poor carry-through properties in baking, but good carry-through in frying. Synergistic with other antioxidants. Less soluble in fats than BHA and BHT.	
Tertiary butylhydroquinone (TBHQ)	Excellent antioxidant in vegetable oils. Does not discolor in presence of metals. Little odor.	

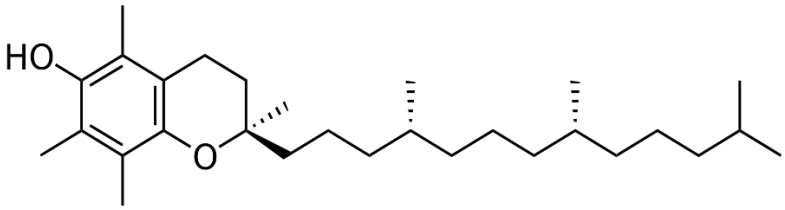
Natural Antioxidants

Natural antioxidants contain tocopherols, vitamin C, carotenoids, and phenolic compounds, which act against the reactive species in the body and helps the body to maintain normal functions (Figure 1:3). They also act as anti-mutagenic, anti-viral, anti-bacterial, anti-inflammatory, and anti-microbial agents. Thus, in recent years, plant extracts have captured the attention of researchers (Miura and others 2002; Jayasinghe and others 2003; Lee and others 2005; Hossain and others 2008; Tavassoli and Djomeh 2011).

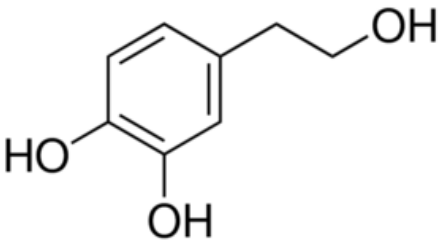
Plant-based natural antioxidants can found from many foods including fruits, vegetables, oilseeds, cereals, legumes, herbs, and spices. Antioxidant activity of natural antioxidants is due to their secondary metabolites which act as bioactive compounds such as beta-carotene, lutein, lycopene, selenium, vitamin A, vitamin C, vitamin E, catechins, flavonoids, and phenolic compounds (Shahidi 1997). Table 1:2 presents the natural food sources of some antioxidants.

Table 1:2-Natural food sources of some antioxidants (Shahidi 1997)

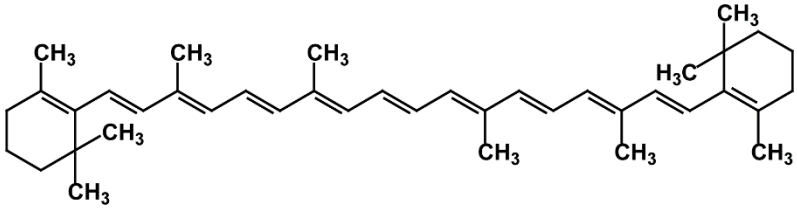
Compounds	**Example of sources**
Vitamin E	Oilseeds, palm oil, nuts, eggs, dairy products, whole grain, vegetables, and cereals
Vitamin C	Fruits and vegetables, berries, citrus fruits, sprouts, green peppers, and potatoes
Carotenoids	Dark leafy vegetables, carrots, sweet potatoes, yams, tomatoes, apricots, kale, turnip green, palm oil, cantaloupes, and citrus fruits
Flavonoids/Isoflavones	Fruits and vegetables, berries, oilseeds, eggplants, peppers, citrus fruits, yams, onions, tomatoes, and cruciferous vegetables
Phenolic acids/Derivatives	Oilseeds and certain oils, cereals, grains, and spices
Catechins	Green tea, berries, and certain oilseeds
Extracts	Extracts from green tea, rosemary, sage, clove, oregano, thyme, cocoa shells, oat, and rice bran



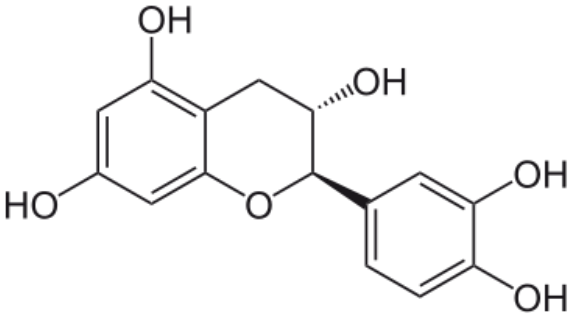
α-tocopherol (Vitamin E)



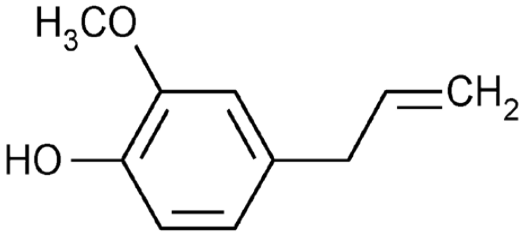
Hydroxytyrosol



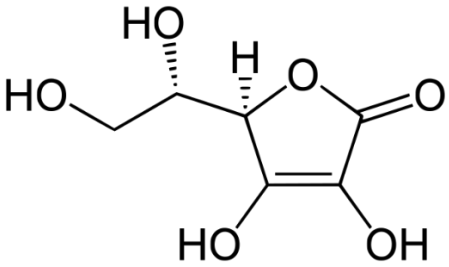
β-carotene



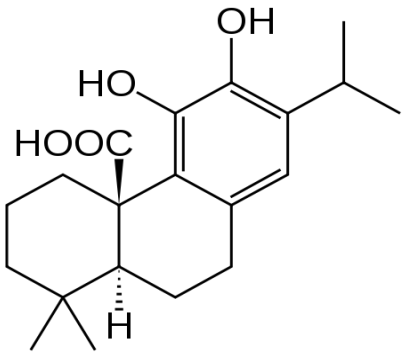
Catechin



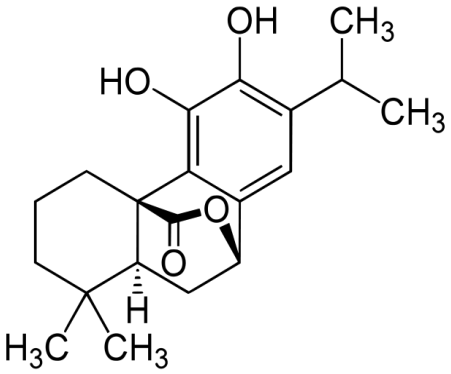
Eugenol



L-ascorbic acid



Carnosic acid



Carnosol

Figure 1:3-Structure of some natural antioxidants

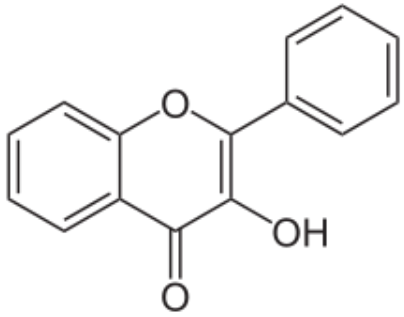
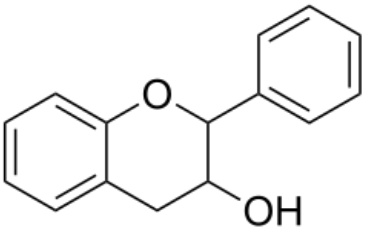
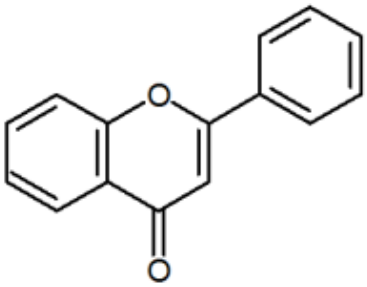
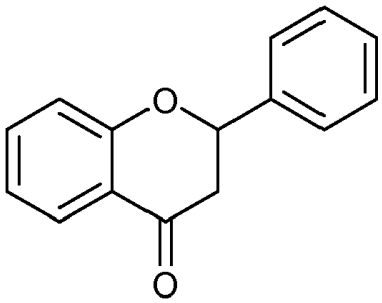
Herbs and Spices as Natural Antioxidants

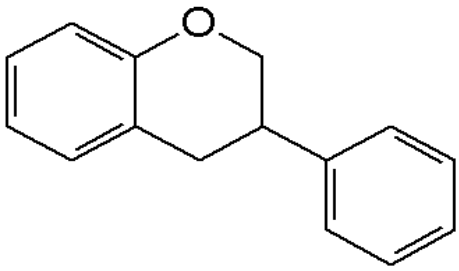
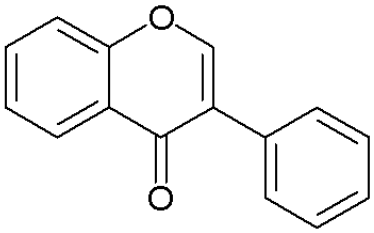
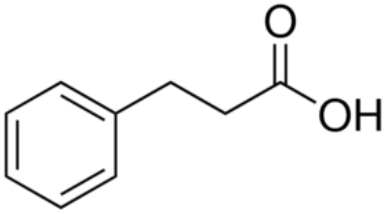
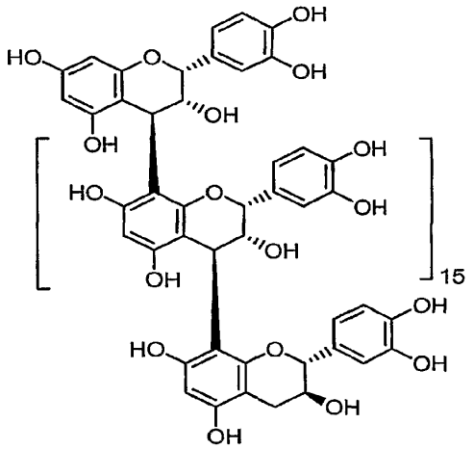
Since ancient times, spices and herbs have been used not only for flavoring foods but also to preserve food, and as well as for their medicinal properties. Spices and herbs are also well-known for their antioxidant properties because they have many phytochemicals such as flavonoids, tannins, phenolic compounds, polyphenolic compounds, phenolic diterpenes, and phenolic acids (Nakatani 1997; Jayasinghe and others 2003; Hossain and others 2008; Amarowicz and others 2009). Phytochemicals are not only antioxidant, but also act as anti-allergic, anti-microbial, anti-bacterial, anti-fungal, anti-inflammatory, antiseptic, antibiotic, and anti-cancer agents. Therefore, natural antioxidants are important disease-fighting compounds (Moure and others 2001; Lee and others 2005; Chitravadivu and others 2009). Neuhouser (2004) reported that the occurrence of cardiovascular diseases and certain forms of cancer can be cured by plant phenolics.

Phenolic compounds present in the leaves, bark, and fruits are secondary metabolites of the plants, and act as bioactive compounds, inhibit the formation of hydroxy radicals, stop free radical chain reactions, and chelate the metals (Zheng and Wang 2001; Miura and others 2002). Polyphenol and flavonoids play a variety of functions in plants and are known as antioxidants in the human diet. They are multifunctional and can act as reducing agents, as hydrogen donors, and as singlet oxygen scavengers (Rice-Evans and others 1996). Table 1:3 summarizes the phenolic phytochemicals with their representing compounds (Fuhrman and Aviram 2002).

The objective of this report to provide an overview of some popular herbs and spices including history, producing regions, botanical description, useful plant parts, chemical compostion, medicinal, and antioxidant properties. In addition, important antioxidant assays are discussed with effects of heat treatments on natural antioxidants and their antioxidant capacity.

Table 1:3-Phenolic phytochemicals (Fuhrman and Aviram 2002)

Class and subclass	**Representing compounds**	**Food or beverage**
Flavonoids	Quercetin	Olives, Onions,
Flavonols	Kaempferol	Kale, Leaf lettuce
	Myricetin	Cranberry, Broccoli, Apple juice Green tea, Black tea, Red wine, Grape juice, Orange juice, and Grapefruit juice
Flavanols	Catechin	Pear, Red wine,
	Epicatechin	Green tea, White wine, and Apple
Flavones	Apigenin	Celery and
	Luteolin Chrysin	Olives
Flavanones	Hesperitin	Grapefruit and
	Hesperidin Naringenin Naringin	Orange

Isoflavans 	Glabridin	Licorice root
Isoflavones 	Genistein Daidzein	Soybean and Soy nuts
Phenolic acids and phenolics Hydroxycinnamic acids 	Caffeic acid Ferulic acid Rosmarinic acid Carnosic acid Gingerol Hydroxytyrosol Oleuropein	Blueberry, Cherry, Pear, Apple, Orange, Grapefruit, Ginger, and Olive oil
Tannins 	Catechin polymers Epicatechin polymers	Red wine, White wine, Apple juice, and Pomegranate

Chapter 2 - Herbs and Spices

Cinnamon

Botanical Name: *Cinnamomum verum* J.

Family: *Lauraceae*.

Synonyms: *Cinnamomum zeylanicum*; Ceylon cinnamon; true cinnamon or real cinnamon.

Common Names: Spanish: canelo de Ceilan; German: ceylon-zimtbaum; French: cannellier; Italian: canella; Hindi: dalchini.

History

Cinnamon is widely used as a spice as well as a traditional medicine. The botanical name *Cinnamomum* is derived from Hebraic and Arabic term "*amommon,*" which means fragrant spice plant, and the word cinnamon is derived from the Greek word "*kinnámömon,*" and Indonesian word "*kayumanis,*" which means sweet wood. Italian called it "*canella,"* which means little tube, which describes cinnamon sticks (Leela 2008a; Brechbill 2012a; Thomas and Kuruvilla 2012).

The Arabs controlled the cinnamon trade for almost 3000 years. Cinnamon is native to Sri Lanka, formerly known as Ceylon, and Malabar coast of India. In the 16th century, the Dutch controlled the island of Ceylon which was under Portuguese control. In 1795, British seized Ceylon from the French, and after 1800, British started cultivating cinnamon in other parts of the world. It is a popular food flavoring and also considered as one of the world's most important spices. Ancient Egyptians used cinnamon during the embalming process because of its pleasant odor and its preservative qualities. The Greeks and Romans used cinnamon as a spice, perfume, and for indigestion, whereas Indian Ayurveda used it for medicines and Chinese herbalists used it to treat fever, diarrhea, and menstrual problems. Nowadays, cinnamon is also used in toothpaste, mouthwash, soap, perfume, cough syrup, nasal sprays, and cola drinks (Leela 2008a; Osborne 2010; Thomas and Kuruvilla 2012; Synan 2013).

Producing Regions

Cinnamon is native to Sri Lanka and India. Now it is cultivated in China, Vietnam, Africa, the West Indies, Zanzibar, and the Seychelles. Sri Lanka accounts for 90% of the world's cinnamon production and it is the major producer and exporter of bark and leaf oil. The cinnamon that grows in Sri Lanka has characteristic flavor and aroma. France imports the highest amount of bark oil followed by the United States of America (Leela 2008a; Brechbill 2012a).

Botanical Description

The cinnamon tree is a tropical, medium-sized, evergreen tree that grows up to 20-60 ft high. It has thick reddish-brown bark with long, lanceolated, bright green and leathery leaves. The flowers are small and yellow in color and arranged in clusters (Figure 2:1; Schoepken2004a). The bark and leaves are highly aromatic, and the bark is used as a spice. After every two years, the trees are cut to just above ground level and bark is harvested from new shoots and then dried. The inner bark is usually used to make cinnamon oil and cinnamon sticks (Figure 2:2; Leela 2008a; Brechbill 2012a).

Figure 2:1-*Cinnamomum verum* J. Presl (Schoepke 2004a)

Useful Plant Parts

The parts of cinnamon tree used include bark, bark powder, bark and leaf essential oil, and oleoresin. The bark has a warm, sweet, and aromatic taste with a pleasant fragrance, while leaves are slightly hot and bitter in taste. The essential oil has a sweet, aromatic, spicy, and clove-like aroma (Leela 2008a; Thomas and Kuruvilla 2012).

Figure 2:2-Cinnamon stick and powder (Gemstone Universe 2014)

Chemical Composition

Cinnamon bark contains up to 2% essential oil with 60-80% cinnamaldehyde as the major constituent (Figure 2:3). This is also responsible for the sweet taste of cinnamon. Other minor constituents are trans-cinnamic acid, o-methoxycinnamaldehyde, eugenol, eugenol acetate, cinnamyl acetate, diterpenes, phenylpropanoids, mucilage, and polysaccharides. Table 2:1 summarizes the active antioxidant chemical in cinnamon. Cinnamon bark oil comes in pale yellow to dark yellow liquid with a characteristic strong warm, sweet, spicy flavor and aroma. The root bark oil contains 60% camphor and is colorless to pale yellowish and brown in color. The leaf oil has 70-90% eugenol and it is cheaper than bark oil. The cinnamon oleoresin is highly viscous and a concentrated dark brown liquid which is obtained by solvent extraction (Leela 2008a; Thomas and Kuruvilla 2012). The nutritional composition and Oxygen radical absorbance capacity (ORAC) values of ground cinnamon are given in Table 2:2.

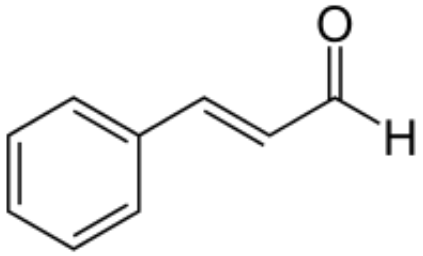


Figure 2:3-Cinnamaldehyde

Table 2:1-Active antioxidant chemicals in cinnamon (USDA 2015b)

Active antioxidant chemicals	**Part of plant**	**Quantity (in ppm)**
1,8-cineole	Bark	165-800
caryophyllene	Bark	135-1,316
cinnamaldehyde	Bark	6,000-30,000
cinnamyl acetate	Bark	510-2,040
eugenol	Bark	220-3,530
mucilage	Bark	20,000-37,000

Table 2:2-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of ground cinnamon (USDA 2015a)

Nutrient	**Units**	**Value per 100 g**
Water	g	10.58
Energy	Kcal	247
Protein	g	3.99
Total lipid (fat)	g	1.24
Carbohydrate, by difference	g	80.59
Fiber, total dietary	g	53.1
Calcium, Ca	mg	1,002
Vitamin C, total ascorbic acid	mg	3.8
Vitamin B-6	mg	0.158
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	15
Vitamin A, IU	IU	295
Vitamin D	IU	0
Fatty acids, total saturated	g	0.345
Fatty acids, total monounsaturated	g	0.246
Fatty acids, total polyunsaturated	g	0.068
H-ORAC	µmol TE/100 g	143,264
L-ORAC	µmol TE/100 g	3,326
Total-ORAC	µmol TE/100 g	131,420
TP	mg GAE/100 g	4,533

Medicinal and Pharmacological Properties

Various *in vitro* studies showed that cinnamon has antimicrobial, antifungal, antibacterial, antioxidant, antitumor, cardiovascular, anti-inflammatory, and immunomodulatory properties (Islam and others 1990; Chao and others 2000; Khan and others 2003; Du and others 2009; Anand and others 2010; Gruenwald and others 2010; Jayaprakasha and Rao 2011; Mandal and others 2011).

Cinnamon extracts, cinnamon bark, and leaf oil all have strong medicinal properties and attributed to the major compound cinnamaldehyde. Traditionally, cinnamon has been used for flatulence, gastrointestinal disorders, diarrhea, and toothache. It is also used to treat arthritis, menstrual disorder, colds, nausea, inflammation, and vomiting. It has carminative and astringent properties. In Ayurvedic medicine, it has been used for bronchitis, colds, congestion, dysentery, oedema, flu, indigestion, liver disorders, muscle tension, headaches, menorrhagia, nausea, and vomiting (Leela 2008a; Anand and others 2010).

Islam and others (1990) reported that cinnamon bark extract was significantly active against 27 strains of *Vibrio cholerae* and *Shigella*. Cinnamon bark oil was also found to have strong antimicrobial properties. Cinnamon bark effectively inhibited gram-positive bacteria such as *Bacillus cereus, Micrococcus luteus,* and *Staphylococcus aureus*; gram-negative bacteria like *Alcaligenes faecalis, Enterobacter cloacae, Escherichia coli,* and *Pseudomonas aeruginosa*; fungi *Aspergillus niger* and *Rhizopus oligosporus*; and pathogenic yeast *Candida albicans* (Chao and others 2000). Sevreal animal studies also showed that cinnamon has hypoglycemic properties (Gruenwald and others 2010). Cinnamon is frequently uses in toothpaste, mouthwash, and other oral hygiene products due to its antibacterial properties (Anand and others 2010).

Antioxidant Properties

Cinnamon also has strong antioxidant properties (Mancini-Filho and others 1998; Shobana and Naidu 2000; Okawa and others 2001; Singh and others 2007; Su and others 2007; Leela 2008a). Intake of cinnamon resulted in a decline in serum glucose, triglyceride, LDL cholesterol, and total cholesterol in type 2 diabetic patients (Khan and others 2003). Ether, methanol, and water extracts of cinnamon showed strong antioxidant activities in β-carotene/linoleic acid system, and it was suggested that cinnamon can be used as natural food preservative to reduce lipid oxidation in a food system (Mancini-Filho and others 1998).

Types of solvent and the extraction method could affect the antioxidant property, and this was explained by Kim and others (1993). In their study, they found that water and 70% methanol extracts had the highest antioxidant activity among 12 different solvents. Water and alcohol extract of cinnamon showed strong antioxidant activity in enzymatic lipid peroxidation, and this antioxidant activity is unaltered by boiling for 30 min at 100°C (Shobana and Naidu 2000). The 50% acetone cinnamon extract also showed highest 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and ORAC value among black peppercorn, nutmeg, rosehip, and oregano leaf (Su and others 2007). Singh and others (2007) evaluated the antioxidant activity of cinnamon volatile oils and oleoresins, and found that oleoresins have strong antioxidant properties, and inhibit primary and secondary oxidation products in mustard oil when added at 0.02%. Cinnamon also showed powerful free radical scavenging activity (Okawa and others 2001; Singh and others 2007). From different literature studies it was concluded that the major compound responsible for strong antioxidant properties is cinnamaldehyde, which is present in the maximum amount (Su and others 2007; Singh and others 2007).

Cinnamon is widely used in the food industry to flavor various foods. In Indian cuisine, it is used as the main ingredient for curries, while in the United States it is often used to flavor cereals and desserts. In Mexico, people use cinnamon with coffee and chocolate. Cinnamon oleoresin is also used to flavor cakes, meats, convenience foods, and savory snacks. It is also used as a colorant in the baking and soap industry, and it is reported that due to synergistic effect cinnamon enhances the sweet sensation of food. Since, cinnamon can serve as a flavoring agent as well as an antioxidant agent in the food system, it can be used as a natural preservative to flavor and to increase the shelf life of the foods (Hirasa and Takemasa 1998).

Clove

Botanical Name: *Syzigium aromaticum* (L.) Merr. and L. M. Perry.

Family: *Myrtaceae*.

Synonyms: *Caryophyllus aromaticus* L.; *Eugenia aromatica* L.; *Eugenia caryophyllus* L.

Common Names: Spanish: clavero; German: gewurznelkenbaum; French: giroflier; Italian: chiodi di garofano; Hindi: laong, lavang.

History

Like other spices, cloves have been used for centuries to cure many diseases, and it also shares a history of greed and bloodshed. Not only the Romans, even the Chinese have used it since 207 BCE. The name clove is derived from the Latin word "*clavus*," the French word "*clou*," and the Spanish word "*clavo*," which means "*nail*," due to its similarity to the shape of a nail. Cloves are native to the Molucca islands, also known as "Spice Islands" (now in Indonesia). Clove is one of the spices from "The Big Four" along with nutmeg, pepper, and cinnamon. It was believed that the clove tree is linked to a new child's fate, so natives used to plant a clove tree whenever a child was born. Wars were also fought in Europe and with native islanders to secure profitable rights to the clove business (Leela and Sapna 2008; Brechbill 2012b; Nurdjannah and Bermawie 2012).

Romans used it as a treasured commodity and used it for fragrance. During the fourth century, Europeans were exposed to clove as a luxury item when it was brought to the continent by Arab traders. During 16th and 17th centuries, it was considered one of the most important spices. In 1816, the Dutch wanted a monopoly on cloves, so they set a fire to burn all clove trees in order to raise the price. Cloves have various medicinal and pharmacological properties, and have been used since ancient times to treat blood circulation, indigestion, toothache, and premature ejaculation. Along with medicinal and culinary uses, it also has been used in cosmetics, perfumes, soaps, and in cigarettes known as "kreteks" (Gladen 2010; Nurdjannah and Bermawie 2012; Brechbill 2012b; Worrall 2012; Razafimamonjison and others 2014; U.S. National Library of Medicine 2015).

Producing Regions

Cloves are native to the Molucca islands and are now commercially cultivated in West Indies, Sri Lanka, India, Indonesia, Madagascar, Brazil, The Philippines, Tanzania (Zanzibar and Pemba islands), and Brazil. Tanzania produces approximately 80% of the world's production, whereas Madagascar exports 11000 tons of cloves and 1500 tons of essential oil every year (Nurdjannah and Bermawie 2012; Razafimamonjison and others 2014).

Botanical Description

Cloves are rich, brown, dried, nail-like unopened flower buds of the tropical evergreen clove tree that grows up to 30 feet, and require a warm and humid climate to flourish. The clove tree may live up to 100 years. Leaves are large, oblong-ovate with glossy green color. It has small white flowers that appear in a terminal inflorescence as shown in Figure 2:5. Flower buds appear pale in color that change into green before finally developing into bright red before harvesting. The buds are generally picked by hand and dried until they turn brown in color. Cloves are about 1/2-1/4 inch long in diameter, with a tapered stem which looks like nails as shown in Figure 2:4. They have unique, strong, warm, aromatic, spicy, sweet, and pungent aroma and flavor (Leela and Sapna 2008; Jirovetz and others 2006; Nurdjannah and Bermawie 2012; Worall 2012; Razafimamonjison and others 2014).

Useful Plant Parts

The parts used include clove as whole or ground, clove bud essential oil, clove leaf essential oil, clove stem essential oil, and oleoresin (Nurdjannah and Bermawie 2012; Razafimamonjison and others 2014).

Figure 2:4-Clove buds

Figure 2:5-*Syzygium aromaticum* (L.) Mer. And L. M. Perry (Schoepke 2004b)
1: flower-bud during flowering, with raised petals (top left); 2: flower-bud in longitudinal section; 3: stamens from different sides; 4: pollen; 5: ovary in cross-section; 6: fruit; 7: same cross-section with rootlets and cotyledons; 8: embryo; 9: a seed flap with rootlets

Chemical Composition

The major constituent of clove oil is eugenol, with a considerable amount of eugenyl acetate and β-caryophyllene. Caryophyllene oxide and α-humulene are also present in smaller amounts. The stem contains the highest amount of eugenol (87.52-96.65%), followed by the leaf (75.04-83.58%) and the clove bud (72.08-82.36%), whereas clove buds have more eugenyl acetate than stem and leaf (Figure 2:6). Table 2:3 summarizes the active antioxidant chemicals in the clove plant (Kramer 1985; Jirovetz and others 2006; Chaeib and others 2007; Fu and others 2007; Pramod and others 2010; Razafimamonjison and others 2014). Cloves also contain tannins (gallotannic acid and methyl salicylate), flavonoids (eugenin, kaempferol, rhamnetin, and eugenitin), triterpenes, and sesquiterpenes. Cloves have a good amount of minerals like potassium, manganese, iron, selenium, magnesium along with vitamin A and beta-carotene

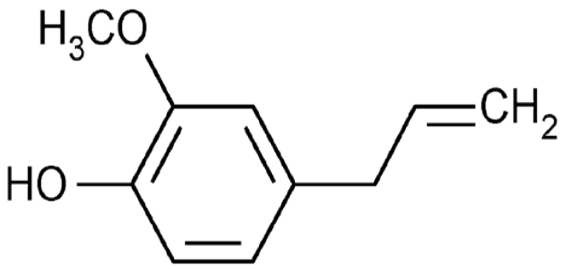
(Leela and Sapna 2008). The nutritional composition and oxygen radical absorbance capacity (ORAC) values of ground cloves are given in Table 2:4.

Table 2:3-Active antioxidant chemicals in clove plant (USDA 2015d)

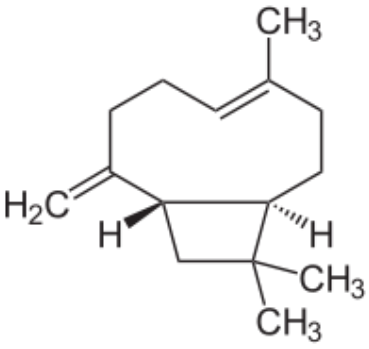
Active antioxidant chemical	**Part of Plant**	**Quantity (in ppm)**
α-humulene	Flower	775-850
Caryophyllene	Flower	74,400-88,160
Eugenol	Flower	108,655
Eugenol	Leaf	8,500-9,000
Eugenol	Stem	8,500-9,000

Table 2:4-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of ground cloves (USDA 2015c)

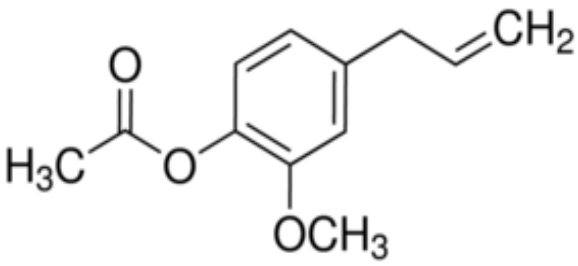
Nutrient	**Units**	**Value per 100 g**
Water	g	9.87
Energy	Kcal	274
Protein	g	5.97
Total lipid (fat)	g	13.00
Carbohydrate, by difference	g	65.53
Fiber, total dietary	g	33.9
Calcium, Ca	mg	632
Vitamin C, total ascorbic acid	mg	0.2
Vitamin B-6	mg	0.391
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	8
Vitamin A, IU	IU	160
Vitamin D	IU	0
Vitamin E	mg	8.82
Fatty acids, total saturated	g	3.952
Fatty acids, total monounsaturated	g	1.393
Fatty acids, total polyunsaturated	g	3.606
H-ORAC	µmol TE/100 g	111,490
L-ORAC	µmol TE/100 g	178,793
Total-ORAC	µmol TE/100 g	290,283
TP	mg GAE/100 g	16,550



Eugenol



β-caryophyllene



Eugenyl acetate

Figure 2:6-Chemical structure of eugenol, β-caryophyllene, and eugenyl acetate (Leela and Sapna 2008).

Medicinal and Pharmacological Properties

Cloves have antifungal, antiseptic, antiviral, antimicrobial, anticancer, anesthetic, antiseptic, antispasmodic, carminative, and stimulating properties (Keene and others 1998; Kalemba and Kunicka 2003; Chaieb and others 2007; Fu and others 2007; Leela and Sapna 2008; Pinto and others 2009; Reichling and others 2009; Pramod and others 2010; Kesin and Toroglu 2011; Kim and Sharma 2011; Machado and others 2011; Timsina and others 2012; Razafimamonjison and others 2014). Cloves and clove oil have been used in treating toothaches for centuries. Eugenol and β-caryophyllene present in clove oil make it antiseptic and anesthetic, which is useful for root canal therapy, temporary fillings, toothache, sore gums, and mouth ulcers. The characteristic aroma of clove oil also helps to eliminate bad breath. Therefore, it is added to various dental products including toothpaste and mouthwash (Markowitz and others 1992; Ghelardni and others 2001; Chaeib and others 2007; Pramod and others 2010; Razafimamonjison and others 2014).

Eugenol, the main constituent of clove oil, also works as an anti-inflammatory agent (0.025mL/kg) and can reduce inflammatory symptoms by 15-30%. Other constituents such as kaempferol and rhamnetin also contribute to anti-inflammatory properties (Öztürk and Özbek 2005; Pramod and others 2010; Miguel 2010). Cloves have been traditionally used to treat indigestion, cough, nausea, headache, stress, vomiting, and blood impurities. Due to its antiviral and blood purifying properties, clove is also useful in boosting the immune system. Halder and others (2011) suggested that clove oil can increase the humoral immune system by decreasing the cell-mediated immunity. Essential oil of clove also showed a strong antimicrobial activity against *S. epidermidis, S. aureus, Salmonella enterica, E. coli,* and *Candida albicans* (Fu and others 2007; Nurdjannah and Bermawie 2012; Razafimamonjison and others 2014). Clove oil is also used for acne treatments, aromatherapy, and premature ejaculation. Other than medicinal uses, it is also used as an insect repellent (Chaieb and others 2007; Nurdjannah and Bermawie 2012; Organic Facts 2015). It is also considered as a strong stress reliever (Singh and others 2009).

Antioxidant Properties

Clove has been shown to have strong antioxidant properties. It exhibits high antioxidant activity in all forms such as ground, extracts, essential oils, and oleoresin (Lee and Shibamoto 2001; Jirovetz and others 2006; Chaeib and others 2007; Yadav and Bhatnagar 2007; Leela and Sapna 2008; Misharina and Samusenko 2008; Dudonne and others 2009; Nurdjannah and Bermawie 2012; Huang and others 2013). Aqueous extract of clove showed powerful antioxidant activities when tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS, ORAC, superoxide dismutase (SOD), and ferric ion reducing antioxidant power (FRAP) methods. A significant direct correlation was also found in phenolic contents and antioxidant activity of cloves (Dudonne and others 2009). Cloves also exhibited the highest DPPH radical scavenging activity and highest FRAP values among mace, cardamom, and licorice (Yadav and Bhatnagar 2007). Clove bud essential oil also showed the highest antioxidant activity when tested against essential oils of lemon, coriander, and grapefruit (Misharina and Samusenko 2008). Clove essential oil showed better DPPH radical scavenging activity than eugenol, BHT, and BHA even at low concentrations. It also acts as an iron chelator and effectively inhibits the hydroxyl radical, and showed high hydroxyl radical scavenging activity. Clove oil significantly ($p \leq 0.01$) reduced the

formation of secondary products of lipid peroxidation and showed higher antioxidant properties than BHT. This study suggested that clove oil can be used as a strong natural antioxidant to reduce lipid oxidation (Jirovetz and others 2006).

Lee and Shibamoto (2001) analyzed the antioxidant activity of aroma extract, which was isolated through steam-distillation of clove buds. At different concentration levels, clove bud extract inhibited hexanal oxidation by 100% for 30 days, and eugenol and eugenyl acetate inhibited hexanal oxidation by 99% for 30 days at a concentration of 500 µg/ml. Eugenol and eugenyl acetate also showed a strong inhibition of malonaldehyde formation by 88% at 160 µg/ml. From the study, they concluded that there is a dose-dependent inhibitory relationship and clove bud extract, eugenol, and eugenyl acetate have strong antioxidant activities as compared to α-tocopherol. Clove oil and extract have powerful antioxidant activity. Thus, it can be used as a natural antioxidant to preserve foods and to treat various degenerative diseases.

Nutmeg and Mace

Botanical Name: *Myristica fragrans* Houtt.

Family: *Myristicaceae.*

Synonyms: *Myristica moschata* Thunb.; *Myristica officinalus* L.; *Myristica aromatica*.

Common Names: (Nutmeg) Spanish: nuez moscada; German: muskatnuss; French: noix muscade; Italian: nos moscata; Hindi: jaiphal; Indonesian: jati-pala.

Common Names: (Mace) French: macis, German: muskatlute; Spanish: macia; Hindi: javatri.

History

Nutmeg and mace are two different parts of the same fruit of the nutmeg tree. The name nutmeg is derived from the Latin word "*nux*," which means nut, and "*muscus*," which means musky. It is native to the Banda Islands but now is cultivated in different parts of the world. It is a very popular and expensive spice and it is also believed that it is abortifacient, so a pregnant woman should avoid nutmeg. High doses of nutmeg (≥0.5 mM) can be toxic (Leela 2008b; Brechbill 2012c).

The Roman writer Pliny talked about nutmeg and mace in the first century. He mentioned a tree bearing fruits with two separate flowers. Around the sixth century, Arabs traded nutmeg to Europe through Venice. In early ages, the Portuguese and the Dutch were predominant traders, and finally, after the Napoleonic War, British gained control over the nutmeg trade. Indian Vedic literature also recommended nutmeg to treat headache, fever, and bad breath. Arabs used it as an aphrodisiac. Nutmeg also has hallucinatory properties because of myristicin compound. Mace aids digestion and also stimulates the appetite. It is considered as a good tonic to remove fatigue and also helps with rheumatic pain (Brechbill 2012c; Rema and Krishnamoorthy 2012).

Producing regions

Nutmeg and mace are native to the Banda Islands in Indonesia but Sri Lanka, China, western Sumatra, Zanzibar, Mauritius, and the Solomon Islands also grow to a smaller extent. Now nutmeg and mace are commercially cultivated in Indonesia, Malaysia (East Indian) and The

Caribbean, Grenada (West Indian). The East Indian nutmeg is superior in quality and flavor than West Indian nutmeg (Leela 2008b; Rema and Krishnamoorthy 2012).

Botanical description

Nutmeg and mace is a seed of a tropical dioecious evergreen tree, which means it has separate male and female plants. The tree grows up to 65 feet in height, yields fruit after 7-8 years from sowing, and can bear fruit for 60 years or longer. The trees can live up to 100 years and could yield up to 20,000 nutmegs a season. It prefers volcanic soil with hot and humid conditions to flourish. It bears pendulous, yellow, smooth, 7-10 cm long fleshy fruits which split into two halves after ripening. The nutmeg is covered with a vivid red lacy covering called mace (Figure 2:7; 2:8). Nutmeg splits into two halves when matured completely. Mace color changes from pale yellow to orange or tan with maturation. Whole dried mace is branched, smooth, and brittle, and is about 40 mm long in size. Nutmeg has a distinctive, pungent, and warm flavor with a slightly sweet taste. Mace from the West Indian is yellowish brown, whereas mace from East Indian is more orange (Leela 2008b; Rema and Krishnamoorthy 2012).

Useful Plant Parts

Nutmeg and mace are generally used as ground spices. Oleoresins, nutmeg butter, and essential oils are also used. Nutmeg oil is usually obtained by steam distillation, and it emerges as colorless to pale yellow or pale green in color. It is highly sensitive to light and temperature. The East Indian nutmeg oil is superior to West Indian nutmeg oil, with better aroma and flavor along with high phenyl propanoid ethers and terpenes. Mace oil is also obtained by steam distillation, and it emerges as a clear dark red liquid with characteristic flavor and odor. It is more expensive than nutmeg oil (Brechbill 2012c; Rema and Krishnamoorthy 2012).

Chemical composition

Nutmeg has 40% moisture with 11% volatile oil and 33.60% nonvolatile ether extract; whereas, mace has 40% moisture with 15.30% volatile oil and 21.98% nonvolatile ether extract. Starch content in nutmeg is 30.20% and in mace it is 44.05%. Nutmeg and mace contain 7-14% essential oil and the major components of essential oils are sabinene and pinene, which are monoterpene hydrocarbons and their major aroma component is myristicin. Depending on the source it contains sabinene (15-50%), α-pinene (10-22%), β-pinene (7-18%), myrcene (0.7-3%),

1,8-cineole (1.5-3.5%), myristicin (0.5-13.5%), limonene (2.7-4.1%), safrole (0.1-3.2%), and terpinen-4-ol (0-11%). (Figure 2:9; Table 2:5 summarizes the active antioxidant chemicals in nutmeg and mace). The seeds contain up to 75% fatty oil known as nutmeg butter, which is semi-solid and reddish brown in color. Nutmeg butter contains 84% trimyristin, 9.8% unsaponifiable constituents, 3.5% oleic acid, 2.3% resinous materials, 0.6% linolenic acid, and formic, acetate, and cerotic acids in traces. Myristicin, safrole, and elemicin are responsible for flavor and pharmacological properties (Leela 2008b; Rema and Krishnamoorthy 2012). The nutritional constituents and ORAC values of ground nutmeg and ground mace are given in Table 2:6.

Figure 2:7-*Myristica fragrans* Houtt. (Schoepke 2004c)

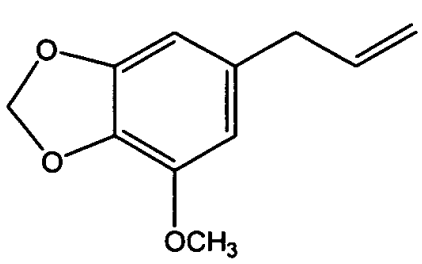
1: stamen column with stamens enlarged; 2: same longitudinal section; 3: same cross-section; 4: pollen; 5: female flower; 6: stamps in longitudinal section; 7: ripe fruit in act of bursting; 8: cross-section of fruit; 9: nut with aril; 10: nut without aril; 11: cross-section of nutmeg; 12: embryo

Table 2:5-Active antioxidant chemicals in nutmeg and mace (Suhaj 2006; USDA 2015g)

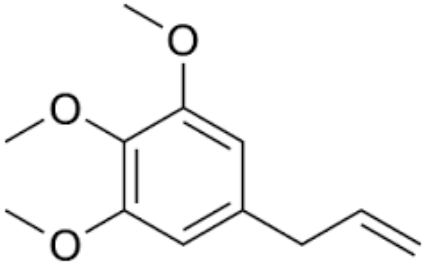
Active antioxidant chemical	Part of plant	Quantity (in ppm)
1,8-cineole	Seed	440-3,520
camphene	Seed	80-640
elemicin	Seed	20-3,500
eugenol	Seed	40-320
gamma-terpinene	Seed	580-4,640
iso eugenol	Seed	140-320
lauric acid	Seed	375-1,600
methyl-eugenol	Seed	20-900
myrcene	Seed	740-5,920
myristic-acid	Seed	60-304,000
myristicin	Leaf	410-620
myristicin	Seed	800-12,800
palmitic-acid	Seed	25,000-128,000
terpinen-4-ol	Seed	600-4,800

Table 2:6-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of ground nutmeg and mace (USDA 2015e & f)

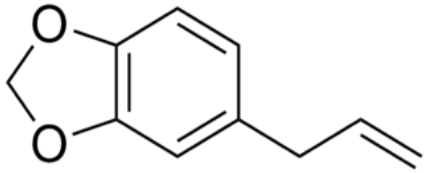
Nutrient	Units	Value per 100 g Nutmeg	Value per 100 g Mace
Water	g	6.23	8.17
Energy	kcal	525	475
Protein	g	5.84	6.71
Total lipid (fat)	g	36.31	32.38
Carbohydrate, by difference	g	49.29	50.50
Fiber, total dietary	g	20.8	20.2
Sugars, total	g	2.99	2.40
Calcium, Ca	mg	184	252
Vitamin C, total ascorbic acid	mg	3.0	21.0
Vitamin B-6	mg	0.160	0.160
Vitamin B-12	µg	0.00	0.00
Vitamin A, RAE	µg	5	40
Vitamin A, IU	IU	102	800
Vitamin D	IU	0	0
Fatty acids, total saturated	g	25.940	9.510
Fatty acids, total monounsaturated	g	3.220	11.170
Fatty acids, total polyunsaturated	g	0.350	4.390
H-ORAC	µmol TE/100 g	12,600	
L-ORAC	µmol TE/100 g	42,625	
Total-ORAC	µmol TE/100 g	69,640	
TP	mg GAE/100 g	567	



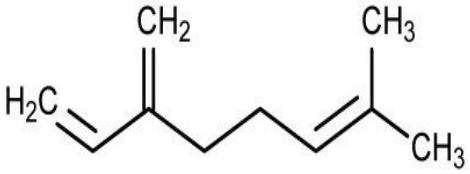
Myristicin



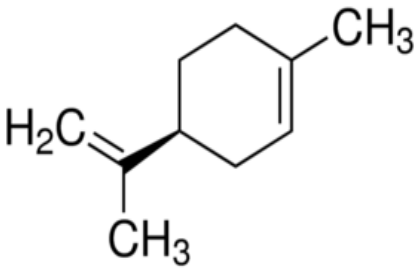
Elemicin



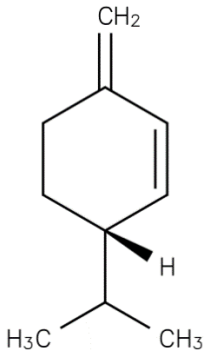
Safrole



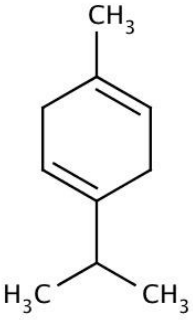
Myrcene



Limonene



β-phellandrene



α-terpinene

Figure 2:8-Volatiles from nutmeg (Leela 2008b)

Figure 2:9-Nutmeg and mace (Stubbs 2010)

Medicinal and Pharmacological Properties

Both nutmeg and mace are used in traditional medicine. In Ayurvedic, Chinese, and Thai medicine, it is used as aphrodisiac, antipyretic, abortifacient, anti-inflammatory, and stomachic. It is also useful to treat flatulence, nausea, vomiting, diarrhea, and stomach cramps as it has carminative and stimulative properties. Essential oil of nutmeg is used in aromatherapy, and it acts as a stress reliever because of myristicin, elemicin, and isoelimicin contents. Myristicin and elemicin are also responsible for hallucinogenic and addictive effects of nutmeg and mace oil. Nutmeg oil can be used for dyspepsia, impotence, insomnia, skin-diseases, and bladder and urinary tract inflammation. Usually, nutmeg butter is used for rheumatism, paralysis, and sprains as an external stimulant. Mace oil is useful in fever, asthma, bowel, and stomach problems (Leela 2008b; Rema and Krishnamoorthy 2012).

Various studies have reported that nutmeg seed extracts, active compounds, and oil have antimicrobial, antifungal, larvicidal, anti-inflammatory, and molluscicidal properties (Narasimhan and Bhake 2006; Mvuemba and others 2009; Muller and others 2010; Rema and Krishnamoorthy 2012; Shafiei and others 2012). Aromatic compounds present in nutmeg and mace are considered to be anticholinergic, antibacterial, hepatoprotective. Morita and others (2003) found that nutmeg has strong hepatoprotective activity among 21 different spices and myristicin is responsible for such activity. Mace oil also acts as an antifungal agent and inhibits aflatoxin formation (Juglal and others 2002). It has been found that nutmeg oil exhibits bactericidal property against *E. coli, Salmonella typhimurium, Shigella boydii*, and *Pseudomonas aeruginosa,* and methanolic extract of mace has strong antibacterial activity against oral bacteria such as *Streptococcus mutans* (Rema and Krishnamoorthy 2012). Cho and others (2008) also suggest that mace inhibits the melanin biosynthesis and so can be used as a skin-whitening agent. Other than medicinal and culinary uses, both nutmeg and mace oil are also used in cosmetics, perfumes, and toiletries.

Antioxidant Properties

Nutmeg and mace have strong antioxidant properties (Chatterjee and others 2007; Sohn and others 2007; Maeda and others 2008; Akinboro and others 2011). Sohn and others (2007) found that mace lignan isolated from nutmeg showed a significant effect against tert-Butyl hydroperoxide (TBHP) and reduced the cell growth inhibition and necrosis induced by TBHP. It

also reduced the malondialdehyde (MDA) formation and intracellular oxygen species caused by TBHP. In addition to antioxidant properties, lignans present in the aqueous mace extract showed radio protective and immunomodulatory effects in mammalian cells (Checker and others 2008). According to Kong and others (2010), nutmeg has strong DPPH radical scavenging activity. Acetone, ethanol, methanol, butanol, and water extracts of nutmeg showed effective antioxidant and antimicrobial properties, and acetone extract showed the highest antioxidant activity with high phenolic content (bio-active compounds of plants). Sabinene was present at the highest amount in acetone extract. β-pinene, α-pinene, myristicin, isoeugenol, p-cymene, carvacrol, eugenol, and β-caryophyllene were also present in acetone extract and are suggested to have strong antioxidant and antimicrobial properties (Gupta and others 2013).

Oregano

Botanical Name: *Origanum vulgare* L.

Family: *Lamiaceae* (*Labiatae*, Mint Family).

Synonyms: *Origanum heracleoticum*; common marjoram; European oregano; winter marjoram; mountain mint; wild marjoram; wintersweet; mexican oregano; rigani; pot marjoram.

Common Names: Spanish: Oregano; German: Gewöhnlicher Dost; French: Origan; Italian: Origano; Turkish: kekik.

History

The name "Oregano" is derived from the Greek word "*oros,*" meaning "mountain," and "*ganos,*" meaning "joy." Therefore, it is commonly called "Joy of the mountains" or "The delight of the mountains" due to its beauty and abundance on the Mediterranean mountains. According to Greek folklore, oregano was created by the goddess Aphrodite and grew on Mount Olympus. It has been traditionally used in Spanish, Mexican, and Italian cuisine as a spice and flavoring agent, whereas the Greeks and Romans used oregano more for medicinal purposes than culinary uses. The Greeks used oregano as a curative agent for dropsy, convulsions, and narcotic poisons. They also used oregano extensively for internal and external fomentations. In addition, they used oregano as a carminative, diaphoretic, emmenagogue, expectorant, and tonic. The herb was also used by the Egyptians as a powerful disinfectant, preservative, and medicine (Kintzios 2002; Seidemann 2005; The Herb Society of America 2005)

Oregano is considered as an herb of love and happiness, and has been used in love potions and spells. It is also used to scent cosmetics because of its volatile contents. There was a custom in Greeks and Roman culture in which the bride and the groom were crowned with oregano wreaths. It was put on graves to give peace to departed spirits. It was also considered a symbol of honor. It is suggested that oregano is beneficial in treating gastrointestinal disorders such as heartburn and bloating, and also for colic, coughs, toothaches, headaches, asthma, menstrual cramps, and nervous complaints. Other than medicinal and culinary uses, Europeans also used oregano to polish furniture and floors, and as an ant repellent. Oregano is also called "the pizza herb" and it is one of the most widely used herbs worldwide. Its popularity in the

United States began in the 1940s after the Second World War when soldiers were returning from Italy and had developed a fondness for oregano used to flavor pizza. (Kintzios 2002, Makri 2002; Seidemann 2005; The Herb Society of America 2005; WebMD 2015).

Producing regions

Oregano is native to Northern Europe, Central Asia, and the Mediterranean region. Now it grows all over the world. Turkey produces over 2/3 of the total worldwide production followed by Mexico, Greece, and other Mediterranean countries. The oregano oil is produced mainly in Russia, Hungary, Italy, and Bulgaria (Kintzios 2002; The Herb Society of America 2005).

Botanical description

Origanum vulgare (oregano) is one of the most famous and economically important culinary herbs of the *Lamiaceae* family. It predominates among 39 species of genus *Origanum*. It is a small, herbaceous, perennial, hardy, and bushy plant that grows up to 2-3 feet tall, with creeping roots. It has multi-branched purplish woody stems with dotted small depressions and leaves that are about 1-4 cm long, grayish to green in color, tiny, opposite, and petiolate. Leaves can be of various shapes including round, oval, and heart-shaped, with shiny/waxy or hairy appearance on the underside, along with a pungent aroma and strong flavor. Oregano is similar to marjoram but has a more pungent flavor and aroma which is not as sweet. The flowers are small, range from white or pink to purple, and appear in corymbs inflorescence from the end of June through August, as shown in Figure 2:11. Each flower produces four small seed-like structures and contains volatile glands which are responsible for the characteristic flavor and aroma of the plant (Figure 2:11). Most oregano varieties prefer full exposure to sunlight because there is a direct correlation with pungency and sunlight. It requires well-drained to dry, sandy, gravelly loamy soil and grows well in pH ranges from 4.5 to 8.7, with an optimum pH of 6.8 (Bosabalidis 2002; Makri 2002; The Herb Society of America 2005).

Useful Plant Parts

The parts used include dried leaves, whole or ground, and essential oil. Essential oil is obtained by steam distillation of the dried flowering herb. *O. vulgare* subsp. *hirtum* contains more than 2% of essential oil. It is yellow to dark brown in color. Essential oil of oregano is rich in phenolic monoterpenoids, mainly carvacrol and/or thymol, with a considerable amount of γ-

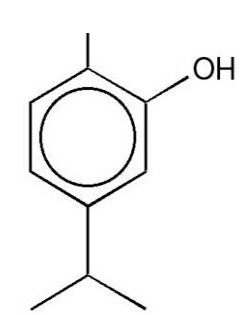
terpinene and p-cymene. *Origanum vulgare* has the best oil quality and yield among all *Origanum* species (The Herb Society of America 2005).

Figure 2:10-*Origanum vulgare* (HGJ 2012)

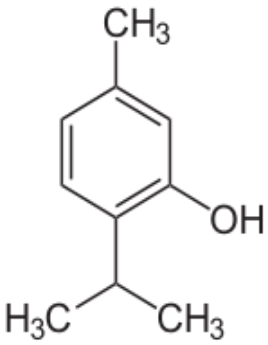
Figure 2:11-Oregano plant (Clipartbest 2015)

Chemical composition

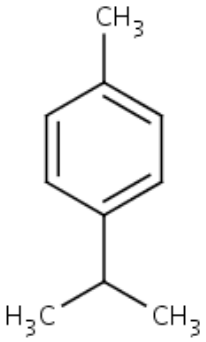
The monoterpenoid phenols carvacrol and thymol are the main constituents responsible for different properties of oregano essential oil, but various other compounds such as γ-terpinene, p-cymene, thymol and carvacrol methyl esters, thymol and carvacrol methyl acetates, p-cymenene, p-cymen-8-ol, p-cymen-7-ol, thymoquinone, thymohrdroquinone, linalool, and borneol are also present at different concentrations. Chemical structures of some compounds are shown in Figure 2:12. Table 2:7 summarizes the active antioxidant chemicals in oregano plant. Different proteins, vitamins, acids, tannins, resins, sterols, bitter compounds, and flavonoids are present in oregano. Other phenolic compounds such as rosmarinic acid derivative, caffeic acid, protocatechuic acid, phenyl glucoside, and 2-caffeyloxy-3-[2-(4-hydroxybenzyl)-4,5-dihydroxy]-phenyl propionic acid are present, which are responsible for antioxidant properties. Flavones including chrysin, negletein, apigenin, luteolin, flavonols kaempferol, galangin, flavanones naringenin, dihydroquercetin are present in *Origanum vulgare* (Skoula and Harborne 2002; The Herb Society of America 2005). The nutritional constituents and ORAC values of dried oregano are given in Table 2:8.



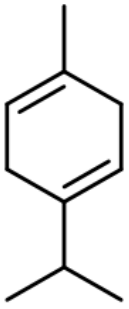
Carvacrol



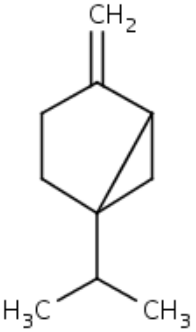
Thymol



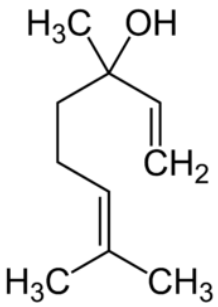
p-cymene



γ-terpinene



Sabinene



Linalool

Figure 2:12-Common compounds present in *Origanum* spp. (Skoula and Harborne 2002)

Table 2:7-Active antioxidant chemicals in oregano plant (USDA 2015i)

Active antioxidant chemical	Part of plant	Quantity (in ppm)
1,8-cineole	Plant	4-96
1-octen-3-ol	Plant	106-530
borneol	Plant	32-252
caffeic acid	Leaf	1,060
caffeic acid	Plant	6,000
carvacrol	Plant	6-640
γ-terpinene	Plant	1-1,476
p-cymene	Plant	3-3,237
rosmarinic acid	Leaf	790
rosmarinic acid	Plant	55,000
sabinene	Plant	50-2,096
tannins	Plant	80,000
thymol	Plant	2-5,000
ursolic acid	Plant	3,100

Table 2:8-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of dried oregano (USDA 2015h)

Nutrient	Units	Value per 100 g
Water	g	9.93
Energy	Kcal	265
Protein	g	9.00
Total lipid (fat)	g	4.28
Carbohydrate, by difference	g	68.92
Fiber, total dietary	g	42.5
Sugars, total	g	4.09
Calcium, Ca	mg	1,597
Vitamin C, total ascorbic acid	mg	2.3
Vitamin B-6	mg	1.044
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	85
Vitamin A, IU	IU	1,701
Vitamin D	IU	0
Fatty acids, total saturated	g	1.551
Fatty acids, total monounsaturated	g	0.716
Fatty acids, total polyunsaturated	G	1.369
H-ORAC	µmol TE/100 g	165,712
L-ORAC	µmol TE/100 g	22,582
Total-ORAC	µmol TE/100 g	175,295
TP	mg GAE/100 g	3,789

Medicinal and Pharmacological Properties

The essential oil of oregano has antibacterial, antifungal, antiviral, spasmolytic, antimutagenic, and anti-inflammatory properties (Barrata and others 1998; Daferera and others 2000; Baričevič and Bartol 2002; Nostro and others 2004; Faleiro and others 2005; Paddock 2008). Oregano is also used to treat colds, coughs, asthma, muscle pain, bronchitis, headaches, allergies, toothache, bloating, itching, insect stings, and menstrual cramps. Essential oil of oregano is used in aromatherapy. Chinese doctors use oregano to treat vomiting, fever, jaundice, diarrhea, and itchy skin. Extracts of oregano also have strong inhibitory effects against both gram-positive and gram-negative bacteria (Barrata and others 1998; Baričevič and Bartol 2002).

Faleiro and others (2005) evaluated the antibacterial and antioxidant activities of oregano essential oil and they found that it was effective against 41 strains of *Listeria monocytogenes*. Thymol, p-cymene, and γ-terpinene are responsible for the strong properties of oregano oil. Some studies also reported that essential oil of Himalayan oregano can even kill the hospital superbug methicillin-resistant *Staphylococcus aureus* (MRSA) due to its strong antibacterial properties (Nostro and others 2004; Paddock 2008). Daferera and others (2000) studied the essential oil of oregano, and reported that carvacrol and thymol have a powerful inhibitory effect against radial growth, conidial germination, and the production of *Penicillium* species. The hypoglycemic effect ($p<0.001$) of aqueous extract of oregano (20 mg/kg) was demonstrated by Lemhadri and others (2004) in rats and suggested that oregano extract can be used in diabetics. A spice mixture (11.25 g) containing oregano was added to hamburgers during cooking and it significantly ($p<0.05$) reduced the malondialdehyde concentration because of poly-phenol compounds present in it, and it suggested the potential health benefits of oregano for atherogenesis and carcinogenesis (Li and others 2010).

Antioxidant Properties

A large number of studies demonstrated the antioxidant properties of oregano extracts, essential oils, and isolated compounds of oregano (Vekiari and others 1993; Barrata and others 1998; Baričevič and Bartol 2002; Houhoula and others 2003; Sanchez-Escalante and others 2003; Govaris and others 2004; Kulisic and others 2004; Cervato and others 2000; Economou and others 2005). According to Tsimidou and Boskou (1994) oregano is rich in carvacrol, and this compound is responsible for higher antioxidant capacities of oregano.

Chen and Ho (1997) also agreed with Tsimidou and Boskou (1994) and found that as an average 55% of the total phenolic compounds in oregano extract is due to caffeic acid, rosmarinic acid, thymol, and carvacol, which possess strong antioxidant activity and also are responsible for oregano's pleasant aroma and flavor. Barrata and others (1998) tested the antioxidant property of oregano essential oil by using egg yolk and rat livers as lipid rich substances. From the study, they concluded that at higher concentration (1000 ppm and 750 ppm) oregano oil has a higher antioxidative effect than BHT and α-tocopherol and carvacrol, thymol, p-cymene, γ-terpinene, and methyl carvacrol were the major constituents responsible for the higher antioxidant activities of oregano. However, water and methanol extracts of oregano was also studied for their strong antioxidant properties by Cervato and others (2000), and they suggested that both extracts can be used as a strong food additive to reduce lipid oxidation because they were rich in polyphenol constituents, which are responsible for high antioxidant properties of extracts.

In another study, meat batter was treated with ethanol and chloroform oregano extracts, and both exhibited effective inhibition of lipid oxidation and it was found that rosmarinic acid could be responsible for the antioxidant effect of oregano. High concentration of rosmarinic acid in ethanolic extracts of oregano also prevented the color deterioration in meat batter. Moreover, when beef patties were treated with 0.1% oregano, they exhibited strong antioxidant properties ($p<0.05$) by inhibiting lipid oxidation which was measured by thiobarbaturic acid (TBA) number. A concentration-dependent effect was observed when oregano was added at lower concentration (Sanchez-Escalante and others 2003).

Oregano also has been used as strong stabilizers in edible oils and ready-to-eat meat products to reduce lipid oxidation (Baričevič and Bartol 2002). Houhoula and others (2003) studied the oxidative stability of cottonseed oil and storage stability of potato chips by using ground oregano and ethanol extract, and they found that both ground oregano and ethanol extract increased the stability of oil by decreasing the oxidation products in frying oils. Ground oregano and ethanol extract efficiently reduced the formation of conjugated dienes, polar compounds, polymerized triglycerides, and dimeric triglycerides. They also showed a protection against conjugated dienes and significantly ($p<0.05$) increased the storage ability of potato chips.

Oregano extracts also exhibited antioxidant effects against lard oxidation and decreased the rate of peroxide formation when added at 0.2 %. Chromatographic and spectrophotometric

analysis of oregano reported that it contains flavonoids (apigenin, eriodictyol, dihydrokaempferol, and dihydroquercetin), which were responsible for high antioxidant activity in lard and in vegetable oils under storage and during frying (Vekiari and others 1993; Economou and others 2005).

The antioxidant effect of oregano oil was studied in breast and thigh turkey meat patties by Govaris and others (2004) and they found that oregano oil significantly ($P<0.05$) reduced the lipid oxidation at all storage periods when meat patties were stored at 4°C for 3, 6, and 9 days. Rojas and Brewer (2007) studied the effects of water-soluble oregano extract in cooked beef and pork. They found that the oregano water extract added at 0.02% was effective at reducing lipid oxidation in vacuum packaged cooked beef samples held at -18°C for 4 months.

Rosemary

Botanical Name: *Rosmarinus officinalis* L.

Family: *Lamiaceae* (*Labiatae*, Mint Family).

Synonyms: *Rosmarinus coronarium*; compass plant; compass weed; polar plant; old man.

Common Names: Spanish: romero; German: rosmarein; French: rosmarin; Italian: rosmarino.

History

Rosemary has quite an interesting history, from fairies and witches to weddings and burials, for the same reason: remembrance. The name "rosemary" is derived from the Latin word "*ros*," meaning "dew," and "*marinus*," meaning "sea," which means "dew of the sea" or "fond of the sea." The ancient Greek called it "*anthos*," which means "The flower of excellence" (Sasikumar 2012; Ad Lunam Labs Inc. 2014).

It has been used since 500 BC. During the Middle Ages, rosemary twigs and leaves were burned to scare away evil spirits and disinfect the surroundings. It was also used to dispel evil spirits and nightmares by placing the leaves under the pillow. Ornaments made of rosemary were used as a symbol of love, intimacy, and fidelity for lovers. Rosemary was also used as an herb of remembrance by placing it on graves and also at funerals. Along with love, friendship, and remembrance, rosemary is also associated with strengthening the memory and recall. Students in ancient Greece wore wreaths of rosemary around their heads or braided it into their hair to help them during examinations. It was also assumed that if rosemary thrived in one's house, the woman rules the house. It was also used as a perfume in baths of ladies in France, Germany, and Turkey. It was also placed in books and among clothes to protect them from moths and vermin (Global Herbal Supplies 2009; Sasikumar 2012; Ad Lunam Labs Inc. 2014; Monterey bay Spice Company 2015; Our Herb Garden 2015).

Producing Regions

Rosemary is native to the Mediterranean regions. It is now cultivated worldwide in Algeria, Spain, France, Russia, China, Portugal, Tunisia, Morocco, Italy, Australia, and the USA

(Sasikumar 2012; Cloverleaf Farm 2015). Major essential oil producing regions are Spain, Tunisia, Morocco, and France (ITC 2014).

Botanical Description

Rosemary is an aromatic, dense, hardy, and an evergreen perennial small shrub which grows up to 6 feet tall or more. Leaves are about 1 inch long, linear, branched, thick, sticky, narrow, and look similar to small pine needles (Figure 2:13). The colors of leaves vary from dark green or blue to white at the underside with a bittersweet, lemony, and slightly piney flavor. The stem is woody, square, and brown. The flowers are small and range from pale purple or bluish to dark blue, and appear in cymosely inflorescence (Figure 2:14). Calyces of the flowers contain most of the volatile components in it (Özacan and Chalchat 2008; Sasikumar 2012; Grieve 2014).

Figure 2:13-Rosemary plant

Useful Plant Parts

The parts used include fresh or dried leaves, whole, chopped, crushed or ground, and essential oil. Essential oil is obtained by steam distillation of the fresh flowering tops. It is colorless to pale yellow, with the odor of rosemary and a warm camphoraceous taste. Rosemary oil from Spain and rosemary oil from Tunisia and Morocco can be sold commercially, and both have different oil composition and yield quality (Grieve 2014).

Figure 2:14-*Rosmarinus officinalis* L. (Schoepke 2004d)

1 and 2: flowers enlarged; 3: flower in longitudinal section; 4: fertile stamens; 5: pollen; 6: infertile stamen; 7: stamp; 8: ovary in longitudinal section; 9: same cross-section; 10: pappus; 11: fruit without calyx; 12 and 13: nutlets from different sides; 14: same in longitudinal section

Chemical Composition

Rosemary leaves and flowers contain the oil glands; however, the most important rosemary oil is obtained from the leaves only. There are many factors such as heredity, part and age of the plant, extraction method, harvest time, environmental, and agronomic conditions, which are responsible for the yield and quality of rosemary oil (Elamrani and others 2000; Ojeda-Sana and others 2013). Rosemary (Spain) and rosemary (Tunisia and Morocco) are two oils sold commercially, and both have different chemical composition, which is as follows:

Rosemary oil (Spain): camphor (17.2-34.7%), α-pinene (10.2-21.6%), 1,8-cineole (12.1-14.4%), camphene (5.2-8.6%), borneol (3.2-7.7%), β-pinene (2.3-7.5%), verbenone (2.2-5.8%),

β-caryophyllene (1.8-5.1%), limonene (2.0-3.8%), α-terpineol (1.2-2.5%), myrcene (0.9-4.5%), p-cymene (0.2-3.4%), bornyl acetate (0.2-2.3%), linalool (0.3-1.0%), and terpinen-4-ol (0.4-0.9%) (Salido and others 2003; Bozin and others 2007).

Rosemary (Tunisia, Morocco): α-pinene (37-40%), camphor (12.41%), 1,8-cineole (58-63%), camphene (5.86%), borneol (6.14%), bornyl acetate (4.13%) , β-pinene (4.84%) (Elamrani and others 2000; Bozin and others 2007; Ait-Ouazzou and others 2011; Kadri and others 2011).

Table 2:9 summarizes the active antioxidant chemicals in rosemary plant and Table 2:10 summarizes the nutritional composition and ORAC values of dried rosemary.

Table 2:9-Active antioxidant chemicals in rosemary plant (Suhaj 2006; Global Herbal Supplies 2009; USDA 2015k)

Active antioxidant chemical	Part of Plant	Quantity (in ppm)
1, 8-cineol	Plant	8,125
α-pinene	Plant	235-4,750
β-caryophyllene	Plant	12-2,075
β-pinene	Plant	17-1,425
β-sitosterol, caffeic acid, camphene	Plant	23-2,350
Camphor	Plant	60-5,800
carnosic acid	Plant	530-9,803
Carnosol	Leaf	530-9,803
Carvacrol	Leaf	0-5
carvone	Plant	16-760
epirosmanol	Leaf	26
γ-terpinene	Plant	4-400
isorosmanol	Flower	17
limonene	Plant	1,950
linalool	Plant	585
myrcene	Plant	25-5,605
p-cymene	Plant	25-950
rosmadial	Leaf	30
rosmarinic acid	Plant	0-25,000
rosmarinic acid	Leaf	3,500
rosmarinic acid	Shoot	0-13,500
terpinen-4-ol	Plant	4-521
ursolic acid	Plant	28,000-41000
verbenone	Plant	10-375

Table 2:10-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of dried rosemary (USDA 2015j)

Nutrient	Units	Value per 100 g
Water	g	9.31
Energy	kcal	331
Protein	g	4.88
Total lipid (fat)	g	15.22
Carbohydrate, by difference	g	64.06
Fiber, total dietary	g	42.6
Calcium, Ca	mg	1,280
Vitamin C, total ascorbic acid	mg	61.2
Vitamin B-6	mg	1.740
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	156
Vitamin A, IU	IU	3,128
Vitamin D	IU	0
Fatty acids, total saturated	g	7.371
Fatty acids, total monounsaturated	g	3.014
Fatty acids, total polyunsaturated	g	2.339
H-ORAC	µmol TE/100 g	112,200
L-ORAC	µmol TE/100 g	53,080
Total-ORAC	µmol TE/100 g	165,280
TP	mg GAE/100 g	4,980

Medicinal and Pharmacological Properties

Rosemary is carminative, cholagogue, sedative, rubefacient, and stomachic. It is used to treat renal colic and dysmenorrhea. It has also been used to increase alertness and to stimulate the growth of hair. It promotes liver function and the production of bile as well as provides relief from muscular pain and joint pain (Al-Sereitia and others 1999; Bozin and others 2007; Global Herbal Supplies 2009; Jiang and others 2011). The rosemary extract, phenolics, and essential oils has antibacterial, antifungal, antiviral, antimicrobial, anti-inflammatory, astringent, and spasmolytic activity (Baratta and others 1998; Moreno and others 2006; Bozin and others 2007; Özacan and Chalchat 2008; Global Herbal Supplies 2009; Jinag and others 2011; Toroglu 2011; Ventura-Martínez and others 2011; Ojeda-Sana and others 2013). Rosemary extract, carnosic acid, and rosmarinic acid also showed anticancer properties in animal and cell culture studies (Moreno and others 2006; Sharabani and others 2006; Bozin and others 2007; Yesil-Celiktas and others 2010). Hsieh and others (2007) found that different extracts of rosemary have potent antiglycative effects and can be used as medicine for the treatment of diabetes, cardiovascular, and other neurodegenerative diseases. Cheung and Tai (2007) reported that ethanolic extract of

rosemary has differential anti-proliferative effects on human leukemia and breast carcinoma cells. Carnosic acid, a major phenolic compound from rosemary, was shown to decrease the viability of the human promyelocytic leukemia cell line, HL-160 and induced G_1 arrest and apoptosis (Wang and others 2008). Several animal studies also concluded that rosemary oil has dose-dependent antinociceptive effect in the pain-induced functional impairment model in the rat (PIFIR) (Martínez and others 2009).

Carnosic acid and carnosol are the major constituents of rosemary extract and are found to have high antibacterial activity against various microorganisms including *Streptococcus mutans, S. salivarius, S. sobrinus, S. mitis, S. sanguinis,* and *Enterococcus faecalis* which initiates dental caries (Bernades and others 2010). Rosemary extract has also been found to be promising as a nutritional strategy for improving meat quality (Bañón and others 2012).

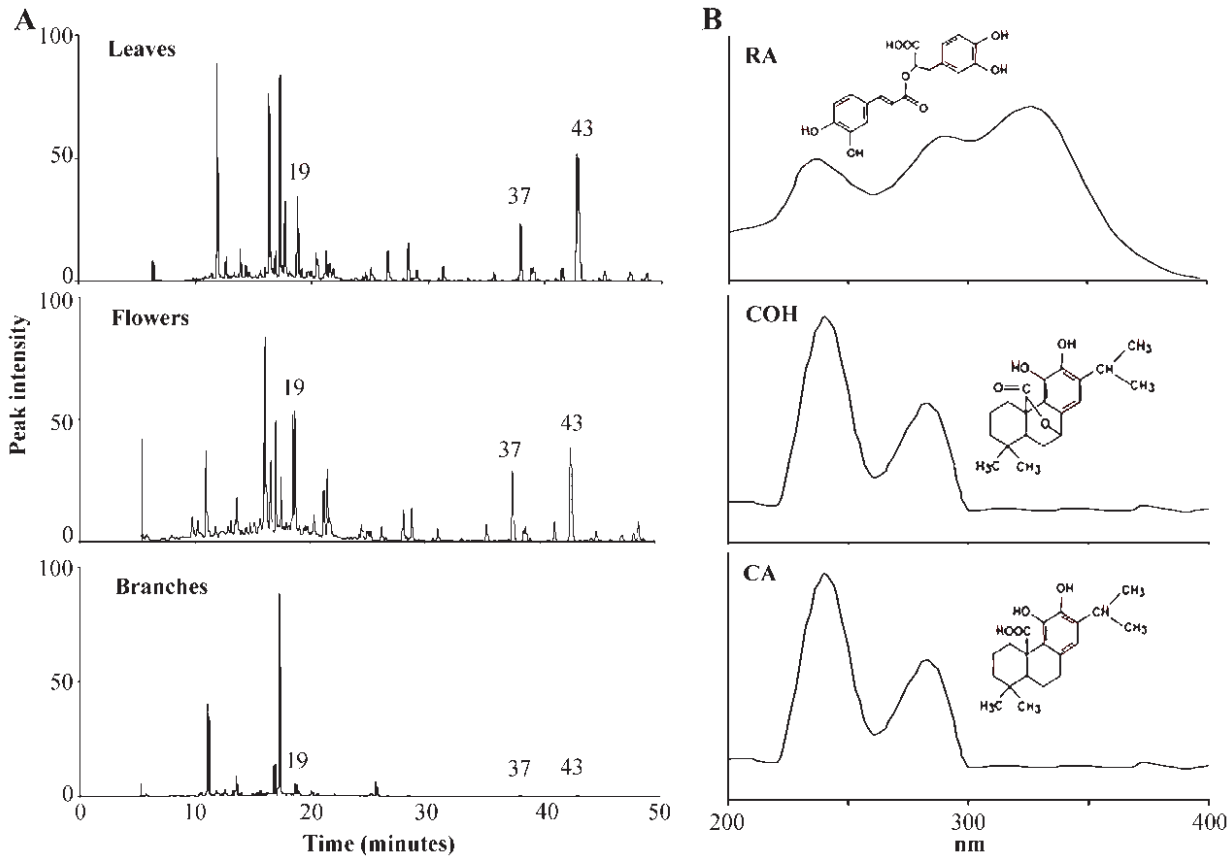


Figure 2:15-HPLC chromatogram of leaves, flowers, and branches extracts obtained from rosemary plant (A). Peaks with Rtime of 19, 37, and of 43min corresponding to rosmarinic acid (RA), carnosol (COH), and carnosic acid (CA) respectively. Spectrum of RA, COH, and CA (B) (Moreno and others 2006).

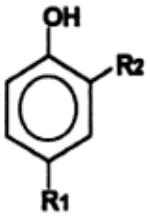
Antioxidant Properties

Rosemary has been shown to have strong antioxidant properties. It exhibits high antioxidant activity in all forms such as ground, extracts, and essential oils (Houlihan and others 1984; Cuvelier and others 1996; Sanchez-Escalante and others 2003; Formanek and others 2003; Hernández-Hernández and others 2009; Beretta and others 2011; Colindres and Brewer 2011; Lara and others 2011; Lagouri and Alexandri 2013)

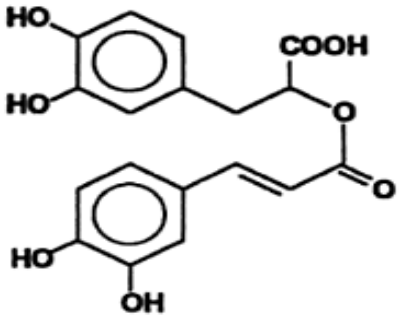
Rosemary essential oil was studied for antioxidant activity by Beretta and others (2011). They isolated essential oil of rosemary at three different plant stages: flowering (aerial parts), post-flowering (aerial parts with seeds and leaves), and vegetative stages (aerial parts with leaves only). From the study, it was concluded that oil collected during the flowering phase has the best antioxidant activity, followed by post-flowering, and then from the vegetative stage. A number of studies have also shown that the antioxidant properties depend on geographical orientation, chemotype, soil type, irrigation conditions, and extraction parameters (Cuvelier and others 1996; Hernández-Hernández and others 2009; Beretta and others 2011). Lara and others (2011) incorporated essential oil of rosemary in irradiated beef patties and found that the addition of natural antioxidants not only significantly reduced the thiobarbituric acid reactive substances (TBARS) value but also significantly increased overall acceptability by improving the color of beef patties.

The major compounds contributing for antioxidant activity of rosemary can be categorized in three groups (Figure 2:16):

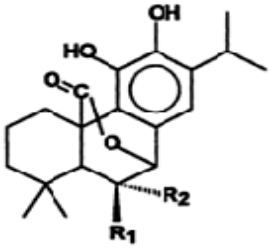
1. Phenolic acids: vanillic, caffeic (3,4-dihydroxycinnamic acid), ferulic, and rosmarinic acid;
2. Phenolic diterpenes: carnosol, rosmadial, carnosic acid, methyl carnosate, rosmanol, epirosmanol, epi-isorosmanol, epirosmanol methyl ether, and epoisorosmanol ethyl ether;
3. Flavonoids: hesperetin, apogenin, genkwanin, cirsimaritin, scutellarein, 4'-methoxytectochrysin, 4',5,7,8-tetrahydroxyflavone, homoplantaginin, and 6-hydrocyluteolin 7-glucoside (Cuvelier and others 1996; Areias and others 2000; Yesil-Celiktas and others 2007)



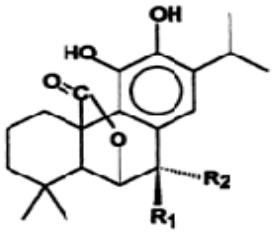
	R_1	R_2
Vanillic acid	COOH	OCH_3
Ferulic acid	-CH=CH-COOH	OCH_3
Caffeic acid	-CH=CH-COOH	OH



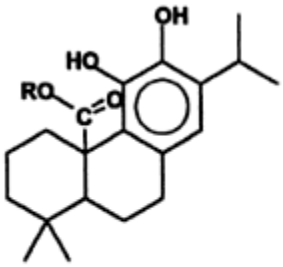
Rosmarinic acid



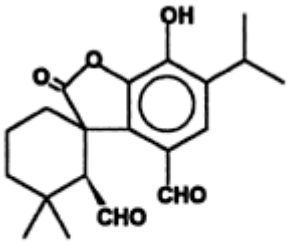
	R_1	R_2
Carnosol	H	H
Epiisorosmanol	OH	H
Epiisorosmanol ethyl ether	OCH_2CH_3	H



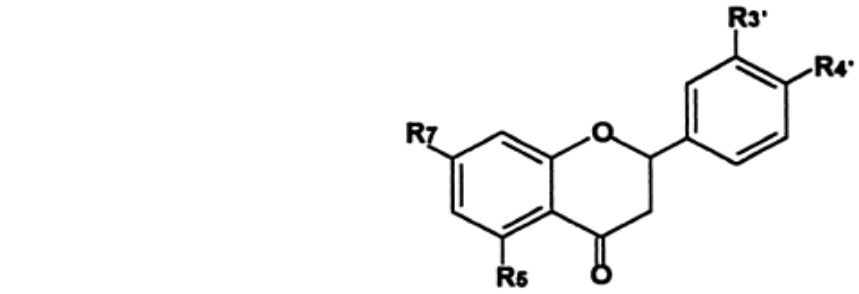
	R_1	R_2
Rosmanol	H	OH
Epirosmanol	OH	H
Epirosmanol methyl ether	OCH_3	H



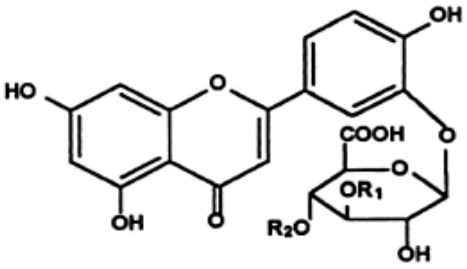
Carnosic acid R=H
Methyl carnosate R=CH_3



Rosmadial



Flavanones	R_5	R_7	$R_{3'}$	$R_{4'}$
Hesperetin	OH	OH	OH	OCH_3
Hesperidin	OH	*O*-rutinose	OH	OCH_3



	R_1	R_2
Luteolin 3′-*O*-β-D-glucuronide	H	H
Luteolin 3′-*O*-(4″-*O*-acetyl)-β-D-glucuronide	H	Ac
Luteolin 3′-*O*-(3″-*O*-acetyl)-β-D-glucuronide	Ac	H

Figure 2:16 -Structures of phenolic compounds identified from rosemary and sage extracts (Cuvelier and others 1996)

Houlihan and others (1984) reported that some components of rosemary extracts such as rosmanol, carnosol, rosmarinic acid, and carnosic acid can become four times more effective than BHA and BHT *in vitro* conditions.The antioxidant activity of phenolic compounds is due to the presence of a primary hydroxyl group and the presence of a second hydroxyl group at *ortho* or *para* position that increases the antioxidant activity through resonance stability and *o-quinone* or *p-quinone* formation. Therefore, rosmarinic acid, a diphenolic compound which is an ester of caffeic acid and 3-(3,4-dihydroxyphenyl) lactic acid shows the highest strong antioxidant activity due to four hydroxyl groups as shown in Figure 2:16 (Lagouri and Alexandri 2013).

Rosemary and Sage were studied for phenolic compounds by Cuvelier and others (1996) and 27 compounds were characterized through high-performance liquid chromatography and ultraviolet spectrum as shown in Figure 2:17. Some structures of phenolic compounds were also identified from extracts as shown in Figure 2:16.

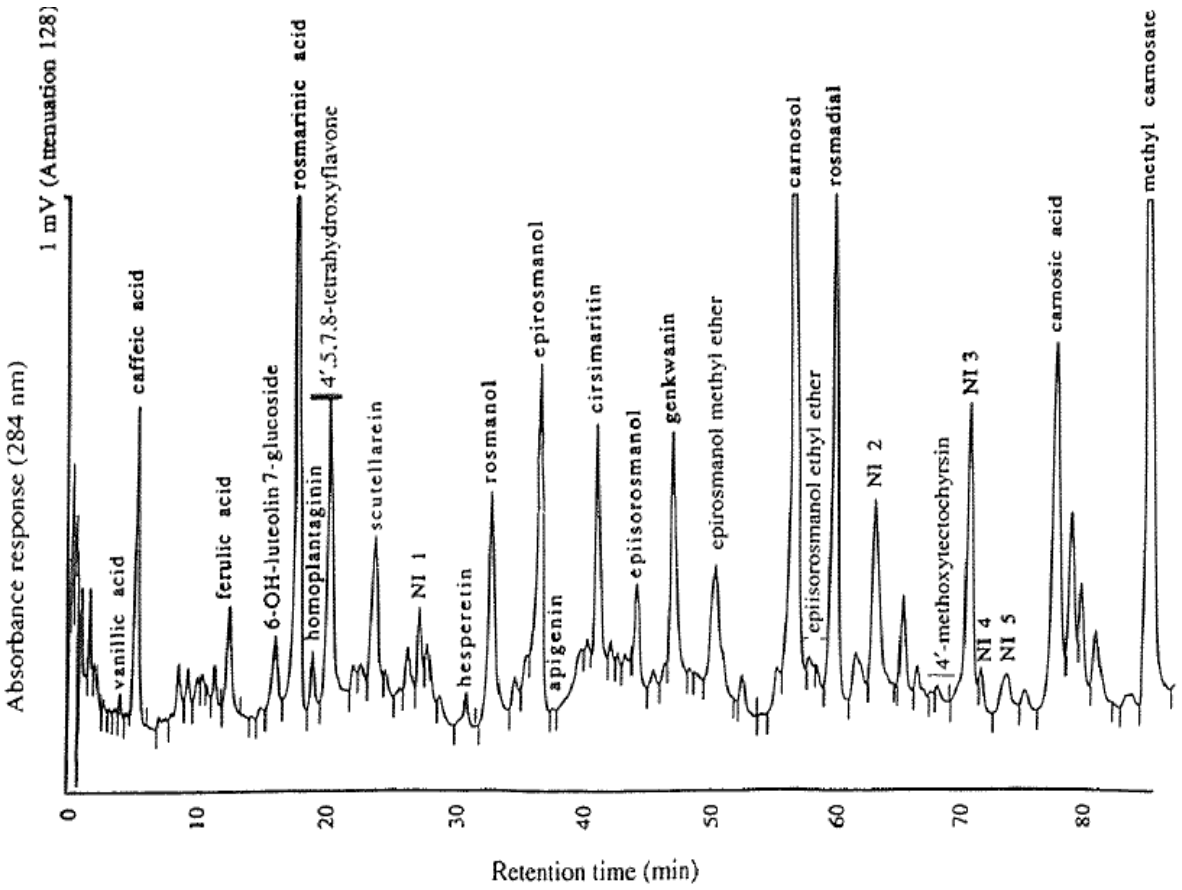


Figure 2:17-High-performance liquid chromatography profile of rosemary and sage extract (Cuvelier and others 1996).

Sanchez-Escalante and others (2003) studied the antioxidant activity of rosemary extracts with or without ascorbic acid in beef patties. Patties were mixed with 2% salt, 0.1% rosemary, and 0.1% rosemary+0.05% ascorbic acid and stored at 2±1°C in modified atmosphere packaging (70% O_2+20% CO_2+10% N_2). In the study, they reported that rosemary extract prevented lipid oxidation in beef patties for 16 days under the conditions tested. This study also agreed with the fact that high antioxidant activity of rosemary is due to carnosol, rosmanol, rosmanidipherol, rosmariquinone, carnosic acid, and rosmarinic acid.

Ethanolic extract of rosemary was studied for antioxidant effect in raw pork batters. The lowest TBARS and higher antioxidant activity ($p<0.01$) values were observed for rosemary-treated samples. This study also found that antioxidant effect depends on the structure of the antioxidant compound, storage temperature, and the initial oxidation state of the sample. Antioxidant activity of phenolic compounds is due to the presence of a primary hydroxyl group

and the presence of a second hydroxyl group at *ortho* or *para* position that increases the antioxidant activity through resonance and *o-quinone* or *p-quinone* formation. Therefore, rosmarinic acid shows high antioxidant activity due to four hydroxyl groups at *ortho* and *para* positions (Sanchez-Escalante and others 2003). Rosemary extract was also analyzed in irradiated ground beef for antioxidant activity, and the study reported that both lipid oxidation and color change were inhibited by rosemary extracts (Formanek and others 2003). Lara and others (2011) also reported that the thiobarbituric acid reactive substances (TBARS) value of pork patties immediately after cooking, ranged from 0.1 to 0.3 mg MA/kg sample and the addition of rosemary extract significantly reduced the lipid oxidation.

In another study, Colindres and Brewer (2011) evaluated the antioxidant effect of oleoresin rosemary in cooked, frozen, and reheated beef patties. Fresh beef, lean and trim were ground and mixed with 2% salt. Oleoresin rosemary was added at 0.2 g kg^{-1} before cooking and patties were cooked to an internal temperature of 71°C. Patties were wrapped in polyvinyl chloride and stored at -18°C for 6 months. After 6 months, there was lower TBARS scores than control. From the study, they found that antioxidant oleoresin rosemary effectively reduced the oxidation of lipid and they also reduced the TBARS to 44% compared to the control samples.

Sage

Botanical Name: *Salvia officinalis* L.

Family: *Lamiaceae* (*Labiatae*, Mint Family).

Synonyms: Sawge; Dalmatian sage; Garden sage; English sage; True sage; Common sage; Ryytisalvia.

Common Names: Spanish: *ierba buena*; German: Salbei; French: *Sauge officinale*; Italian: *Salvia officinale*.

History

Sage has one of the longest histories for its medicinal properties. The genus *Salvia* comes under one of the most widely spread members of the *Lamiaceae* family. The name of the genus "*Salvia*" is derived from the Latin word "*salvere*," which means "to save." In French, the word "sage" means "wise" and in Rome it is considered the sacred herb (Dweck 2000).

It has been used in many ways, such as the flowers being used as rouge, essential oil used in perfumery, the seed oil used as an emollient, the roots used as a tranquilizer, and the leaves used for varicose veins. The ancient Egyptians used sage to increase the fertility of women, whereas Romans used it for promoting menstruation and also as an anesthetic. The Greeks used it to treat ulcers and sores and also to heal wounds. A sage tea has been used from ancient times to treat fever, sore throats, coughs, and to excite the nervous system. It was also considered as a useful medicine to treat typhoid, liver, and kidney complaints (Dweck 2000).

Sage has been used effectively as carminative, spasmolytic, antiseptic, bactericidal, astringent, wound healing agent, memory-enhancing agent, antidepressive, in skin and hair care, insecticidal, and as a mouthwash against throat and mouth inflammation. It has also been used to scare away evil spirits and was considered as an effective antidote against snakebite. Sage leaves are also used to make tooth powder with sea salt, and is considered to be the best agent to darken and tone the hair. It is used also for rheumatism, to strengthen the nervous system, and to sharpen the senses. Therefore, because of various medicinal properties, the International Herb Association named sage as the "Herb of the Year" in 2001 (Baricevic and Bartol 2000; Dweck 2000; Karamanos 2000).

Producing regions

Sage is native to the Mediterranean region. It is cultivated worldwide, but abundantly found in southern Europe, in South-East Asia, and in Central and South America. Croatia produces Dalmatian sage oil and smaller quantities are also manufactured in France, Bulgaria, Turkey, and Germany (Kaousou and others 2000; Ulubelen 2000).

Botanical Description

Sage is an evergreen, perennial, medicinal, and culinary shrub which grows up to 2 feet tall. Leaves are about 2 inches long, oblong or spear-shaped, grayish green, shiny, and covered with fine hairs (Figure 2:18). It has spindle-shaped roots with woody branches. Flowers are large, attractive, and appear in verticillaster inflorescence in violet, blue, or pale-blue colors as shown in figure 2:19. Flowering starts from mid-March to June. All aerial parts are covered with glandular hairs which give a silver color to plant. All parts of the plant have a strong aroma because volatile components are present in the glandular hairs of aerial parts. The maximum concentration of volatile compounds are found in the leaves followed by the flowers and stems (Karamanos 2000; Karousou and others 2000).

Useful Plant Parts

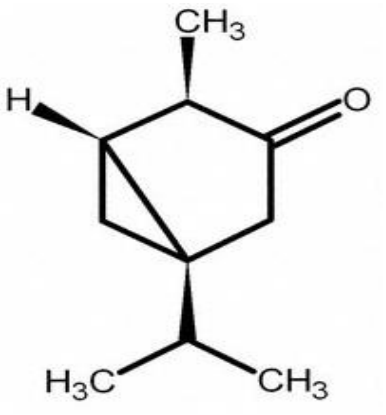
Sage parts used include fresh or dried leaves, whole, chopped, ground, rubbed, or minced, plus essential oil, and oleoresin. The essential oil is clear, colorless to pale yellow, and is generally obtained by steam distillation of partially dried leaves. The yield of oil is about 1.3-3.6% which varies with environmental and geographical conditions. Sage oil has a strong, warm aroma with a bitter and astringent taste (Karousou and others 2000).

Figure 2:18-*Salvia officinalis* L. (Schoepke 2004e)

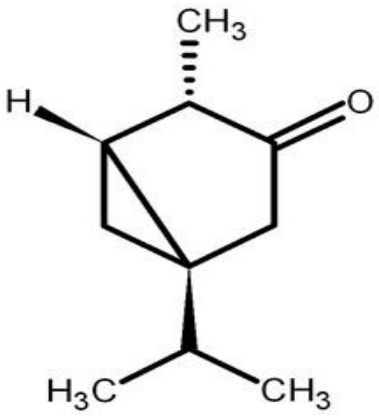
Figure 2:19-Sage plant (Blazel 2012)

Chemical composition

The glandular hair of the aerial plant contains the oil glands, which are rich in flavonoids, terpenoids, and monoterpenoids, while roots are rich in diterpenoids. The major constituents of sage oil are α-thujone (15-50%), β-thujone (3-9%), camphor (4-20%), 1,8-cineol (5-8%), camphene (5-6%), α-pinene (4-5%), limonene (1-2%), linalyl acetate (2%), borneol (2%) β-pinene, α-humulene, and β-caryophyllene (Figure 2:20). The major phenolic compounds present in sage are rosmarinic acid, caffeic acid, carnosol, and carnosic acid (Giannouli and Kintzios 2000; Karaousou and others 2000; Ulubelen 2000). Table 2:11 summarizes the active antioxidant chemicals present in sage plant. Nutritional constituents and ORAC values of ground sage are given in Table 2:12.



α-thujone



β-thujone

Figure 2:20-Chemical structure of α-thujone and β-thujone

Table 2:11-Active antioxidant chemicals in sage plant (USDA 2015m)

Active antioxidant chemical	Part of plant	Quantity (in ppm)
1, 8-cineol	Plant	390-6,288
α-humulene	Leaf	110-616
α-pinene	Plant	7-1,540
α-thujone	Leaf	200-10,172
β-caryophyllene	Leaf	500-760
β-pinene	Plant	20-1,540
borneol	Shoot	7,000
Camphene	Leaf	20-18,592
Camphor	Leaf	9,324
Limonene	Plant	39-2,380
Linalol	Plant	3,500
Linalyl acetate	Plant	6,048
Rosmarinic acid	Plant	30,000
Rosmarinic acid	Shoot	2,000-5,800
Tannin	Plant	20,000-80,000

Table 2:12-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of ground sage (USDA 2015l)

Nutrient	Units	Value per 100 g
Water	g	7.96
Energy	Kcal	315
Protein	g	10.63
Total lipid (fat)	g	12.75
Carbohydrate, by difference	g	60.73
Fiber, total dietary	g	40.3
Calcium, Ca	mg	1,652
Vitamin C, total ascorbic acid	mg	32.4
Vitamin B-6	mg	2.690
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	295
Vitamin A, IU	IU	5,900
Vitamin D	IU	0
Vitamin E (α-tocopherol)	mg	7.48
Fatty acids, total saturated	g	7.030
Fatty acids, total monounsaturated	g	1.870
Fatty acids, total polyunsaturated	g	1.760
H-ORAC	µmol TE/100 g	98,714
L-ORAC	µmol TE/100 g	21,214
Total-ORAC	µmol TE/100 g	119,929
TP	mg GAE/100 g	4,520

Medicinal and Pharmacological Properties

Sage has been studied for various medicinal and pharmacological properties including antibacterial, antiviral, antiseptic, renal, anti-inflammatory, cardiovascular, anti-mutagenic, antispasmodic, hypoglycemic, memory-retention, and hepatoprotective activities (Farag and others 1986; Dweck 2000; Li 2000; Baricevic and others 2001; Eidi and others 2006; Imanshahidi and Hosseinzadeh 2006; Riznar and others 2006; Bozin and others 2007; Longaray Delamare and others 2007). It contains bactericidal compounds which are useful for sore throat, and because of high tannin contents it can be used as an astringent. High tannin content and antimicrobial activities are useful in dental care preparations as it reduces the growth of plaques, inhibits gingival inflammation, and also has useful effects on caries prophylaxis (Baricevic and Bartol 2000; Dweck 2000; Li 2000).

It is suggested that a sage tea after a meal is good for digestion, and also considered as a good nerve and blood tonic. Sage tea is also used for excessive sweating, and to cure cold and cough. Animal studies indicated that sage extracts have a hypoglycaemic effect when it effectively reduced the serum glucose in diabetic rats in 3h (Baricevic and Bartol 2000; Eidi and others 2005). Animal studies also concluded its depressant action and hypotensive activity on the central nervous system (Baricevic and Bartol 2000). Essential oil of sage also showed strong antibacterial activity against *Escherichia coli, Salmonella typhi, Salmonella enteritidis, Shigella sonnei, Bacillus cereus, Bacillus megatherium, Bacillus subtilis, Aeromonas hydrophila, Aeromonas sobria, and Klebsiella oxytoca,* and antifungal activity against six fungi (Bozin and others 2007; Longaray Delamare and others 2007). Some studies reported that gram-negative bacteria are less sensitive to sage oil as compared to gram-positive bacteria (Baricevic and Bartol 2000). *In vitro* studies confirmed the antispasmodic effect of sage extract. Sage extract reduced the muscle contraction by 60-80%, induced by acetylcholine, histamine, serotonin, and barium chloride (Baricevic and Bartol 2000). From various studies, it is concluded that polyphenolic compounds such as rosmarinic acid, carnosol, carnosic acid, ursolic acid, apigenin, and caffeic acid are responsible for the medicinal and pharmacological properties of sage (Farag and others 1986; Imanshahidi and Hosseinzadeh 2006; Longaray Delamare and others 2007).

Antioxidant Properties

The strong antioxidant properties of sage can be used for the treatment and prevention of various degenerative diseases as well as in the food industry to reduce lipid oxidation and to increase the shelf-life of the foods (Wong and others 1995; Wang and others 1998; Deans and Simpson 2000; Lu and Yeap Foo 2001; Bandoniene and others 2002; Exarchou and others 2002). Chang and others (1977) found that the yield of essential oil increases with the polarity of the solvent. The methanol extract showed the highest antioxidant activity in lard when stored at 60°C up to 12 days, followed by chloroform, acetone, ethyl ether, benzene, and hexane extract (Table 2:13).

Table 2:13-Antioxidant activity of different sage extracts (Chang and others 1977)

	Peroxide value (meq/kg) Prime steam lard stored for days at 60^{0}C			
Additives (0.02%)	**0**	**4**	**8**	**12**
Control, no additive	2.25	8.02	23.09	56.41
Methanol extract	2.25	2.94	3.53	4.39
Chloroform extract	2.25	3.43	4.06	4.38
Acetone extract	2.25	3.31	4.61	6.16
Ethyl ether extract	2.25	3.16	4.24	5.23
Benzene extract	2.25	3.95	7.46	13.68
Hexane extract	2.25	7.55	21.48	61.87

Bozin and others (2007) found that sage essential oil possesses stronger ($p<0.05$) antioxidant activity than BHT, and according to them oxygenated monoterpenes (α- and β-thujone, bornyl acetate, camphor, and menthone) and mono and sesquiterpene hydrocarbons are responsible for the neutralization of DPPH radicals. In another study, Miura and others (2002) isolated a new abietane diterpenoid, 12-O-methyl carnosol, along with 11 abietane diterpenoids, 3 apianane terpenoids, 1 anthraquinone, and 8 flavonoids from the sage leaves. They also evaluated antioxidant activity by the oil stability index method, and found that carnosol, rosmanol, epirosmanol, isorosmanol, carnosic acid, and galdosol have high antioxidant activity as compared to α-tocopherol. The strong antioxidant activities of these compounds were attributed to the presence of an *ortho*-dihydroxy group in molecule. These conclusions are in collaboration with the findings of Wang and others (1998) and Lu and Yeap Foo (2001).

Turmeric

Botanical Name: *Curcuma longa* L.

Family: *Zingiberaceae* (Ginger Family).

Synonyms: *C. domestica* Vale.; *Amomum curcuma* Jacq.; *C. domestica* Loir., Indian saffron, curcuma, yellow ginger, yellow root.

Common Names: Spanish: curcuma; German: Gelbwurz; French: terre merite; Chinese: yu-chin; Hindi: haldi; Sanskrit: haridra; Thai: kamin.

History

Turmeric, the "golden spice" or the "spice of life," has been used for thousands of years in medicine, food, and as a dye. The ancient Indo-European people highly valued it for its medicinal properties. The genus name *Curcuma* is derived from the Arabic and Hebrew word "*kurkum*" and the name turmeric is derived from Latin word "*terramerita,*" which means "deserved earth" or "meritorious earth," and refers to the yellow color of ground turmeric, which looks like a mineral pigment. In Sanskrit, turmeric has at least 55 different names and because of its brilliant yellow color it is also known as "Indian saffron" or "yellow root" (Ravindran 2007; Prasad and Aggarwal 2011).

Turmeric is native to Southeast Asia and by the 7th Century it reached China and later reached to West Africa and Jamaica in the 13th Century and the 18th Century respectively. By the late 20th Century, it became popular in western countries for its potential health benefits. Marco Polo also described turmeric as a plant having properties similar to saffron, but it's not true saffron. Turmeric has a long history of medicinal use. It was an important herb in Ayurvedic and Unani medicine. It was around 500 BCE that turmeric emerged as an important part of Ayurvedic medicine. In Ayurveda, it has been used as a stomachic, tonic, blood purifier, and in the treatment of skin diseases. Greek physician Dioscorides also used turmeric as a medicinal plant (Ravindran 2007; Remadevi and others 2007; Prasad and Aggarwal 2011).

In Indian culture, the importance of turmeric goes far beyond medicine. The Hindu religion sees turmeric as auspicious and sacred. It is used to worship the Sun God and was also worn by people as a part of the purification process. It is also used for protection from evil spirits. Buddhists monks used turmeric to dye their robes because of its yellow color. Nowadays,

pharmacy and cosmetic companies are conducting various studies so that turmeric can be used in drugs and beauty products for its beneficial properties (Nahar and Sarker 2007; Ravindran 2007; Remadevi and others 2007; Chempakam and Parthasarathy 2008; Prasad and Aggarwal 2011; Sasikumar 2012).

Producing Regions

It is native to Southeast Asia and is extensively cultivated in different parts of India, including Kerala, Tamilnadu, Karnataka, and West Bengal. India is the largest producer, consumer, and exporter of turmeric. India produces approximately 90% of total world production with an annual production of 843,000 tons. Indian turmeric is valued as the best in the world because of its highest curcumin content. It is also grown in Pakistan, Myanmar, Vietnam, Korea, some South Pacific islands, Cambodia, Thailand, China, Taiwan, Sri Lanka, Indonesia, Malaysia, Nepal, Japan, Philippines, Madagascar, Peru, some Caribbean islands, and Central America (Ravindran 2007; Prasad and Aggarwal 2011; Sasikumar 2012).

Botanical Description

Turmeric is an erect, annual or perennial herb that grows up to 1-3 feet in height and produces both flowers and a rhizome. The leaves are long, dark green in color, lanceolate-shaped, and tapered at both ends. The dull yellow flowers appear in cylindrical inflorescence with a central spike of 10-15 cm long. The rhizome has an appearance similar to ginger and length of the rhizome is approximately 1-3 inches with a diameter of 1 inch. The rhizomes grow symbolically and the color of rhizome is yellowish brown with a dull orange flesh, while the dried powder is yellow in color (Figure 2:21). It has a peculiar fragrant odor with a slight bitter acrid taste. There are about 60 varieties available in India with its inherent properties (Ravindran and others 2007; Chempakam and Parthasarathy 2008; Sasikumar 2012).

Useful Plant Parts

Turmeric is used as dried rhizome powder or whole. It is also used fresh. Turmeric oleoresin and turmeric oil are also used occasionally in the food and perfume industries. Ground turmeric is the main part of curry powder in developing countries. Turmeric extracts are also used as a coloring agent for cotton, silk, and wood (Balakrishnan 2007; Prasad and Aggarwal 2011; Sasikumar 2012).

Figure 2:21-Turmeric plant (Schneider Illustration 2014)

Chemical Composition

Turmeric rhizome contains 11-13% moisture, 5-6.6% curcumin, 3-5% volatile oils, 47.5 mg iron, 3 g sugars, and <0.5% extraneous matter. Curcumin is the major component responsible for the yellow color of turmeric. Other than curcumin, minor constituents such as curcuminoids, curcumin 1,7-*bis* (4-hydroxy-3-methoxy-phenyl)-1,6-hepatadiene-3,5-dione, and demethoxy curcumin [4-hydroxy-cinnamoyl (4-hydroxy-3-methoxycinnamoyl) methane and *bis*-demethoxy curcumin [*bis*-(4-hydroxy-cinnamoylmethane] are also responsible for imparting yellow color (Figure 2:22). The essential oil of turmeric contains turmerone (60%), curlone (18-21%), *ar*-turmerone (19-25%), zingiberene (25%), cineole, forneol, sabinene, and α-phellandene. Turmeric oil is pale yellow in color with a peppery and aromatic odor. The chemical compounds tumerone, *ar*-turmerone, and zingiberene are responsible for characteristic aroma. Turmeric is also considered a good source of ω-3 fatty acid and α-linolenic acid (2.5%). The Indian variety Allepey contains the highest amount of curcumin and is considered to be the best variety (Nahar and Sarker 2007; Premavalli 2007; Chempakam and Parthasarathy 2008; Prasad and Aggarwal 2011; Sasikumar 2012). The nutritional composition and ORAC values of ground turmeric are

shown in Table 2:14. Table 2:15 summarizes the active antioxidant chemicals present in turmeric plant.

Table 2:14-Nutritional composition and oxygen radical absorbance capacity (ORAC) values of ground turmeric (USDA 2015n)

Nutrient	Units	Value per 100 g
Water	g	12.85
Energy	kcal	312
Protein	g	9.68
Total lipid (fat)	g	3.25
Carbohydrate, by difference	g	67.14
Fiber, total dietary	g	22.7
Calcium, Ca	mg	168
Potassium	mg	2,080
Vitamin C, total ascorbic acid	mg	0.7
Vitamin B-6	mg	0.107
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	0
Vitamin A, IU	IU	0
Vitamin D	IU	0
Fatty acids, total saturated	g	1.838
Fatty acids, total monounsaturated	g	0.449
Fatty acids, total polyunsaturated	g	0.756
H-ORAC	µmol TE/100 g	44,776
L-ORAC	µmol TE/100 g	82,292
Total-ORAC	µmol TE/100 g	127,068
TP	mg GAE/100 g	2,754

Table 2:15-Active antioxidant chemicals in the turmeric plant (Suhaj 2006; USDA 2015o)

Active Antioxidant Chemical	Part of Plant	Quantity (in ppm)
ar-tumerone	Rhizome	5,800
Ascorbic acid	Rhizome	293
bis-demethoxycurcumin	Rhizome	67-27,000
Curcumin	Rhizome	9-38,888
Cyclo-isoprenemyrcene	Rhizome	8,500-29,750
Demethoxycurcumin	Rhizome	500-11,000
p-coumaric acid	Rhizome	345
tumerone	Rhizome	1,800-43,200
Zingiberene	Rhizome	750-18,000

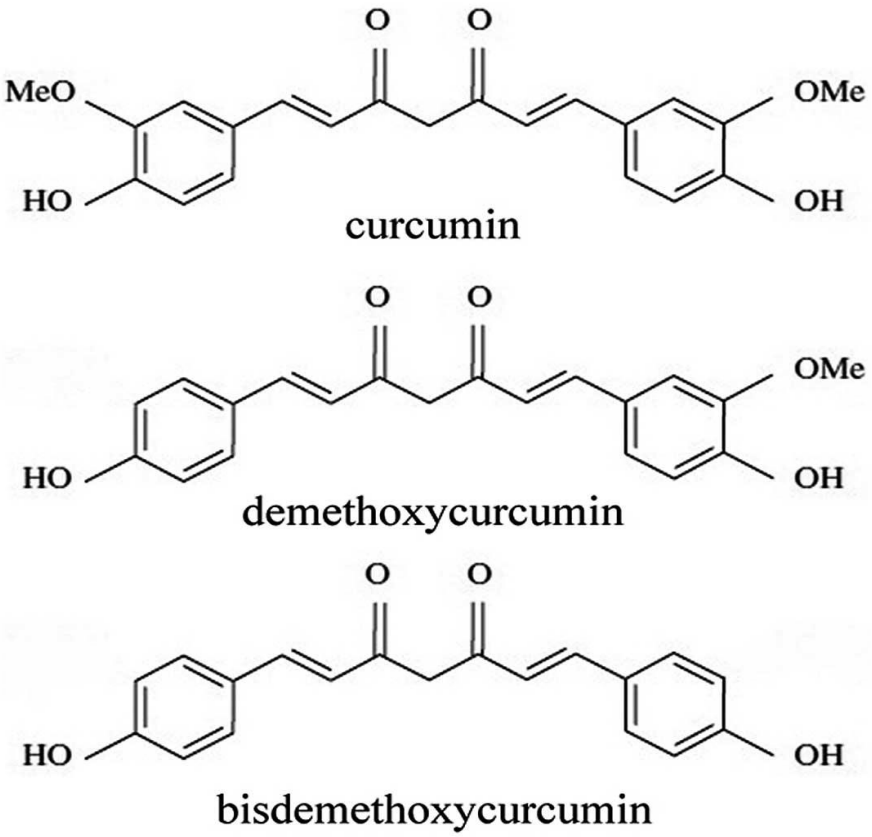


Figure 2:22-Chemical structure of curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Nahar and Sarker 2007; Xu and others 2012)

Medicinal and Pharmacological Properties

Turmeric has long been used in the traditional medicinal systems like Ayurveda, Unani, and Chinese systems to treat a wide variety of conditions, including asthma, bronchial hyperactivity, rheumatism, cough, sinusitis, sprains, cuts, injuries, swellings, skin diseases, insect bites, flatulence, indigestion, diarrhea, anorexia, bruises, and liver disorders. Turmeric also has good healing properties, and is usually used as a paste to heal the wounds and to protect from bacterial infection. In some parts of Bangladesh, turmeric paste is also put on the umbilical cord after childbirth (Remadevi and others 2007; Sarker SD and Nahar L 2007; Chempakam B and Parthasarathy VA 2008; Prasad and Aggarwal 2011; Sasikumar B 2012).

Turmeric is considered to have antibiotic, antifungal, anti-venomous, antiviral, anti-inflammatory, antibacterial, antirheumatic, choleratic, hypocholesterolemic, cytotoxic, spasmolytic, antidiabetic, antiseptic, antitoxic, anti-HIV, and anti-Alzheimer's properties. *In vitro*

and *in vivo* studies showed that turmeric is significantly effective against *Bacillus cereus, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Staphylococcus* spp., *Salmonella typhi, Aspergillus niger, Paramaecium caudatum, Trichophyton gypseum, Helicobacter pylori, Trichophyton longifusus, Mycobacterium tuberculosis,* and *Cornebacterium diphtheria*. Turmeric constituents are also effective against various plant pathogens such as *Ralstonia solanacearum, Xanthomonas oryzae, Helminthosporium sacchari,* and *Rhizoctonia solani*. (Srivastava and others 1995; Srimal 1997; Maheshwari and others 2006; Sarker and Nahar 2007; Ahmed and Gilani 2009; Prasad and Aggarwal 2011; Sindhu and others 2011; Sasikumar 2012; Marathe and others 2013).

Bundy and others (2004) found that turmeric extract significantly reduced the Irritable Bowel Syndrome in healthy adults and a reduction in the abdominal pain/discomfort score was also observed. The yellow pigment of turmeric, curcumin, has various medicinal properties. It easily breaks down fats and toxins and creates clear bile flow, therefore protecting the liver from alcohol-induced damage. Various animal studies showed that turmeric can be effective against various human diseases such as diabetes, obesity, neurologic and psychiatric disorders, and cancer. Curcumin has strong anti-inflammatory property and can be used to treat various inflammatory problems, including arthritis, liver, and gall bladder disorders. *In vivo* and *in vitro* studies of turmeric also shown to have potential health benefits in memory retention and in the prevention of Alzheimer's disease (Aggarwal and others 2007; Prasad and Aggarwal 2011; Sasikumar B 2012).

Curcuminoids, a group of phenolic compounds, also have antioxidative effects and have been used to treat a wide variety of cancers, including those of the colon, breast, lung, mouth, prostate, and esophagus. Ahmed and Gilani (2009) suggested that curcuminoids (a mixture of curcumin, bisdemethoxycurcumin, and demethoxycurcumin) have a powerful acetylcholinesterase inhibitory and memory enhancing activities. Therefore, turmeric, curcumin, and its analogs have strong health benefits, and can be useful for the treatment and prevention of various diseases (Premavalli 2007; Remadevi and others 2007).

Antioxidant Properties

In Asian countries, turmeric powder is a main ingredient in curry powder and it is used with other spices to make different dishes including meat, soup, and vegetable dishes. Turmeric

powder is used as a natural preservative in pickles, while oleoresin is generally used in brine pickles. Turmeric powder, oil, extracts, and oleoresin all have powerful antioxidant properties (Scartezzini and Speroni 2000; Sreekanth and others 2003; Balakrishnan 2007; Saker and Nahar L 2007; Chempakam and Parthasarathy 2008; Prasad and Aggarwal 2011; Sasikumar 2012). Kaur and Kapoor (2002) evaluated antioxidant activity of 36 vegetables through β-carotene and linoleic acid system, and turmeric was found to have the highest antioxidant activity (92%) among them. In a DPPH assay and a lipid peroxidation inhibition test, turmeric showed IC_{50} of <30µg/ml and IC_{50} of <32µg/ml, respectively. It also displayed 20% inhibition of the *in vitro*-induced OH^- attack to deoxyglucose (Ramos and others 2003). Turmeric methanolic extract also showed 50% peroxynitrile scavenging activity at IC_{50} of 1.7µg/ml (Kim and others 2003).

Sreekanth and others (2003) assessed the antioxidant property of "Smoke Shield" in human and animal models. "Smoke Shiels" is a formulation which contains supercritical carbon-dioxide turmeric extract, post-supercritical hydroethanolic turmeric extract with extracts of green tea and spices. Combination of all increase the activity of turmeric because of synergistic effect."Smoke Shield" showed 50% inhibition of superoxide radicals induced by photoreduction of riboflavin and 50% inhibition of hydroxyl radicals induced by Fenton reaction. The addition of "Smoke Shield" also reduced the lipid peroxidation in serum, the liver, and in the kidneys. Moreover, it increased the antioxidant enzymes, including catalase and superoxide dismutase, in mice blood, livers, and kidneys. Glutathione-S-transferase activity was also found to increase in livers and kidneys. These results suggested that "Smoke Shield" has strong antioxidant activity and can be used as an effective chemoprotective agent. It is suggested that in the reduction of lipid oxidation, curcumin has stronger antioxidant activity than alpha-tocopherol, pine bark extract, grape seed extract, and BHT (Sreejayan and Rao 1993). A mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin was also found to have a powerful antioxidant effect. Sometimes, turmeric is also used as oil preservative. Ramaswamy and Banerjee (1948) evaluated that turmeric as a dye has powerful antioxidant properties on coconut oil, groundnut oil, cottonseed oil, and sesame oil. This is due to the phenolic compounds present in turmeric dye. Turmeric is also reported to have strong antioxidant activity (at 10% concentration) in salted cooked fish, as it prevented lipid oxidation (Ramanathan and Das 1993). A dose-dependent relation was found in free radical scavenging activity and curcuminoids concentration (Balakrishnan 2007).

ORAC Values of Herbs and Spices

Table 2:16-Oxygen radical absorbance capacity (ORAC) values of herbs and spices

Herbs and Spices	Hydrophilic-ORAC	Lipophilic-ORAC	Total-ORAC
Cinnamon	143,264 µmol TE/100 g	3,326 µmol TE/100 g	131, 420 µmol TE/100 g
Clove	111,490 µmol TE/100 g	178, 793 µmol TE/100 g	290, 283 µmol TE/100 g
Nutmeg	12, 600µmol TE/100 g	42,625 µmol TE/100 g	69,640 µmol TE/100 g
Oregano	165, 712 µmol TE/100 g	22,582 µmol TE/100 g	175,295 µmol TE/100 g
Rosemary	112, 200 µmol TE/100 g	53,080 µmol TE/100 g	165,280 µmol TE/100 g
Sage	98,714 µmol TE/100 g	21,214 µmol TE/100 g	119,929 µmol TE/100 g
Turmeric	44,776 µmol TE/100 g	82,292 µmol TE/100 g	127, 068µmol TE/100 g

Chapter 3 - Antioxidant Assays

There are several assays to evaluate the total antioxidant capacity of a food system. Food has a complex matrix; therefore, one-dimensional assay may be inadequate to obtain a proper idea of the antioxidant capacity of a particular food system. So, for better results, more than one method can be used to measure the total antioxidant capacity. The most commonly used methods to determine total antioxidant capacity can be divided into two major groups on the basis of chemical reactions: assays based on single electron transfer (SET) reaction and assays based on hydrogen atom transfer (HAT) reaction. In the SET based assays, there is a change in color because the oxidant is reduced, which is monitored spectrophotometrically. The degree of color change is directly proportional to the antioxidant concentration present in the sample. In the HAT based assays, substrate (probe) and antioxidant compete for free radicals generated through the azo compound decomposition (Huang and others 2005; Prior and others 2005; Somogyi and others 2007).

The electron transfer reaction assays include the trolox equivalent antioxidant capacity (TEAC) assay, the ferric ion reducing antioxidant power (FRAP) assay, the cupric ion reducing antioxidant capacity (CUPRAC) assay, the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation radical scavenging capacity assay, the N,N-dimethyl-p-phenylenediamine (DMPD) assay, the Folin-Ciocalteau reagent (FCR) assay, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay. These reactions contain two components: antioxidants and oxidants which act as a probe. Color change of the sample is an indication of the presence or absence of antioxidants, and when color change stops, it is determined as an endpoint of the reaction. The graph is plotted using the change of absorbance on the y-axis and antioxidant concentration on the x-axis. The slope of the curve represents the antioxidant capacity. The results are expressed as Trolox equivalents (TE) or Gallic acid equivalents (GAE) (Huang and others 2005).

Hydrogen atom transfer assays include the crocin bleaching assay, the total radical-trapping antioxidant parameter (TRAP) assay, the inhibition of induced low density lipoprotein auto-oxidation (LDL), and the oxygen radical absorbance capacity (ORAC) assay. These assays have the following components: (1) an azo radical initiator; (2) ultraviolet or fluorescence molecular probe for monitoring; (3) antioxidant; and (4) reaction kinetic parameters for

quantification of antioxidant capacity. Other methods not included in these two groups are superoxide anion radical scavenging capacity assay, hydroxyl radical scavenging assay, hydrogen peroxide scavenging capacity assay, singlet oxygen scavenging capacity assay, and peroxynitrite scavenging capacity assay (Frankel and Meyer 2000; Huang and others 2005).

Oxygen Radical Absorbing Capacity (ORAC) Assay

The ORAC assay depends on free radical damage to a fluorescent probe, generally caused by an oxidizing reagent resulting in a loss of fluorescent intensity over time. This method was developed by Cao and others in 1993. In this assay, they used the indicator protein β-phycoerythrin (β-PE) isolated from *Porphyridium cruentum* as a fluorescent probe and measured antioxidant activity against peroxyl radical induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The fluorescence decay of β-PE was used as an indication of damage from its reaction with the peroxyl radical.

Ou and others (2001) introduced an improved version of ORAC by replacing β-PE with a more stable and less expensive synthetic nonprotein fluorescent probe, fluorescein (FL) (3',6'-dihydroxyspiro[isobenzofuran-1[3H], 9'[9H]-xanthen]-3-one). In this method, LC/MS was used to detect oxidized FL products which were induced by a peroxyl radical, and a hydrogen atom transfer mechanism was used to determine the process. Loss of fluorescence due to oxidative damage is measured kinetically and the AUC (area under the curve) is calculated as the integral of the area under the curve. The resultant antioxidant capacity is expressed as micromoles of trolox equivalents per unit of the sample. The improved assay provides a direct measure of the hydrophilic and lipophilic chain breaking antioxidant capacity versus peroxyl radicals. Huang and others (2002) also developed high-throughput ORAC assay by using a microplate reader and a robotic handling system. Schematic illustration of ORAC assay is given in Figure 3:1 (Somogyi and others 2007)

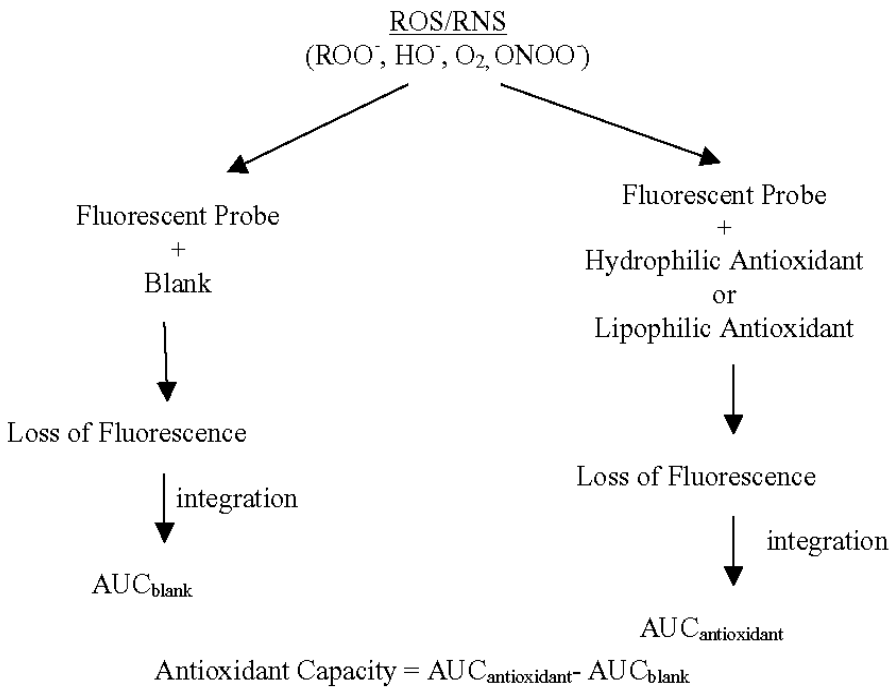


Figure 3:1-Schematic illustration of oxygen radical absorbing capacity (ORAC) assay (Somogyi and others 2007)

Total Phenol Assay by Folin-Ciocalteu Reagent (FCR)

This is the most popular assay to assess total phenolic compounds. Originally, this assay was introduced for tyrosine and later it was modified to assess total phenols in wine. This assay evaluates the change in color from a yellow Folin-Ciocalteu reagent color to dark blue in the presence of antioxidants, and measures the absorbance at 750-765 nm (Huang and others 2005). Gallic acid is generally used as a standard and results are expressed in GAE equivalents. It is a very simple, popular, and inexpensive method. It measures the reducing capacity of the sample, and a direct correlation is also found between the total phenolic contents and the total antioxidant capacity.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Capacity Assay

This is a spectrophotometric decolorization assay introduced by Blois (1958). The DPPH (2,2-diphenyl-1-picrylhydrazyl) is a dark colored crystalline powder composed of a stable free radical which shows maximum absorbance at 517 nm and reduced absorbance when radicals are scavenged or reduced (Figure 3:2). DPPH has a deep purple color in solution and changes to

yellow or colorless when it encounters a proton-donating substance such as an antioxidant and a radical species (Figure 3:3).

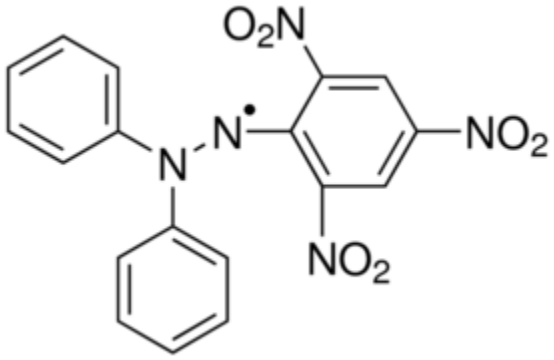


Figure 3:2-2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Huang and others 2005)

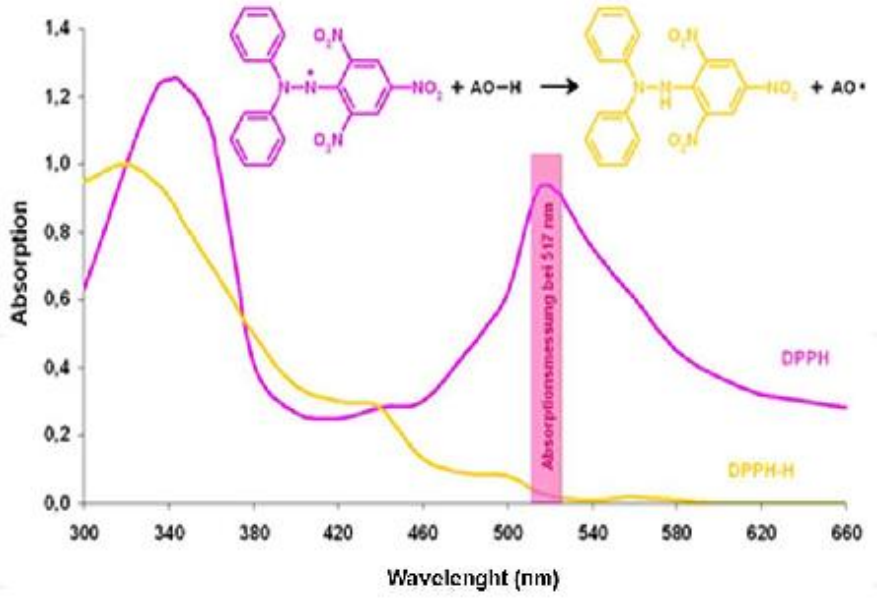


Figure 3:3-DPPH radical scavenging activity at 517 nm (Pérez and Aguilar 2013)

The data is commonly reported as EC_{50}, which is the AH concentration required to reduce the initial DPPH concentration by 50%. A lower absorbance of reaction mixture indicates a higher free radical scavenging or antioxidant activity, and higher absorbance indicates less antioxidant activity (Huang and others 2005).

The percentage of DPPH scavenging activity is calculated as

%DPPH Scavenging Activity = 100 x [Abscontrol-Abssample]/[Abscontol]

This method is based on the reduction of alcoholic DPPH solutions at 517 nm in the presence of hydrogen donating antioxidants due to the formation of non-radical form DPPH-H by the reaction (Erkan and others 2008).

$$DPPH\bullet + AH \rightarrow DPPH\text{-}H + A\bullet$$

$$DPPH\bullet + R\bullet \rightarrow DPPH\text{-}R$$

There are several changes proposed to compare the data obtained from different laboratories or from the same group at different times. Sanchez-Moreno and others (1998) introduced a new parameter, "antiradical efficiency" (AE), to explain the DPPH scavenging capacity:

$$AE = 1/EC50 \text{ X } TEC50$$

where, EC_{50} was the required amount of an antioxidant to decrease the initial $DPPH^{\bullet}$ concentration by 50% and T_{EC50} was the time required to reach the steady state for EC_{50}.

Cheng and others (2006) developed the RDSC (relative DPPH radical scavenging capacity) assay that allows comparing results obtained in different laboratories even in different concentrations, because it is independent of either sample or initial DPPH radical concentrations. The DPPH method is simple, rapid, reproducible, sensitive, sample-polarity independent, and does not require expensive reagents or instruments. It has some limitations as this method does not provide information about the type of protected lipid substrate and is not specific with respect to the scavenged radical species (Blois 1958; Brand-Williams and others 1995; Frankel and Meyer 2000; Huang and others 2005; Kedare and Singh 2011).

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Figure 3:4). The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue-colored ferrous complex Fe^{II} tripyridyltriazine (Fe^{II}-TPTZ) from a colorless Fe^{III}-TPTZ compound by the action of electron-donating antioxidants under acidic (pH 3.6) conditions. The FRAP assay is based on a redox reaction in which antioxidants act as reductants and ferric ions act as oxidants. The increase in

absorbance is directly correlated to the total ferric reducing power of the tested sample. The FRAP assay was originally developed to measure "antioxidant power" of blood plasma, but this assay has also been used to evaluate the total antioxidant capacity of the different food system. The FRAP assay is simple, speedy, inexpensive, and highly reproducible. The major drawback of this assay is, that it cannot measure the antioxidant capacity of the compounds that contain thiol groups or those which react with Fe (II) (Benzie and Strain 1996; 1999; Huang and others 2005).

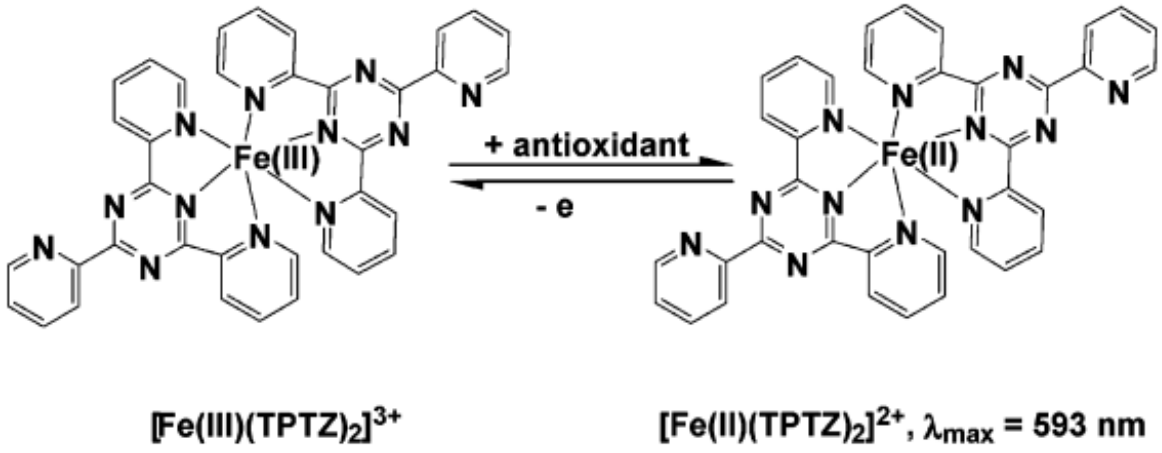


Figure 3:4-FRAP assay (Huang and others 2005)

ABTS Cation Radical ($ABTS^{\bullet+}$) Scavenging Capacity Assay

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) assay is a decolorization method applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, carotenoids, hydroxycinnamates, and plasma antioxidants. ABTS free cation radical ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium persulfate and then by reduction in the presence of hydrogen donating antioxidants. $ABTS^{\bullet+}$ has a blue/green color with maximum absorption spectra at 734 nm. ABTS radical cation is produced by reacting ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the analysis, $ABTS^{\bullet+}$ solution is diluted in deionized water or ethanol to an absorbance of 0.7(±0.02) at 734 nm. Plant extract of 100µL is mixed with 3 ml of $ABTS^{\bullet+}$ solution and absorbance is read after 10 min incubation at 734 nm. The result is expressed as the trolox equivalent antioxidant capacity (TEAC). This spectrophotometric assay is simple, rapid, is suitable for antioxidants in food components, and it is more reactive than DPPH radicals.

Another advantage of this assay is that it can be used at different pH values; therefore, the effect of pH on the antioxidant capacity of selected sample can be analyzed. The only disadvantage of this assay is the non-physiologically unstable radicals (Huang and others 2005; Prior and others 2005; Dudonne and others 2009).

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

The CUPRAC assay was introduced by Apak and others (2004). This method is based on the reduction of copper (II)-neocuproine [Cu(II)-Nc] to copper(I) by the antioxidants present in the sample. The reduction of Cu^{2+} to Cu^{+} has a maximum absorbance at 450 nm (Figure 3:5).

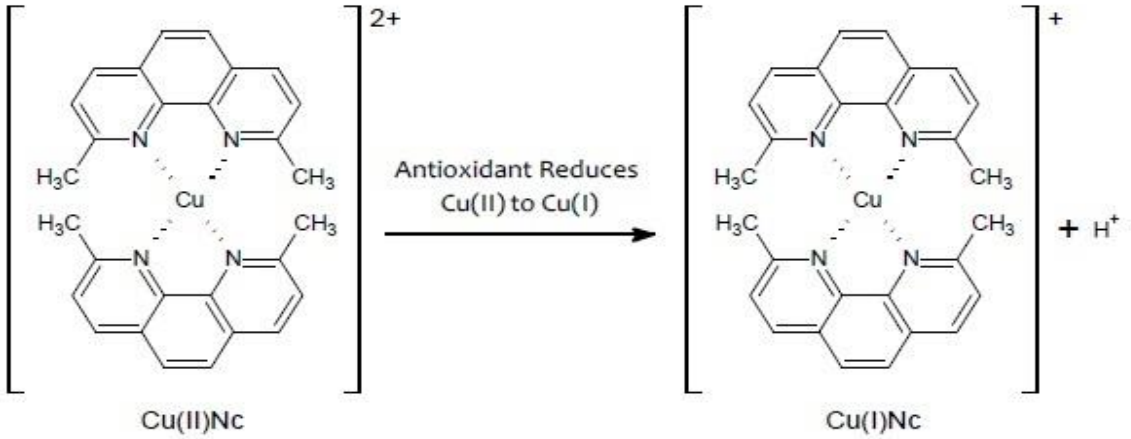


Figure 3:5-The CUPRAC reaction (Özyürek and others 2011)

This method is rapid, reliable, selective, and suitable for a large variety of antioxidants. It has lower redox potential than ferric method and reacts to a much broader range of thiol antioxidants than the FRAP method. It is much more stable than chromogenic radical reagents such as ABTS and DPPH. It can measure lipophilic and hydrophilic antioxidants, and it measures the antioxidant capacity at nearly physiological pH (i.e. pH 7) (Apak and others 2010; Özyürek and others 2011).

Table 3:1 summarizes different assays, their reaction mechanism, method of quatification, and source of free radical for individual assay.

Table 3:1-Mechanism of chemical assays commonly used to evaluate antioxidant capacity of food components

Assay	**Source of Free Radical**	**Reaction Mechanism**	**Method of Quantification**
ORAC	Dissolve AAPH in buffer to form peroxyl radicals	HAT	β-phycoerythrin Fluorescein
FCR	Mixture of sodium tungstate, sodium molybdate, concentrated hydrochloric acid, phosporic acid.	SET	Measure absorbance at 750-765 nm
DPPH	Dissolve DPPH in ethanol	SET	Measure the absorbance at 517 nm
FRAP	Fe (III)/tripyridyltriazine complex	SET	Measure the absorbance at 593 nm
ABTS	Oxidize ABTS with potassium persulfate	SET	Measure the absorbance at 734 nm
CUPRAC	Copper (II)-neocuproine [Cu(II)-Nc]	SET	Measure absorbance at 450 nm

Chapter 4 - Effects of Heat Treatments on Natural Antioxidants

Oxidation of lipids causes rancidity and reduces the shelf life and nutritional quality of the food products. Meat and meat products are highly susceptible to oxidation due to their complex physical structures and chemical compositions. Therefore, antioxidants are added to prevent deterioration from the lipid oxidation. Synthetic antioxidants are widely used; however, there are some toxicity and health concerns associated with them. As a result, consumers are becoming increasingly conscious about the food additives and a preference for natural additives has encouraged the development of plant-based natural antioxidants. Herbs and spices are traditionally added to dishes for their flavoring and preserving characteristics. They are commonly added as whole, fresh chopped, ground paste, or as extract and usually cooked at around 100°C. Herbs and spices also contain excellent amounts of phenolic compounds that have significant antioxidant effectiveness (Table 4:1; 4:2; 4:3; 4:4, 4:5). It is well known that food processing conditions such as cooking at higher temperatures make compositional and structural changes to foods and it is assumed that herbs and spices are also consumed after thermal treatments. Therefore, the effectiveness of herbs and spices as antioxidants could either be significantly altered or remain unchanged by temperature changes (Erickson 2002; Reische and others 2002; Brewer 2011). There is very limited information available in this area. Therefore, the aim of this chapterwas to summarize the available data that focused on the thermal decomposition of natural antioxidants.

Table 4:1-Selected antioxidant compounds identified in selected herbs (Brewer 2011)

	Phenolic diterpenes						Phenolic acids		
Herbs	Rosmanol	Epi-, isorosmanol	Rosmadial	Rosmaridiphenol	Carnosic acid	Carnosol	Rosmarinic acid	Simple phenolic acids	Caffeic acid
Rosmary	X	X		X	X	X	X	X	X
Oregano							X	X	
Sage	X	X	X		X	X	X	X	

Table 4:2-Selected antioxidant compounds identified in selected herbs (Brewer 2011)

	Phenylpropanoids				Volatiles		
Herbs	Thymol	Carvacrol	p-Cymene	Flavonoids	1,8-cineole	α-Thujene	α,β-pinene
Rosmary	X	X			X		X
Oregano	X	X	X	X		X	X
Sage				X	X	X	X

Table 4:3-Selected antioxidant compounds identified in selected spices (Brewer 2011)

	Phenolic acids					
Spices	Vanillic acid	Caffeic acid	Gallic acid	p-Hydroxybenzoic acid	p-Hydroxybenzaldehyde	p-Coumaric acid
Cinnamon	X	X	X	X	X	X
Clove		X	X	X	X	X
Nutmeg	X	X	X			
Turmeric	X	X				X

Table 4:4-Selected antioxidant compounds identified in selected spices (Brewer 2011)

Phenylpropanoids				
Spices	Carvacrol	Cuminaldehyde	Cinnamaldehyde	Eugenol
Cinnamon			X	X
Clove	X		X	X
Nutmeg	X			X
Turmeric		X		X

Table 4:5-Selected antioxidant compounds identified in selected spices (Brewer 2011)

Volatiles									
Spices	α,β-Pinene	α,β-Carophyllene	Curcumin	Argentaene	Camphene	Linalool	Piperine	Limonene	Cymene
Cinnamon		X			X	X	X	X	X
Clove		X				X			X
Nutmeg	X			X	X	X		X	X
Turmeric	X		X						X

Effects of Heat Treatment

Tomaino and others (2005) studied the effect of thermal treatment on antioxidant activity and chemical composition of spice essential oils. Cinnamon, clove, nutmeg, and oregano essential oils were incubated at 80, 100, 120, and 180°C for 3 h and immediately cooled in ice bath and used to analyze radical-scavening activities. Clove oil showed the highest ($p<0.05$) free radical-scavenging activity at room temperature, followed by cinnamon, nutmeg, and oregano. This agreed with the fact that eugenol with electron-repelling group at the ortho position is responsible for higher activities. After heating at 180°C, a significant ($p<0.05$) positive effect was found in nutmeg oil but there was no change in other essential oils (Table 4:6). Nutmeg oil showed significantly ($p<0.05$) higher DPPH free radical-scavenging activity with a change in its chemical constituents. Safrole and myristicin contents were found to be elevated while a reduction was observed in α-pinene, β-pinene, and sabinene levels. It is assumed that the heating process increased the safrole and myristicin levels, which are responsible for higher free radical-scavenging activity of nutmeg oil. Therefore, thermal treatments may also increase the antioxidant activity by releasing bound antioxidants or by the formation of new compounds with antioxidant properties.

Table 4:6-Change in DPPH free radical-scavenging activity of essential oils kept at room temperature or exposed to different temperature for 3 h (Tomaino and others 2005)

Percent Remaining DPPH					
Essential oils	Room Temp.	80^0C	100^0C	120^0C	180^0C
Clove	34.8±2.92	36.6±3.92	40.4±3.58	37.72±3.85	40.3±3.82
Cinnamon	55.3±4.58	53.3±4.56	51.9±4.82	49.24±3.95	44.7±4.16
Nutmeg	51.8±4.78	51.2±4.32	45.1±3.94	44.26±4.14	26.6±1.96
Oregano	51.8±4.71	53.0±4.62	57.50±4.48	57.2±4.93	48.4±4.53
Standard: Eugenol	43.5±3.65	47.2±3.58	49.8±4.72	49.4±3.56	44.6±4.28

Data are expressed as means±SD of three experiments and student's t-test used for paired data. $P < 0.05$ vs. the respective room temperature.

In another study, Khatun and others (2006) evaluated the antioxidant activity of spices by using ethanol as an extraction solvent. The ethanol extract of cloves, cinnamon, nutmeg, mace, and turmeric shown to have antioxidative potential such as DPPH radical-scavenging and peroxy radical-scavenging activities. Before heating, cloves showed the highest radical-scavenging activity (1353.3±103.0 µmol Trolox eq./g) followed by cinnamon (364.0±10.3 µmol Trolox eq./g), nutmeg (50.9±4.6 µmol Trolox eq./g), mace (18.1±0.9 µmol Trolox eq./g), and turmeric (9.6±0.7 µmol Trolox eq./g). These results agreed with Shobana and Naidu (2000), where they reported that clove has higher antioxidant activity than cinnamon. A significant change was observed in the DPPH radical-scavenging activity after the heat treatment (100°C) of spices, where cloves, mace, and turmeric showed an increase in the activity. Change in the DPPH radical-scavenging activity is presented in Table 4:7. Turmeric antioxidant activity increased by three times after 6 h of heating ($p<0.001$), whereas no change was found in cinnamon. They also found that nutmeg is coagulated after heating and that might have decreased the extraction ability of nutmeg, resulting in a decreased radical-scavenging activity. The difference in nutmeg activities reported in the previous study (Tomaino and others 2005) could be attributed to the differences in the heating methods and extraction solvents used.

Table 4:7-Change in DPPH radical-scavenging activity of some spices after heating at 100°C (Khatun and others 2006)

Spices	DPPH radical-scavenging activity (µmol Trolox eq/g)			
	Heating at 100°C			
	0h	1h	3h	6h
Clove	1353±103.0	1536.4±132.2 (1.1)*	1572.1±120.7 (1.2)*	1671.1±157.2 (1.2)*
Cinnamon	364.0±10.3	356.5±29.4	354.5±33.3	303.3±27.8
Nutmeg	50.9±4.6	28.7±1.6 (0.6)**	38.5±3.5 (0.8)**	45.3±2.6
Mace	18.1±0.9	19.6±1.4	22.2±1.1	23.9±0.9*
Turmeric	9.6±0.7	14.5±0.3 (1.5)*	23.3±1.2 (2.4)**	28.3±0.4 (2.9)***

The values are the means±SD of three experiments. *$p<0.05$, **$p<0.01$, ***$p<0.001$

The most likely explanation for higher antioxidant activity is the main active constituents of spices such as eugenol (cloves, cinnamon), myristicin (mace, nutmeg), curcumin (turmeric) are fat soluble and here ethanol was used as the extraction solvent. Therefore, due to thermal treatment, cell walls were disrupted and active components passed into the cell, leading to an increase in the solubility of active constituents that cause an increase in the antioxidant activities (Khatun and others 2006). This suggestion is further confirmed by Shobana and Naidu (2000). They reported that thermal treatments liberate the bound antioxidants and increase the antioxidant activity. Maeda and others (1992) also suggested that the release of active components might be possible due to disruption of the cell walls and sub cellular compartments when they studied the effects of heating on various vegetables. Dewanto and others (2002) also agreed with the concept and found that heating decomposed the cell structure and released lycopene content from the insoluble portion and increased the antioxidant activity of tomato.

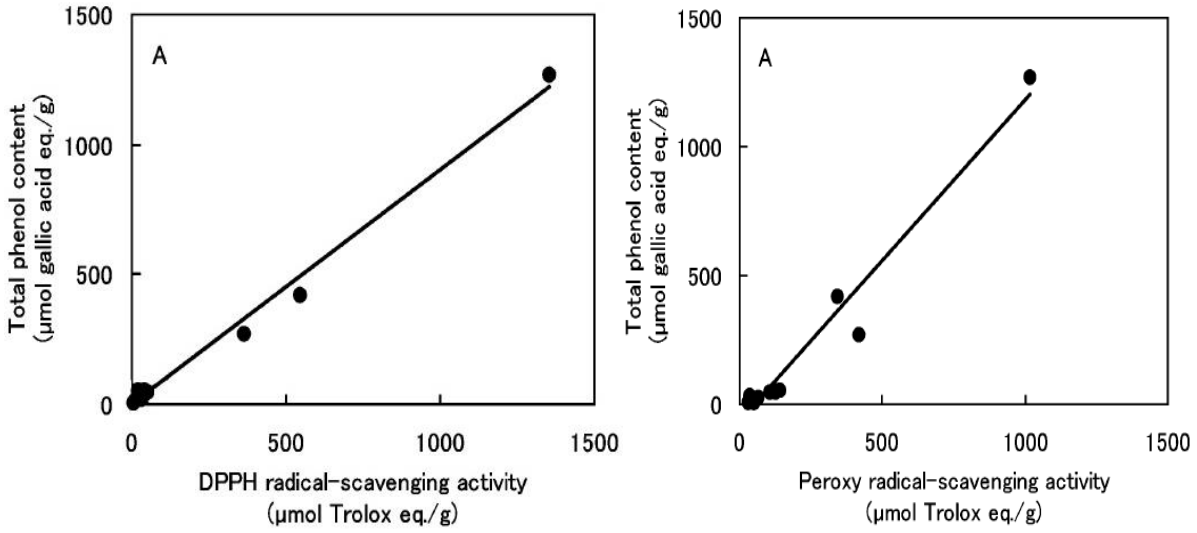
Khatun and others (2006) also evaluated the peroxy radical-scavenging activity of spices and, in the study clove (1018.7±99.2 µmol Trolox eq./g) showed the highest peroxy radical-scavenging activity (in both raw and heating samples) followed by cinnamon and other spices. After heating a significant change was observed in mace and turmeric activity and there was no change in the activity of cloves, cinnamon, and nutmeg (Table 4:8). Gulcin and others (2004) also quantified same level of superoxide radical-scavenging activity of ethanol and water extract of clove buds and this activity was powerful than BHA, BHT, and tocopherol.

Table 4:8-Change in peroxy radical-scavenging activity of some spices after heating at 100°C (Khatun and others 2006)

Spices	Peroxy radical-scavenging activity (µmol Trolox eq/g)			
	Heating at 100°C			
	0h	1h	3h	6h
Clove	1018.7±99.2	1054.7±83.7	1235.7±16.4	1215.8±120.4
Cinnamon	417.4±19.2	469.7±39.6	455.9±9.1	420.0±27.7
Nutmeg	104.3±6.8	84.9±4.6	105.6±5.5	117.4±9.2
Mace	58.6±4.8	68.3±4.3 (1.2)*	81.1±8.0 (1.4)**	74.8±6.6 (1.3)*
Turmeric	48.4±3.7	88.2±7.5 (1.8)**	112.1±11.1 (2.3)**	142.6±13.0 (2.9)**

The values are the means±SD of three experiments. *p<0.05, **p<0.01, ***p<0.001

When Khatun and others (2006) studied the total phenol content (TPC), a direct correlation was also found between TPC and DPPH (R^2=0.99) or peroxy radical-scavenging activity (R^2=0.97) (Figure 4:1). Results were compared with various studies and active components of spices were considered as major polyphenolic compound and responsible for higher antioxidant activities. Plant phenolic compounds act as free radical scavengers and metal ion chelators and are considered potent natural antioxidants. Several studies have reported their unexceptional role in cancer, chronic diseases, and coronary heart diseases. In their study, cloves showed the highest amount of polyphenol contents (1267.0±125.0µmol gallic acid eq./g) followed by other spices (Table 4:9).



(a) (b)

Figure 4:1-Correlation between DPPH (a) and peroxy radical-scavenging activity (b) and total phenol content (Khatun and others 2006)

A significant change was also observed in TPC in mace and turmeric after heating (Table 4:9). Turmeric TPC was increased by 4 times, whereas a significant negative change was measured in nutmeg. It is considered that flavonoids are present as glycosides, which may change into their aglycone and sugar by hydrolyzing through an enzyme, acid or heat treatment. Some aglycone are better antioxidants than their glycoside form, such as quercetin (aglycone) and have more potent antioxidant activity than their glycoside (rutin) (Shahidi and others 1992). Therefore, glycoside might be hydrolyzed because of heating and an increase was found in antioxidant activity. Simultaneously, some compounds degraded into lesser active forms and

showed a decrease in the activity. Some studies also reported that the activity of some of the constituents of the spices also depends on pH and it might be considered as a decrease or increase in the activity.

Table 4:9-Change in total phenol content of some spices after heating at 100°C (Khatun and others 2006)

Spices	Total phenol content (µmol Gallic acid eq/g)			
	Heating at 100°C			
	0h	1h	3h	6h
Clove	1267.0±125.0	1226.0±45.0	1335.0±85.0	1395.0±135.0
Cinnamon	272.5±10.0	279.0±20.0	259.0±15.0	210.5±10.0
Nutmeg	49.0±5.0	35.0±1.0 (0.7)*	45.0±5.0	45.0±5.0
Mace	20.0±2.0	25.0±0.5	30.0±1.0 (1.5)*	30.0±0.5 (1.5)*
Turmeric	14.5±1.0	25.0±2.5 (1.7)*	47.0±2.5 (3.3)**	59.0±1.0 (4.1)***

The values are the means±SD of three experiments. *p<0.05, **p<0.01, ***p<0.001

Effect of cooking on TPC, total flavonoid content (TFC), and total antioxidant capacity on cloves and cinnamon was also investigated by Raj and Arulmozhi (2013). In the study, cloves and cinnamon were heated at 100°C for 1 and 2 h by using water bath as heating medium and results were compared with the fresh samples (Table 4:10).

Table 4:10-Total phenolic and total flavonoid content of spices (Raj and Arulmozhi 2013)

Spices	TPC (mg GAE/g of dry powder)			TFC (mg catechin eqw/g of w)		
	Fresh	1 h	2 h	Fresh	1 h	2 h
Cinnamon	4.9±0.7 a*	4.8±1.23a*	4.3±1.5a*	36±1.2a*	42±1.3a*	51±1.7a
Clove	9.5±1.2b*	10.5±1.37b*	11.0±0.9b*	32±2.4b*	32±1.7b	52±3.1b

Values are mean±SD of five samples in each group. A-G1 vs G2; b-G2 vs G3 *p<0.05

After 1 and 2 h of heat treatment, a significant (p<0.05) increase was found in the TPC of cloves, whereas TFC increased for both cloves and cinnamon. A significant increase in phenols

and flavonoids shows that they are relatively stable under the heat treatment and health benefits remain unchanged after the heating. Total antioxidant capacity of cloves significantly increased after 1 and 2 h of cooking, whereas for cinnamon it was decreased during 1 h but significantly ($p<0.05$) increased after 2 hour of heating. Khatun and others (2006) also reported that clove and cinnamon contain 8.3 mg GAE/g and 5.8 mg GAE/g of TPC and they retained 75% and 172.41% respectively after 1 h of heating. The difference may be due to the solvent and extraction method as well as cooking method.

Shobana and Naidu (2000) investigated the antioxidant activity of Indian spices using water and alcohol (1:1) as an extraction solvent. Among the spices, cloves showed highest (IC50 value: 0.28±0.005 mg) antioxidant activity followed by cinnamon (IC50 value: 1.00±0.02 mg) and pepper (IC50 value: 5.50±0.03 mg). After heating at 100°C for 30 min, cloves, cinnamon, pepper extracts showed much higher ($p<0.05$) antioxidant activity as compared to the untreated samples. It indicates that bioactive compounds of spices are thermally stable and increase the antioxidant activity after heating. The possible reason for an increase in antioxidant activity could be the release of bound antioxidants due to heat treatment. The antioxidant activity was retained even after heat treatment, indicating that the spice constituents are resistant to thermal denaturation.

In another study, Temitope and others (2005) analyzed the effect of thermal treatment on antioxidant activity of some spices. They heated the spices at 100°C for 1 and 2 h and methanol was used as extraction solvent. In total phenol and total flavonoids determination, turmeric showed the highest amount with 41.00±1.02 mg GAE/g fresh wt. and 27.96±0.47 mg catechin eqv/g fresh wt. respectively. TPC for turmeric was significantly ($p<0.05$) increased after 1 h of heating(58.31±1.27 mg GAE/g fresh wt) and total flavonoid content for turmeric was also increased after heating for 1 and 2 h (45.00±7.57 mg GAE/g fresh wt and 33.80±2.88 mg GAE/g fresh wt). These results are in agreement with Stewart and others (2000) where they reported that levels of free flavonoids increase after the heat treatment. Turmeric also showed highest antioxidant and free radical-scavenging activity among all spices before and after heating. It is considered that curcumin (major bioactive compound of turmeric) is usually fat soluble and methanol was used as extraction solvent in this study. Therefore, after heating there was a disruption of cell wall and bound antioxidant released, and an increase in the total phenol content

and antioxidant activity of turmeric. In addition to this study, other groups have also reported that there is linear correlation between total phenol content and antioxidant activity.

Table 4:11-DPPH results of turmeric powder and oil (Tiwari and others 2006)

Spice	Before heating	After heating (120°C, 1h)
Turmeric powder	10.81%	6.01%
Turmeric oil	1.46	3.16

When turmeric powder and oil was analyzed for their antioxidant activity before and after heating, a decrease in the antioxidant activity was found in turmeric powder when heated at 120°C for 1 h, whereas a significant increase was found for turmeric oil (Table 4:11; Tiwari and others 2006). Although resistant to thermal denaturation, different forms of spices respond to it differently. For example, enhanced antioxidant activity was observed when turmeric oil was heated, whereas turmeric powder exhibited reduction in the activity. These findings suggest that effect of heat treatment on antioxidant activity varies with crop type, with individual spice, with heating process, and with solvent extraction. It is assumed that the food matrix also affects the antioxidant activity. Therefore, in order to make valid conclusions, more research into the antioxidative activity concerning all types of food composition will be very useful and required (Tiwari and others 2006).

Horváthová and others (2007) evaluated the effect of heat treatment and storage on the antioxidative activity of oregano through antiradical activity (DPPH), reducing power, thiobarbituric acid (TBA) number, and TPC. Structural changes in the plant matrix produced by the food processing influence the antioxidant activity. Inactivation of active antioxidant compounds due to high temperature negatively affects the antioxidant activity. However, some compounds alter into more active compounds such as endogenous glycosides and glycosyl transferases conversion of quercetin into the monoglucoside and aglycone form as well as some inhibition of enzymes increase the antioxidant activity. Maillard reactions also produce some intermediates and Maillard reaction products (MRP's) that contribute to the increased antioxidant activity of the final product.

The methanol extract of oregano showed highest ($p<0.05$) DPPH antiradical activity followed by other spices used in the study, whereas heating at 130°C for 5 min decreased the

antioxidant activity of oregano by 6% and by 12% after 6 months of storage. Untreated samples stored for 6 months showed no change in antioxidant activity. Thermal treatments significantly reduced the TBA number and it can be related to the formation of MRP's. After 6 months there was a 15% decrease in oregano TBA number. In the case of reducing power, oregano showed the highest ($p<0.05$) tendency to reduce Fe^{3+} to Fe^{2+} than other spices and thermal treatments also affected the reducing power. During the first two months of storage, a reduction was observed in the reducing power and then it increased. Oregano also showed highest TPC but thermal treatments decreased 5% of TPC in oregano (Horváthová and others 2007). The impact of various cooking methods such as simmering, stewing, stir frying, grilling, and storage conditions such as vinegar maceration, cold maceration, and freezing was studied by Chohan and others (2008). Simmering of cinnamon (from 53±2.6 to 2,916.9±148.4 µmol/g) and stewing of cloves (from 225±21.3 to 1473±30.5 µmol/g) significantly increased ($p<0.001$) the antioxidant capacity while grilling of sage (from 625±0.5 to 238±21.3 µmol/g) and stir-frying of rosemary (from 331.3±6.5 to 76±7.6 µmol/g; 4 fold reduction) decreased ($p<0.001$) the antioxidant capacity. Freezing and cold maceration have a preservative effect on antioxidant capacity, whereas vinegar maceration also showed a decrease ($p<0.05$) in antioxidant capacity. Change in the antioxidant capacity was supported by other studies which concluded that thermal retention of spices varies with the individual spice and cooking conditions (Choi and others 2006).

In a different study, Takamura and others (2002) examined the radical-scavenging activity of various vegetables and from the study, they concluded that an increase in the antioxidant activity after cooking was due to thermal disruption of the cell wall and subcellular compartments that release some antioxidant compound. Heating process also causes the inactivation of oxidase, and/or the activation of inactive compounds that might decrease antioxidant activity in some vegetables. They also suggested using salt with the cooking water because sodium chloride inhibits the release of active components from the tissue. In different cooking methods, microwave cooking was found to have higher antioxidant activities than boiling, because a large amount of water-soluble antioxidants may be lost due to leaching and diffusion into the cooking liquid, resulting in loss of activity (Boari and others 2013). Takamura and others (2002) reported a decrease in the radical-scavenging activity of curry paste and cooked curry and suggested that decomposition and evaporation of spice active constituents are

possible because they were heated at high temperature with butter. Hence, an efficient cooking method is necessary to get maximum benefits of antioxidant compounds.

Degradation of carnosic acid, carnosol, rosmarinic acid, and a mixture of three was studied by Zhang and others (2012) by using HPLC. Several degradation products also formed by exposure to light. Ethanolic standards of carnosic acid, carnosol, rosmarinic acid, and a mixture of three exposed to six different conditions such as -10°C, 4°C in dark, room temperature with light exposure, room temperature in dark, 40°C with light exposure, and 40°C in dark. The effect of temperature and light was observed for 13 days. As shown in Figure 4:2, rosmarinic acid did not degrade either by itself or in the mixture, whereas carnosic acid was fairly stable than carnosol degradation. Carnosic acid by itself and in the mixture was quite stable at -10 and 4°C in dark, while carnosol was completely degraded at day four when exposed to 40°C with light exposure. Temperature and light exposure speeded up the degradation of carnosol. Carnosol degradation was in the following order in terms of rate of degradation: 40°C with light exposure > 40°C in dark > room temperature with light exposure > room temperature in dark > 4°C in dark > -10°C in dark. Carnosol in a mixture showed a slower degradation and this might because of other antioxidants present in the mixture. Degradation of carnosic acid stored in dark was higher than when it exposed to light at the same temperature. A similar pattern was observed for degradation in the mixture or by itself for carnosic acid.

Rosmanol, epirosmanol, epirosmanol ethyl ether major degradation products were also observed in HPLC chromatograms of carnosol and effect of temperature also noticed during the formation of these degradation products. Rosmanol was present in highest amount when stored at -10°C. Rosmadial and 11-ethoxy-rosmanol semiquinone also appeared as degradation products in carnosol HPLC chromatograms. When carnosol completely degraded and stored at 40°C with light exposure, formation of 11-ethoxy-rosmanol semiquinone was also noticed. In the case of carnosic acid, only carnosic acid quinone was observed as a degradation product when stored at -10°C in dark for 13 days. Carnosic acid degradation pathway is shown in Figure 4:3. When stored at -10°C in dark for 13 days carnosic acid was completely converted into carnosic acid quinone. Carnosic acid quinone also considered as intermediate during oxidation of carnosic acid. Carnosol, rosmanol, epirosmanol, and epirosmanol ethyl ether were collected as degradation products of carnosic acid. 5,6,7,10-tetrahydro-7-hydroxy-rosmariquinone also

derived as degradation product of carnosic acid. The formation of this product was accelerated by exposure to light. The light-induced carnosic acid degradation product was noticed when solution exposed to light and had strong UV absorption at 260 nm (Zhang and others 2012). Degradation products of the mixture were similar to carnosol and carnosic acid degradation and rosmarinic acid was also stable in the mixture. In the mixture, carnosic acid maintained the levels of carnosol. From the study, Zhang and others (2012) concluded that the degradation of solutions increased with temperature and exposure to light, as well as new degradation products were observed during the study.

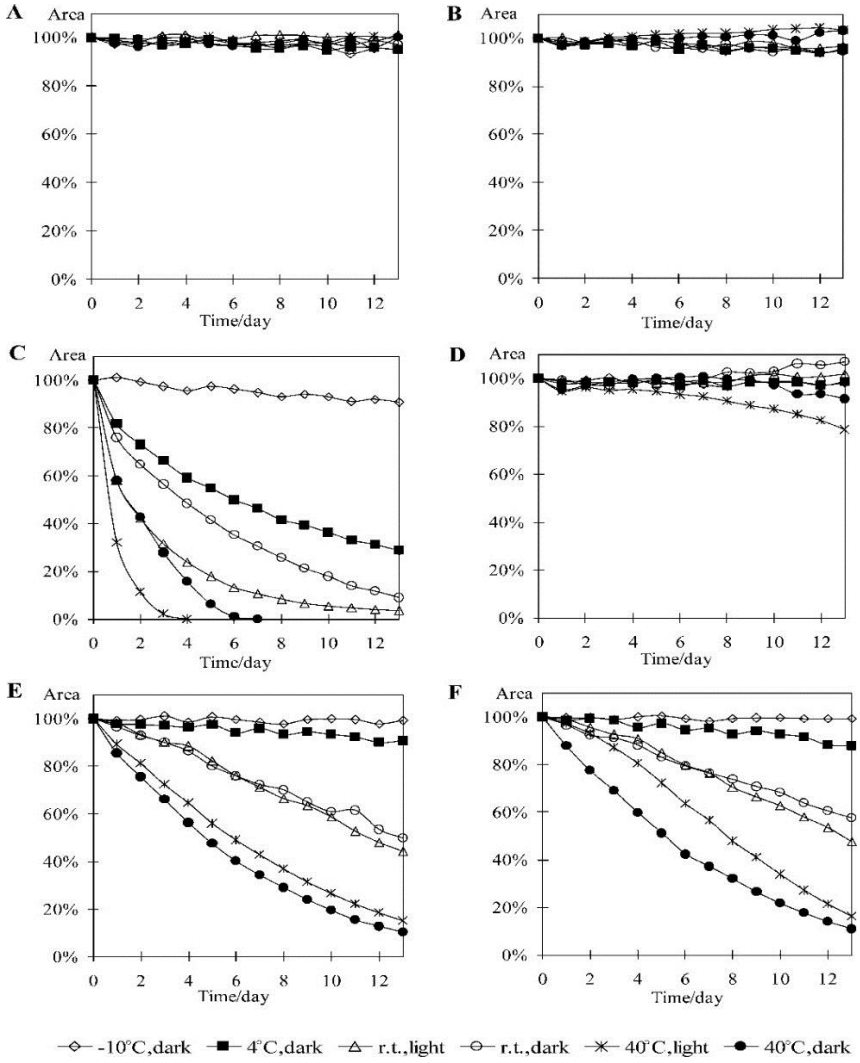


Figure 4:2-Degradation profile of the ethanolic solution of (A) rosmarinic acid by itself, (B) rosmarinic acid in mixture, (C) carnosol by itself, (D) carnosol in the mixture, (E) carnosic acid by itself, and (F) carnosic acid in the mixture under different storage conditions (Zhang and others 2012)

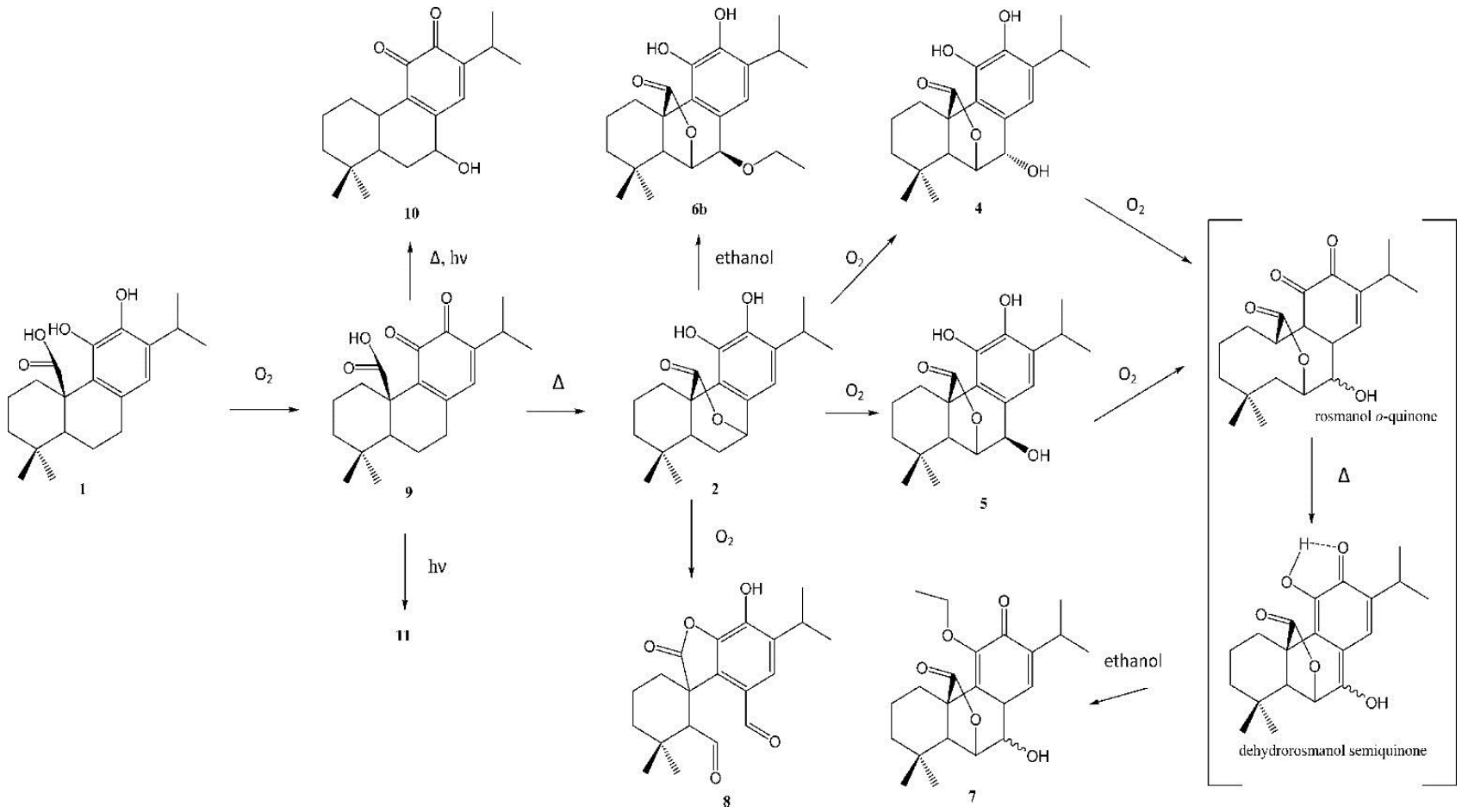


Figure 4:3-Proposed degradation pathway of carnosic acid in ethanol solution where (1) is carnosic acid, (2) carnosol, (4) rosmanol, (5) epirosmanol, (6b) epirosmanol ethyl ether (7) 11-ethoxy-rosmanol, (8) rosmadial, (9) carnosic acid quinone, (10) 5,6,7,10-tetrahydro-7-hydroxy-rosmariquinone, and (11) the light induced degradation product (Zhang and others 2012)

Maillard Reaction Products

The Maillard reaction plays an important role in the food industry and reaction products are of great importance for color and flavor of foods. MRPs also act as non-nutrient antioxidants, produced at various steps such as degradation of Amadori compounds to amino reductones. MRPs are shown to have antioxidant properties by chelation and scavenging of oxygen species (Chiu and others 1991; Antony and others 2000). Therefore, overall antioxidant properties remain unchanged or even enhanced after the thermal treatments due to formation of MRPs. Figure 4:4 represents the changes in color and antioxidant properties of tomato puree when heated at 95°C. It shows that with browning, antioxidant activity of tomato puree increased with the increase in heating time. This suggests the development of MRPs and those act as chain breakers, oxygen scavengers, and metal chelating agents, and increase the antioxidant activity of the final product (Nicoli and others 1997).

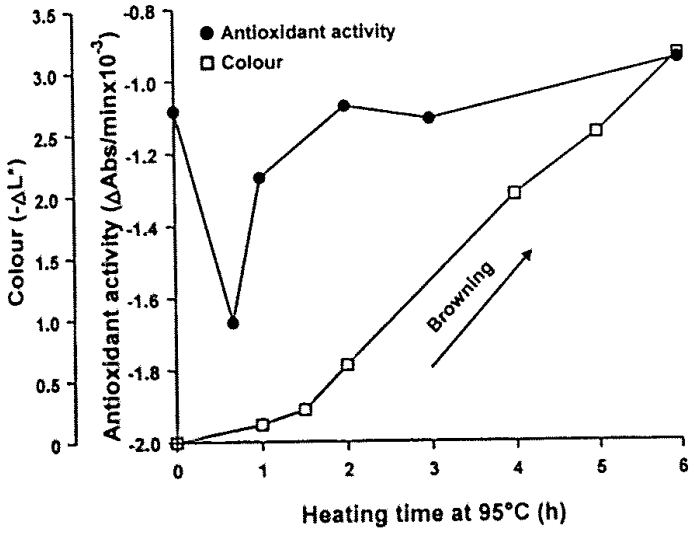


Figure 4:4-Changes in color and in antioxidant activity of a tomato puree in relation to heating time at 95°C (Nicoli and others 1997)

In another study, Serpen and others (2012) used QUENCHER, a method independent of soluble and insoluble fractions of food to analyze the total antioxidant capacity (TAC) of beef, chicken, pork, and fish. In the study, meat samples were cooked at 180°C for 5, 10, 15, and 20 min and stored at -20°C. The TAC of all meat samples were evaluated by the ABTS and DPPH methods and expressed in mmoles of Trolox per kg of dry weight (Figure 4:5). The total ABTS

scavenging capacity of raw samples was in the range of 25.9±1.0 mmol Trolox Eq. /kg dry wt. to 51.7±1.2 mmol Trolox Eq./ kg dry wt. and a higher ABTS scavenging activity ($p<0.05$) was found in raw chicken among all meat samples. There was no significant difference in all meat samples when DPPH radical was used to measure TAC. Various studies reported that proteins and peptides can scavenge free radicals and chelate prooxidative metals and due to this ability meats have antioxidant capacity. Carnosine and anserine are histidine dipeptides, present in chicken meats and due to dipeptides chiken showed stronger ABTS activity (Chan and Decker 1994).

The total DPPH scavenging capacity of raw samples was in the range of 19.1±1.8 mmol Trolox Eq. /kg dry wt. to 31±0.9 mmol Trolox Eq./ kg dry wt. Chicken and fish meats antioxidant capacity was significantly ($p<0.05$) different in both assays where as there was no significant difference in raw pork and beef analysis. It might be because of ability of radicals to scavenge different types of antioxidants. DPPH is a hydrophobic radical and polar macromolecular antioxidant has less interaction with hydrophobic radicals as well as DPPH is more selective radical than ABTS. Due to characterstics of DPPH radical, low TAC values were observed.

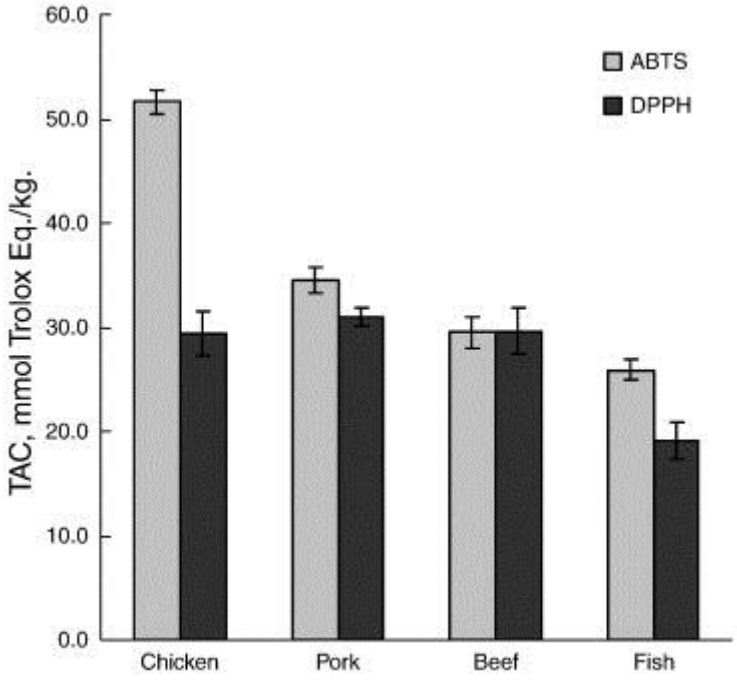


Figure 4:5-Total Antioxidant Capacity (TAC) of raw meats determined using ABTS and DPPH as antioxidant probes (Serpen and others 2012)

It also has been determined that TAC of the most meat samples was increased at the beginning and after long-term heating (Figure 4:6; 4:7). Thermal treatment modifies the physical properties of secondary and tertiary proteins. Modification of protein structure might influence the antioxidant properties of proteins resulting in an increase in the antioxidant activity. Unfolding of protein structures increases the capacity to scavenge free radicals. In addition, it is well known that pro-longed heating causes the Maillard reaction and its products, and there are several reports on antioxidative properties of MRPs. Therefore, MRPs can be a possible reason for an increase in the antioxidant activity of meat samples on pro-long heating.

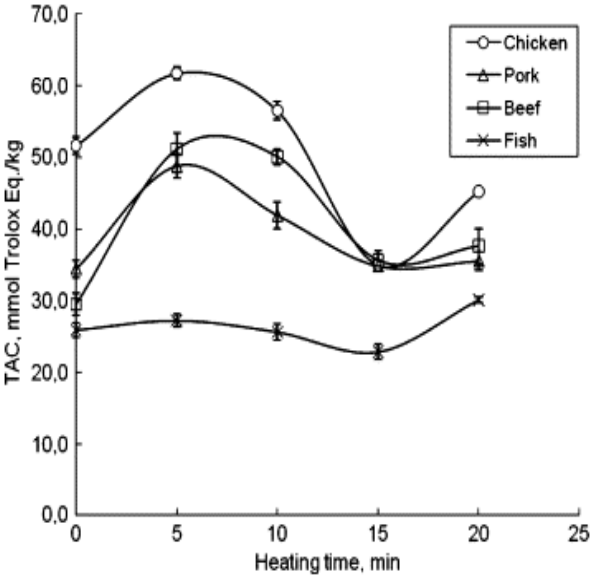


Figure 4:6-Effects of time at 180^{0}C on TAC values of meats measured using ABTS as the antioxidant probe (Serpen and others 2012)

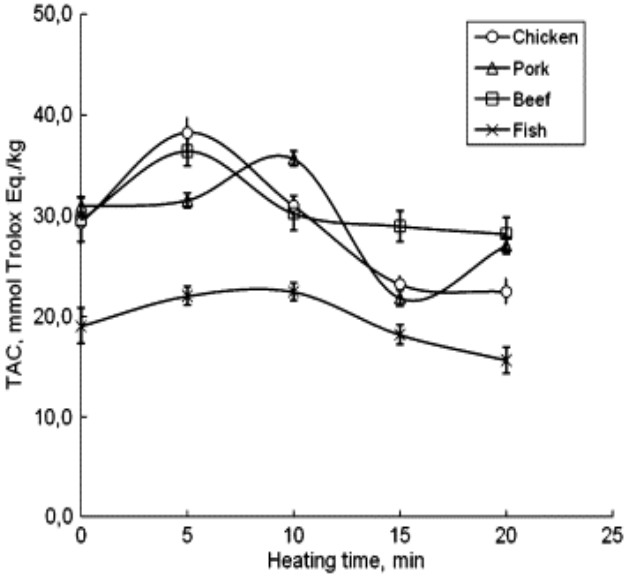


Figure 4:7-Effects of time at 180^{0}C on TAC values of meats measured using DPPH as the antioxidant probe (Serpen and others 2012)

Condensation reactions between amino acids and reducing sugars or lipid oxidation products result in MRPs and exhibit antioxidative activities. Various factors such as the type of reactants, temperature, pH, water activity, intermediate products, and oxygen availability also affect the production and properties of final MRPs. Yilmaz and Toledo (2004) reported that melanoidins showed antioxidant properties through metal chelation or scavenging oxygen radical, and MRPs from histidine shown to have the highest antioxidant activity. The ORAC values for peroxy radical scavenging range from 0.50 to 0.63 µmol of Trolox/mg His while, histidine and glucose MRPs also showed copper ion binding ability (Table 4:12).

Table 4:12-Antioxidative properties of Maillard reaction products obtained from model systems reported in the literature (Yilmaz and Toledo 2004)

Model System Sugar-amino acid	Mode of antioxidative property	References
Glu-His	Copper chelator Oxygen radical scavenger Peroxyl radical scavenger	Bersuder and others (2001) Lingnert and others (1983) Lingnert and Eriksson (1980a,b)
Glu-Lys	Copper chelator DPPH radical scavenger Oxygen radical scavenger Peroxyl radical scavenger	Wijewickreme and others (1997), Dittrich and others (2003), Wijewickreme and Kitts (1998) Morales and Jimenez-Perez (2001) Bressa and others (1996) Wijewickreme and others (1999)
Glu-Gly	Copper chelator Oxygen radical scavenger Peroxyl radical scavenger Fe2+ chelator	Dittrich and others (2003) Wagner and others (2002) Yoshimura and others (1997) Yoshimura and others (1997)
Fru-Lys	Copper chelator hydroxyl radical scavenger	Wijewickreme and others (1997) Wijewickreme and others (1999)
Lac-Lys	Peroxyl radical scavenger	Monti and others (1999)

Summary

Herbs and spices are abundant sources of polyphenolic compounds which are responsible for their strong antioxidant capacities. Polyphenolic compounds are resistant to thermal denaturation and due to thermal stability antioxidant capacity remain unchanged or even enhanced. Cooking at higher temperature degrades the cell wall and increase the solubility of active antioxidants and their antioxidant capacity. Heat treatments also produce some additional compounds which possess antioxidant capacity such as MRPs. As well as, degradation product of phenolic compounds also shown to have antioxidant property. Therefore, antioxidant capacity of final products remain unchanged or increased and health benefits can also be retained after heat treatments.

Chapter 5 - Future Research and Concluding Remarks

Research on the thermal decomposition of natural antioxidants suggest that food processing steps such as thermal treatments not only retain the antioxidant activity, but also showed a significant increase in the antioxidant activity. It indicates that bioactive polyphenolic components of herbs and spices are resistant to thermal treatments and potential health benefits can also be retained after the thermal treatments. Based on the literature search, the most likely explanations for higher antioxidant activity are as follows:

1. Heating processes cause the thermal destruction of cell walls and subcellular compartments and helps into release of bound antioxidant constituents;
2. Thermal treatments also induce the formation of more active non-nutrient antioxidant compounds through Maillard reaction called as Maillard reaction products (MRPs);
3. Transformation of antioxidants into more active compounds, such as deglycosylation of quercetin;
4. Thermal inactivation of some oxidative enzymes.

The antioxidant activity of these herbs and spices are thermally stable and cooking processes may even improve nutritional value by enhancing their antioxidant activities. Therefore, these dietary herbs and spices can be used as a potential source of plant-based natural antioxidants in foods. It also suggests that thermal stability of spices varies with individual spice, cooking conditions, solvents used, and extraction methods. Antioxidant activity of any extract depends on the types of solvent used because different antioxidants have different polarity.

Further investigations are still required to elucidate the effect of heating on the individual polyphenol components of herbs and spices as well as a change in the antioxidant activity due to different temperature or cooking methods, solvent extractions, and type of spices are also needs to be analyzed. MRPs also have different antioxidant activity which depends on their origin and food composition. Excessive heating of MRPs cause negative effects, therefore before final recommendations it is necessary to further analyze the toxicity of prolong heated MRPs.

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