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5 Natural products as antioxidants

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Abstract

The addition of synthetic antioxidants to oils and/or foods is one of the most efficient ways to prevent lipid oxidation. However, the safety of synthetic additives has been questioned stimulating the evaluation of naturally occurring compounds with antioxidative properties. Although there is no assurance of the safety of natural antioxidants, there is some comfort knowing that such antioxidants are purified from natural products that have been consumed for generations.

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Phenolic compounds in plants are recognized as important compounds in conferring stability against oxidation. Natural antioxidant phenolics can be classified into a lipophilic group, tocopherols, and a hydrophilic group, including simple phenolics, phenolic acids, anthocyanins, flavonoids and tannins. Even though chemists have elucidated the structures of thousands of phenolics, there are still many compounds that have not yet been fully characterized and they are referred as phenolic extracts. In this ways berry extracts, aromatic plant extracts, essential oils and their components are gaining interest because of their relatively safe and wide acceptance by consumers. Many authors have reported antioxidant and radical-scavenging properties by berries, spices and essential oils. In this work we reviewed the most important groups of natural antioxidants, with some peculiarities related to chemical composition.

Introduction

According to modern theory of free radical biology and medicine [1], reactive oxygen species are involved in several disorders. The harmful action of the free radicals can, however, be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers [2-6] and neurodegenerative diseases [7]. Therefore, plant derived antioxidants are now receiving a special attention [8,9]. A large number of phenolic compounds present in vegetable foods, such as fruits and nuts, have been reported to possess good antioxidant properties. Moreover, the essential oils and various extracts of aromatic plants have been of great interest for their potential antioxidative effects for the preservation of the foods from the toxic effects of the oxidants [10-13].

Antioxidant properties of phenolic compounds

The term “phenolic” is used to define substances that possess one or more hydroxyl groups (OH) substituents bonded onto an aromatic ring. Compounds that have several or many phenolic hydroxyl substituents are often referred to as polyphenols. Due to its chemical structure phenolic compounds have the ability of phenoxide ion delocalize. The phenoxide ion can lose a further electron to form the corresponding radical which can also delocalize. In reference to this property, phenolic compounds have radical scavenging and antioxidant activity [14].

Phenolics are large and heterogeneous groups of secondary plant metabolites that are distributed throughout the plant kingdom. They have been implicated in a number of varied roles including UV protection, pigmentation,

disease resistance, and nodule production. Phenolics have a wide variety of structures. Flavonoids, tannins, and phenolic acids are the main phenolic compounds [14-17].

Due to toxicological concerns of synthetic antioxidants [18,19], there have been increasing interests in identifying phenolic compounds in plants to minimize or retard lipid oxidation in lipid-based food products. Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs [17].

Hundreds of natural phenolic compounds have been reported to possess high antioxidant properties. Their use in foods, however, is limited by certain requirements not the least of which is adequate proof of safety. Only a few of them can be commercially applied in foods. The main lipid-soluble antioxidants currently used in food are monohydric or polyhydric phenols with various ring substitutions. For maximum efficiency, primary antioxidants are often used in combination with other phenolic antioxidants or with various metal sequestering agents, e.g. tocopherols with citric acid and isopropyl citrate [20]. Most important commercially available natural antioxidants are tocopherols (vitamin E), ascorbic acid (vitamin C) and rosemary extract (21, 22). Compounds such as β-carotene, ascorbic acids have demonstrated to have antioxidant and synergistic activity in despite of their non-phenolic structure [23, 24].

It is generally accepted that lipid oxidation is a typical free radical chain process consisting of initiation, propagation and termination steps.

1. Initiation: thermal or photochemical homolytic cleavage of a lipid substrate (RH, i.e, an unsaturated fatty acid).

$$RH \xrightarrow[In]{} R\cdot + H\cdot$$

2. Propagation: two different reactions can be visualized:

2.1 Addition of molecular oxygen to lipid radicals (R•) and formation of peroxyl radicals (ROO•).

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

2.2. Abstraction of a hydrogen atom from RH by ROO• to generate a lipid hydroperoxide (ROOH) and another radical R·.

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

3. Termination: a radical – radical coupling reaction that interrupt the free radical chain propagation process.

$$\left.\begin{array}{l} R\cdot + R\cdot \\ R\cdot + ROO\cdot \\ ROO\cdot + ROO\cdot \end{array}\right\} \text{Non–radical products}$$

It is well known that phenolic antioxidants (PhH) can react with ROO• to generate ROOH and a relatively unreactive phenoxyl radical (Ph•).

$$ROO\cdot + PhH \longrightarrow ROOH + Ph\cdot$$

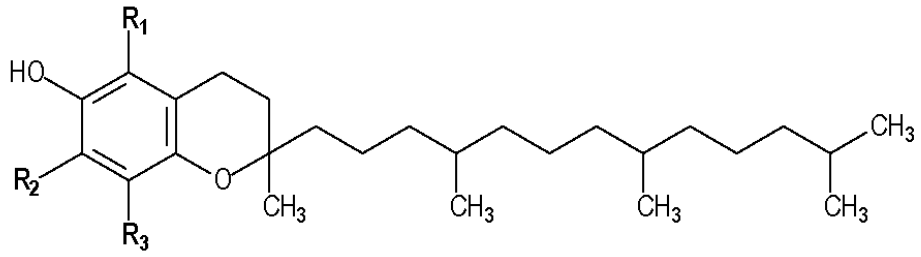
It was also established that Ph· can subsequently undergo chain temination reactions with ROO• to give non-radical products [25].

$$ROO\cdot + Ph\cdot \longrightarrow \text{Non–radical products}$$

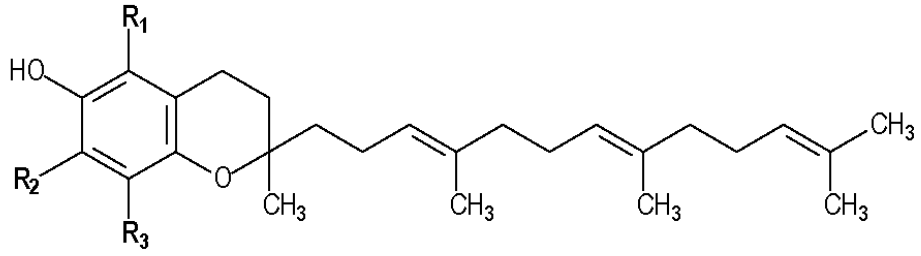
Thus, phenolic substances behave as chain-breaking antioxidants by competing with the substrate (RH) for the chain – carrying peroxyl radicals.

Natural phenolic compounds with antioxidant properties can be classified into a lipophilic group (tocopherols mainly) or a hydrophilic group (phenolic acids and flavonoids).

Tocopherols and tocotrienols are the most important lipid-soluble antioxidants present naturally in vegetable oils [26-28]. Both tocopherols and tocotrienols share the same ring structure, but tocotrienols have unsaturated carbon chains.



Tocopherol structure

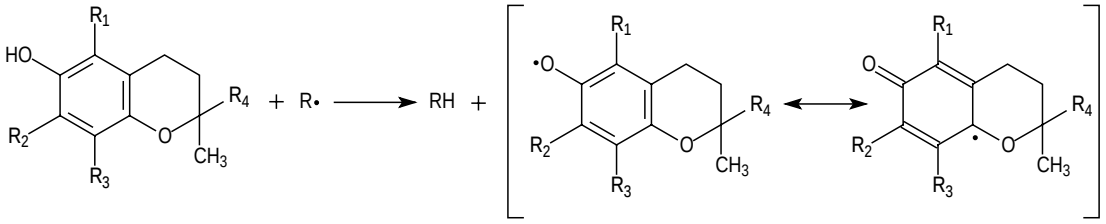


Tocotrienol structure

Position of methyl group on aromatic ring	Tocopherol structure	Tocotrienol structure
R_1, R_2, R_3	α-tocopherol	α-tocotrienol
R_1, R_3	β-tocopherol	β-tocotrienol
R_2, R_3	γ-tocopherol	γ-tocotrienol
R_3	δ-tocopherol	δ-tocotrienol

A relatively high proportion of the tocopherols present in crude vegetable oils survives the oil processing steps and remains in sufficient quantities to provide oxidative stability in the finished products. As antioxidants, tocopherols exert their maximum effectiveness at relatively low levels, approximately equal to their concentration in vegetable oils.

Tocopherols interrupt free radical chain reaction by capturing free radicals generated during oil autooxidation.



Resonance – stabilized radical

If used at a very high concentration, tocopherols may actually act as prooxidants [20,29,30]. Thus, Marinova et al. [31] found that at high concentration (200 – 2000 ppm), α-tocopherol may participate in two side reactions:

a) Decomposition of hydroperoxides

$$\alpha\text{-TocH} + ROOH \longrightarrow \alpha\text{-Toc}\cdot + RO\cdot + H_2O$$

b) Spontaneous oxidation

$$\alpha\text{-TocH} + O_2 \longrightarrow \alpha\text{-Toc}\cdot + HO_2\cdot$$

Although there is some controversy about the antioxidant capacity of the different tocopherol homologs, most studies show a relative antioxidant activity in the order δ > γ > β > α [32,33]. This order, however, may be altered by effects of temperature, substrate composition and tocopherol concentration [31,33-36]. Hence, the optimum tocopherol concentration to inhibit peroxide formation should be established for each specific and well defined system.

Another group of phenolic substances with antioxidant properties are the phenolic acids. Among them, gallic acid, a natural product arising from tannin

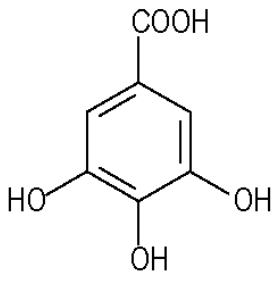
Table 1. The tocopherol composition (mg/kg) determined in some commercial oils.

Oil source	α-Toc	β-Toc	γ-Toc	δ-Toc	Ref.
Canola	103-170	134	174-403	10,3-41	37,38
Sunflower	622-723	19-41	5-65	---	31,37,39,40
Soybean	81-151	27-33	545-624	207-300	31, 37,41
Walnut	15-29	1-8	207-355	30-62	42
Peanut	49-304	1-41	99-389	---	43
Olive	104-425	1-3	5-30	---	37, 44
Jojoba	11-23	---	24-41	---	45, 46
Wheat germ	984-1845	386-739	231-681	105	47
Safflower	386-477	8-9	2-44	10	48
Palm	3-185	---	4-36	---	43,49
Sesame	12-113	3-6	244-400	5-32	40
Corn	134-344	14-31	412-729	36-39	37, 40
Cottonseed	402-573	1,5-40	317-572	7,5-10	50

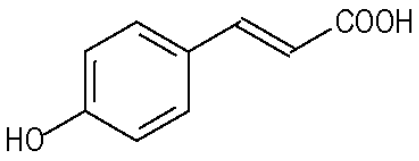
hydrolysis, has attracted considerable interest. Novel antioxidative food additives are being developed using gallic acid as a starting compound [51-53]. Strlic et al. [54] demonstrated that in Fenton-type systems containing Fe (III) / H_2O_2, gallic acid at a molar ratio of GA:Fe > 2:1 has an antioxidative role due to its pronounced HO• scavenging properties. However, at a molar ratio of GA:Fe < 2:1 the overall role is prooxidative due to the promotion of HO• production.

Monohydroxylated phenolic acids, such as vanillic, caffeic, sinapic, siringic, ferulic and coumaric acids are relatively ubiquitous. Their antioxidant capacities have been demonstrated in several "*in vitro*" and "*in vivo*" studies [55-57].

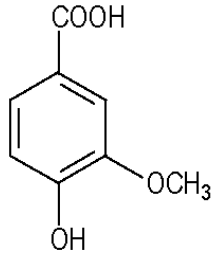
Hydroxycinnamic acids such as *p*-coumaric acid are abundant in higher plants and are found with various hydroxyl and methoxyl substitutions and may exist in esterified forms or bound to proteins [58-60]. As an antioxidant, *p*-coumaric acid is an effective radical inhibitor *in vitro* [61-62] but contains only moderate inhibitory properties against lipid peroxidation [63, 64].



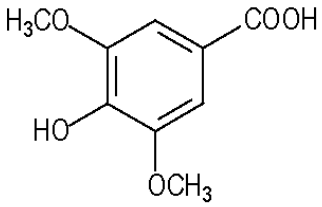
Gallic acid



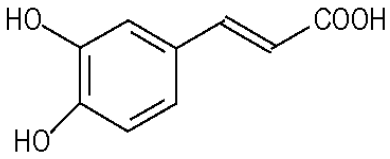
p – Coumaric acid



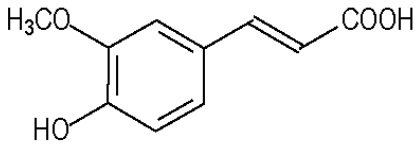
Vanillic acid



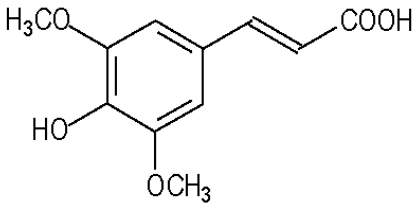
Siringic acid



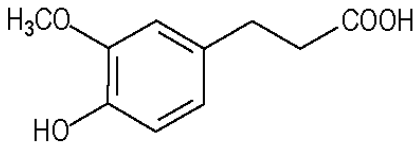
Caffeic acid



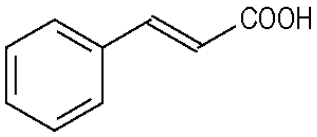
Ferulic acid



Sinapic acid



Dihydroferulic acid



Cinnamic acid

The antioxidant activity of phenolic acids is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and synglet oxygen quenchers. In addition, some phenolic acids have a metal chelation potential.

Small berries are abundant source of fenolic acids, ranging from 284 mg/kg to 5418 mg/kg [65]. They are hydroxylated derivatives of benzoic acid and cinnamic acid. Hydroxy cinnamic acids are the predominat class of phenolic acids comprising from 67.1% (blue-berry honeysuckle) to 96% (black mulberry) [65]. According to Häkkinen et al. [66] strawberry, red raspberry, and cloudberry possess high contents of ellagic acid, rowanberry contains high amounts of ferulic acid, and bilberries are especially rich in hydroxycinnamic acid derivatives. Overall, berries exhibit high antioxidant activity. Furthermore, berries with strong colour such as bilberries, black currants, cowberries,

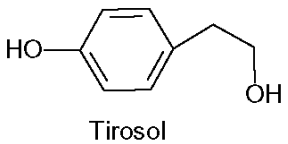
cranberries, and crowberries showed stronger antioxidative properties in the oxidation of methyl linoleate (MeLo) than those of berries with light color such as cloudberries and strawberries [67].

Antioxidant activities of extracts from different berries were also measured by Kähkönen et al. [68]. They demonstrated that the formation of MeLo – conjugated diene hydroperoxydes was inhibited over 90% by crowberry, rowanberry, cloudberry, cranberry, and whortleberry. Raspberry and black currant were somewhat less active with inhibitions of 88 and 83%, respectively. Red current and strawberry gave the least active berry extracts in this study, with inhibition of 60 and 57%. On the other hand, Wang et al. [69] showed that fresh strawberry extracts have 15 times higher antioxidant capacity than Trolox in an artificial peroxyl radical model system. Constantino et al. [70] reported that extracts of blackberries, black and red currants, blueberries and black and red raspberries possess a remarkably high scavenging activity toward chemically generated superoxide radicals.

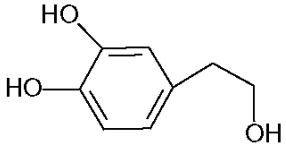
Olive (*Olea europea* L.*)* fruits and oils provide a rich source of natural phenolics, including tocopherols and other phenolic compounds, which not only protect them from oxidation but also contribute to oil flavour and aroma. The antioxidant potential of olive phenolic compounds has been analyzed extensively [71-76]. The amount of phenolics in virgin olive oils ranges from 150 to 700 mg/kg depending on variety, fruit maturity, environmental conditions and extraction method [76-82].

The extraction method plays a critical role on phenol content of olive oils. Phenols present in olive fruits are soluble in water and oil, depending on their partition coefficients and extraction temperatures. Addition of water to olive paste to be extracted, alters the partition equilibrium between liquid (oil and water) phases and causes a reduction of phenol concentration through dilution in the aqueous phase [81]. In a recent work [76] olive oils from different genotypes extracted with either centrifugation or pressure systems, showed significant variations in their phenol contents. Differences between genotypes were also remarkable. Pressure extracted oil from Manzanilla cultivar was noteworthy for its higher content of phenolic compounds (588 mg/kg.). Accordingly, this oil had the highest oxidative stability.

Phenolics of olive fruits are characterized by the presence of a number of secoiridoid compounds derived from *p*-hydroxyphenylethanol (*p*-HPEA, or tyrosol) and 3,4-dihydroxyphenylethanol (3,4-DHPEA, or hydroxytyrosol).



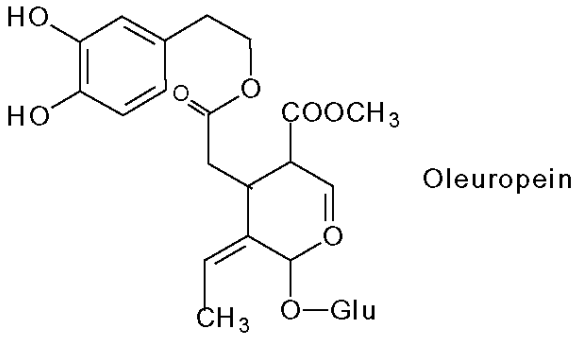
Tirosol



Hydroxytyrosol

The importance of 3,4-DHPEA and derivatives (3,4-DHPEA-EA and 3,4-DHPEA-EDA) as the most active antioxidant compounds in virgin olive oils has been demonstrated by different authors [56,75,83,84]. All these compounds bear two hydroxyl groups linked to an aromatic ring on the orto position and they have shown much stronger antioxidant activities than α-tocopherol [85]. When 3,4-DHPAE derivatives were used in association with α-tocopherol, a synergistic effect was observed [86].

Oleuropein, a secoiridoid glucoside, may reach concentrations of up to 140 mg/g on a dry matter basis of olive fruit [87].



Oleuropein

The glucose moiety in oleuropein increases antioxidant activity in the MeLo model, conferring a lower solubility of hydrophylic oleuropein compared to the secoiridoid derivatives [75]. This is in accordance with the phenomenon of polar paradox reported by Porter [88], that is, hydrophilic antioxidants being more effective than lipophilic antioxidants in bulk oils.

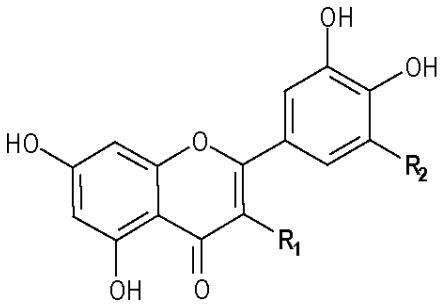
Flavonoids, natural polyhydroxylated aromatic compounds, are widely distributed in the plant kingdom, including fruits and vegetables. About 2/3 of the polyphenols we obtain in our diet are flavonoids. It is estimated that humans consume on the average 1 g / day of flavonoids [89].

Flavonoids display pronounced biological effects. Various investigations have established a relationship between the structure of different flavonoids and their relative efficiencies as antioxidants [90-93]. This effect is commonly associated with their ability to scavenge reactive free radicals, including hydroxyl, peroxyl and superoxide radicals (93-95), and/or to deactivate catalytic metals due to complexation [96,97]. Furthermore, flavonoids can inhibit lypoxygenase and cyclooxygenase enzymes [90].

Studies have also found both an increased antioxidant capacity of human blood plasma following ingestion of flavonoids, and a decreased level of LDL cholesterol oxidation [98].

One factor that seems influence the antioxidant capacity of a flavonoid is the degree of hydroxylation on the “B” ring. Bors et al. [94] concluded that for maximal radical scavenging activity a flavonoid molecule needs to meet the following characteristics: a) 3’,4’-dihydroxy structure on the “B” ring,

b) 2,3-double bound in conjunction with a 4-oxo group in the “C” ring and a 5-hydroxyl group on the “A” ring. The flavonoids luteolin, quercetin, myricetin, and their glycosides meet these structural requirements.

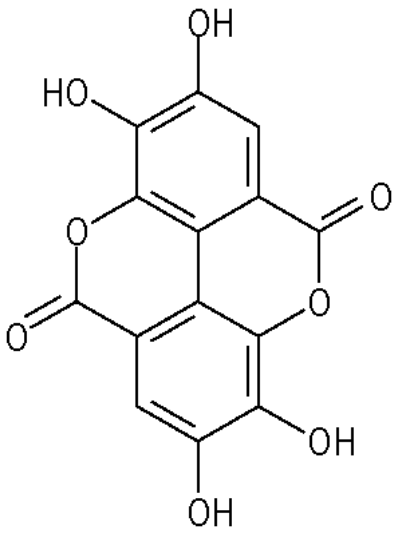


Flavonoids	R_1	R_2
Luteolin	H	H
Quercetin	OH	H
Myricetin	OH	OH
Quercitrin	*O*-rhamnose	H
Isoquercitrin	*O*-glucose	H
Rutin	*O*-rutinose	H
Myricitrin	*O*-rhamnose	OH

Phenolic antioxidants in nuts

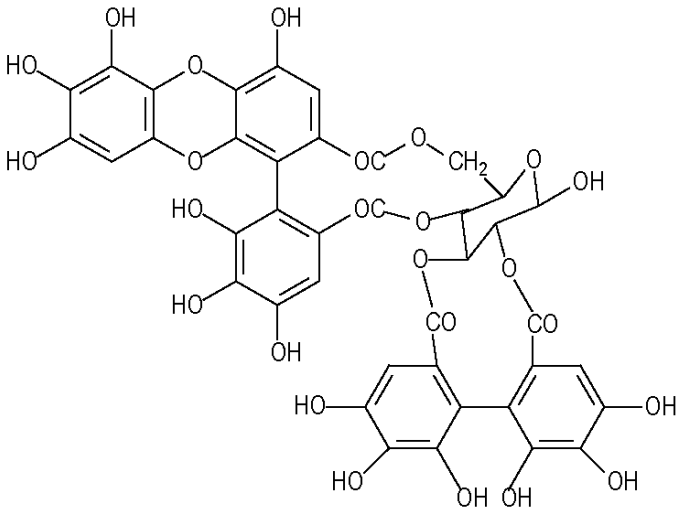
Walnut, the seed of *Juglans regia* L., is appreciated as a highly nutritious food. It is very rich in oil (up to 70%) which is abundant in polyunsaturated (linoleic and linolenic) fatty acids [99]. Although these fatty acids are prone to oxidation, walnut oil is readily preserved. This implies that the nut contains antioxidants inhibiting lipid autooxidation. Besides tocopherols, which are lower than in other nuts, walnut fruit had larger amounts of polyphenolics. They are found in higher concentration in the seed coat, the skin that lines the pulp of the nut.

Polyphenolics in walnut seed include the monomers ellagic acid, gallic acid and methyl gallate, which when present as polymers and bound to sugars are known as hydrolyzable tannins, and comprise the majority of the polyphenolics present [100,101].



Ellagic acid

More recent data from Anderson et al. [57] and Fukuda et al. [102] show that walnut polyphenolics are principally of the non-flavonoid type and fall into the category of ellagitannins.



Glansrin, an ellagitannin polyphenol present in walnut

Increasing evidences show that walnut consumption has advantageous effects on human serum lipid profiles, with a decrease in total and LDL cholesterol, and an increase in HDL cholesterol [103-106]. *In vitro* data on polyphenolics walnut extracts used at expected physiologic concentrations, demonstrate their potent antioxidant capacities with plasma and LDL lipids [57].

Ellagitannin polyphenols were evaluated for their antioxidant effects using superoxide-dismutase (SDO) - like activity and 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging activity (102). Although the structure-activity relationships are not clear, ellagitannins isolated from walnut hulls could be the most effective antioxidant compounds in preserving walnut oil stability.

Phenolic antioxidants present in peanut

Peanut is a principal agricultural plant in the world. Argentina is a major producer and exporter of peanuts, along with China, India and USA, among others [107]. Naturally-occurring phytochemicals in peanuts (*Arachis hypogaea L.*) and other oilseeds, such as tocopherols, carotenoids and polyphenolics, may have a role in slowing or preventing lipid oxidation due to their antioxidative nature [30,108].

Peanuts consist of peanut kernels, peanut skins, and peanut hulls [109,110]. The edible parts of peanut are the kernels and the protective skin. The kernels are used to make peanut butter, roasted snack peanuts, peanut confections and peanut oil. The skin and hull becomes the waste of above industry, and is mainly used as animal feed and fertilizers [111,112].

Peanut kernels contain approximately 50-55% oil with 30-35% and 45-50% of the oil being linoleic and oleic acids, respectively, which becomes susceptible to development of rancid and off-flavors through lipid oxidation [30,113-115].

The major lipid-soluble antioxidants found in peanut and in other oilseeds are tocopherols [26-28,116].

In addition, numerous polyphenolics have been identified in peanuts [117-119]. These compounds may contribute to purported health benefits of peanuts. The stability over time of many polyphenolics was studied by Talcott et al. [108]. Fajardo et al. [120] demonstrated a stress-elicited synthesis of free and bound phenolics in peanuts, with *p*-coumaric and ferulic acids as the major compounds identified.

Recently, resveratrol (*trans*-3,5,4'-trihydroxystilbene) was other phenolic antioxidant found in peanuts and peanut products [121-123]. Stilbenes are produced by peanut plant as a defense response to some exogenous stimuli, particularly, a fungal challenge [124-126]. Earlier research on resveratrol in the 1970s and 1980s [124] indicated that the compound was only found in peanuts that had been inoculated and incubated with microorganisms, making them inedible. But more recent works [122] have demonstrated that resveratrol is present, even without any indication of fungal attack. In edible peanuts and peanut products, resveratrol was found at levels of 0.02-7.87 μg/g [121,122,127]. Resveratrol was also described in grapes, grape skins and grape products, such as wines in levels of 0.031-7.17 ppm [127,128]. It has been shown to possess cancer chemopreventive activity in mice and to act as an antioxidant and antimutagen [129]. It is also associated with reduced risk of cardiovascular disease by inhibiting or altering platelet aggregation and coagulation, or modulating lipoprotein metabolism [130-133].

Other compounds potentially present in roasted peanuts, such as Maillard-derived compounds, were shown to be effective suppressants of rancidity in model and food systems, while proteins and protein hydrolysate were also reported to be antioxidants in model systems [59,110,134-136].

The peanut skin has a pink-red color and astringent taste, and is typically removed before peanut consumption. However, peanut skin is reach in phenolic antioxidant compounds.

In earlier works, a flavone and a flavanone were purified from peanut skin by Masquelier and Blanquet [137]. Proanthocyanidines were described as the major phenolic and antioxidant compounds in peanut skins (138). Lou et al. [139] have identified six A-type procyanidines.

Some works have been undertaken to study antioxidant compounds obtained from Argentinean peanut skins [140-142]. In these studies, the content of phenolic compounds was found between 120-150 mg/g. Ethanolic extracts exhibited high radical-scavenging and antioxidant activity as

demonstrated in sunflower oil [140] and in peanut products [141]. In addition, resveratrol was also found in peanut skins in levels higher than that of peanut kernels [122,123].

In other studies, the antioxidant activity and major antioxidant principles of peanut seed skins were also isolated and identified as ethyl protocatechuate (3,4-dihydroxybenzoic acid ethyl ester) by Huang et al [110].

Yu et al. [143] have identified other phenolic compounds such as chlorogenic, caffeic, coumarin and ferulic acids, epigallocatechin, epicatechin, catechin galate and epicatechingalate.

Peanut hulls were found to exhibit marked antioxidant activity and antimutagenic effect. Several studies on the antioxidant components from peanut hulls have been performed [144-151]. Duh et al. [144] extracted and identified luteolin as the major flavonoid compound in peanut hulls with antioxidant activity similar to BHA and higher than α-tocopherol. Yen et al. [145] described the relationship between antioxidant activity of methanol extracts and maturity of peanut hulls and reported that the total phenolic content increased with maturity. Yen and Duh [147] reported a marked radical-scavenging effect of methanolic extracts of peanut hulls and these authors also found that the Spanish peanut cultivar had higher total phenolic content than other peanut cultivars [148]. Finally, Duh and Yen [151] reported that antioxidant compounds of methanolic extracts from peanut hulls had antioxidant efficacy in soybean and peanut oils.

Antioxidant activity of aromatic plants

Consumer interest in natural food additives, have reinforced the interest in natural antioxidants. Herbs and spices are harmless sources for obtaining natural antioxidants.

A very important compound in herbs of *Lamiaceae* family is rosmarinic acid, showing high scavenging DPPH potential, this being related to the presence of four hydroxyl groups in its molecule [152]. Oregano is particularly rich, 55000 ppm, in this compound and peppermint and lemon balm also contain high amounts, about 30000 and 37000 ppm, respectively [153].

Curcuma zedoaria (Berg.) Rosc. (*Zingiberaceae*) has long been used as a chinese folk medicine. The essential oil of its dried rhizome was moderate to good in antioxidant activities by three different methods, good in reducing power and excellent in scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical but low in chelating effect on ferrous ion. Although epicurzerenone and curzerene were found with moderate to good antioxidant activity, the compounds 5-isopropylidene-3,8-dimethyl-1(rH)-azulenone was responsible for better antioxidant properties [154]. However, natural curcuminoids were also isolated from *Curcuma longa* and showed reducing antioxidant activities [155].

Table 2. Aromatic plant extracts with antioxidant activity.

Species	Antioxidant sources	Antioxidant activity	Ref.
Achillea millefolium subsp. millefolium	WE	IC_{50} (μg/ml). Extract. DPPH 45.6, hydroxyl 407.3, LP 892.67. BHT, DPPH 19.3, hydroxyl 32.0, LP 17.8	158
Anthemis nobilis	AE	Antioxidant test: rapeseed oil at 40^{0}C. PV. low effective (0.2% w/w)	159
Baccharis grisebachii	*p*-Coumaric acid trans-ferulic acid quercetin	% Free radical scavengers: p-coumaric acid (35% 10μg/ml) quercetin (97% 10μg/ml)	160
Balsamita major	AE	Antioxidant test: rapeseed oil at 40^{0}C. PV. low effective (0.2% w/w)	159
Citrus grandis	EAE	% Scavenging effect: Albedo extract: 80%, flavedo extract: 80%. BHT 80%.	161
Coriandrum sativum	Extract of high density (0.73-0.83g/ml) CO_2	Moderate activity	162
Cuminum cyminum	ME	Protection factor (Conc. 0.02%) 1,1	163
Cyclotrichium origanifolium	HE	% Inhibition of β-carotene-linoleic acid assay 62.5. BHT 96	164
Cyclotrichium origanifolium	AME	% Inhibition of β-carotene-linoleic acid assay 70.9. BHT 96	164
Cyclotrichium origanifolium	WE	% Inhibition of β-carotene-linoleic acid assay 76.9. BHT 96	164
Eucalyptus globulus	EAE	EC_{50} of 0.15 g/l	165, 166
Eucalyptus sp.	Gallic acid ellagic acid, eucalyptone macrocarpals	Low activity	167
Foeniculum vulgare	ME	Protection factor (Conc. 0.02%) 1,4	163
Foeniculum vulgare	WE EE	Water extract: 100 μg 99.1% inhibition of peroxidation in linoleic system. α tocopherol (36.1%)	168
Geranium purpureum	ME	Protection factor (Conc. 0.02%) 1,5	163
Hierocloe odorata	AE	Antioxidant test: rapeseed oil at 40^{0}C. PV. was most effective (0.2% w/w)	159
Hipophae rhamnoides	AE	Antioxidant test: rapeseed oil at 40^{0}C. PV. low effective (0.2% w/w)	159
Jasminun officinalis	ME	Protection factor (Conc. 0.02%) 0.8	163
Laurus nobilis	AME	IC_{50} mg/ml. Extract 0.55, BHT 0.1	156
Lavandula angustifolia	EAE	DPPH (% inhibition) 2.5	169
Lavandula angustifolia	ME	DPPH (% inhibition) 35.4	169
Matricaria recutita	EAE	DPPH (% inhibition) 6.4	169
Matricaria recutita	ME	DPPH (% inhibition) 44.7	169

Table 2. Continued

Melissa officinalis	DFH	DPPH 93 % (Conc. 0.41%)	153
Mentha x piperita	DFH	DPPH 92 % (Conc. 0.41%)	153
Nepeta cataria	ME	Protection factor (Conc. 0.02%) 1,6	163
Ocimum basilicum	AME	IC_{50} mg/ml. Extract 0.5, BHT 0.1	156
Ocimum sanctum	EE	IC_{50}, 16 μg inhibited LP. IC_{50}, 6 μg inhibited LP.	170
Origanum dictaminus	ME	Protection factor (Conc. 0.02%) 1,2	163
Origanum vulgare	DFH	DPPH 93 % (Conc. 0.41%)	153
Origanum vulgare ssp *hirtum*	HE	Moderate	171
Origanum vulgare ssp. *vulgare*	ME	It behaved as a strong free radical scavenger IC_{50} = 9.9 μg/ml. It inhibited the 32% of linoleic acid oxidation at 2mg/ml	172
Origanum vulgare	AME	IC_{50} mg/ml. Extract 0.32, BHT 0.1	156
Pimenta dioica	HE	It inhibited autoxidation of linoleic acid in a water-alcohol system	173
Pinus nigra subsp. pallsiana	Turpentine exudes	500 μg of exudes showed 91% inhibition on peroxidation of linoleic acid emulsion	174
Pistacia lentiscus.	Ursolic acid oleanolic acid	Satisfactory antioxidant activity in sunflower oil	175
Rosmarinus officinalis	AME	IC_{50} mg/ml. Extract 0.2, BHT 0.1	156
Ruta graveolens	ME	Protection factor (Conc. 0.02%) 1,6	163
Salvia aethiopis	ME	% β-carotene-linoleic acid. BHT 96.0. Extract: 29.0	176
Salvia caespitosa	ME	% DPPH. BHT 18.8. Extract: 41.3. % β-carotene-linoleic acid. BHT 96.0. Extract: 55.9	176
Salvia candidissima subsp. candidissima	ME	% DPPH. BHT 18.8. Extract: 49.7. % β-carotene-linoleic acid. BHT 96.0. Extract: 62.3	176
Salvia cryptantha	ME	Low efficient in free radical scavenging and LP inhibition	164
Salvia euphratica subsp. euphratica	ME	% DPPH. BHT 18.8. Extract: 20.7. % β-carotene-linoleic acid. BHT 96.0. Extract: 59.1	176
Salvia glutinosa	EAE	DPPH (% inhibition) 16.2	169
Salvia glutinosa	ME	DPPH (% inhibition) 91.5	169
Salvia hypargeia	ME	% DPPH. BHT 18.8. Extract: 34.6. % β-carotene-linoleic acid. BHT 96.0. Extract: 69.2	176
Salvia multicaulis	ME	Low efficient in free radical scavenging and LP inhibition	164
Salvia officinalis	EAE	DPPH (% inhibition) 91.7	169
Salvia officinalis	ME	DPPH (% inhibition) 92.3	169
Salvia officinalis	WE	Liver antioxidant potential	177
Salvia officinalis	Polyphenols	Luteolin glycosides were more active than apigenin glycosides	178

Table 2. Continued

Salvia officinalis	AE	Antioxidant test: rapeseed oil at 40^{0}C. PV. was most effective (0.2% w/w)	159
Salvia officinalis	AME	IC_{50} mg/ml. Extract 0.1, BHT 0.1	156
Salvia plebeia	Compounds isolated	Lard 100^{o}C. Conc. 0.02%. β-sitosterol: 5.3; 2'-hydroxy-5'-methoxybiochanin A: 5.2, BHT: 4.2	179
Salvia pratensis	EAE	DPPH (% inhibition) 17.2	169
Salvia pratensis	ME	DPPH (% inhibition) 93.0	169
Salvia sclarea	EAE	DPPH (% inhibition) 21.5	169
Salvia sclarea	ME	DPPH (% inhibition) 92.4	169
Salvia sclarea	ME	% DPPH. BHT 18.8. Extract: 23.4 % β-carotene-linoleic acid. BHT 96.0. Extract: 63.5	176
Satureja hortensis	AME	IC_{50} mg/ml. Extract 0.31, BHT 0.1	156
Satureja hortensis.	EE	Stabilization of sunflower oil at frying temperature	171
Syzygium cumini	WE	IC_{50} (μgml^{-1}). Hydroxyl radical: 428, DPPH 168, LP 222.	180
Tanacetum vulgare	AE	Antioxidant test: rapeseed oil at 40^{0}C. PV. low effective (0.2% w/w)	159
Terminalia catappa	ME	0.1 mg ml^{-1} excellent activity as scavenging effects	181
Thymus spathulifolius	ME	IC_{50} (μg/ml). Extract. DPPH 16.15. BHT 19.8	182
Thymus vulgaris	AME	IC_{50} mg/ml. Extract 0.2, BHT 0.1	156

Abbreviations: Extracts: methanol (ME), ethanol (EE), ethyl acetate (EAE), n-hexane (HE), water (WE), acetone (AE), aqueous-methanol (AME). Dry and Fresh herbs (DFH). Lipid peroxidation (LP). Peroxide value (PV), Conc.: Concentration.

Acid treatment may have effect on antioxidative efficacy of polyphenolic phytochemicals [156]. The ethanolic extracts of *Ocimum sactum* L. and *Alpinia galanga* showed good heat stability (80^{o}C, 1 h). At neutral and acidic pH, *O. sactum* extracts had high antioxidative stability, whereas *A. galanga* extracts showed higher antioxidative stability at neutral than at acidic pH ranges. Holy basil and Galangal extracts exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity [157].

In dried aromatic plant material the significant losses of both ascorbic acid and carotenoids were found in comparison with the fresh aromatic herbs. Ascorbic acid is easily decomposed during post-harvest treatment of leafy tissue and carotenoids are sensitive to oxidation radiation. However, the dried aromatic herbs are rich sources of antioxidants, in prticular from the group of phenolic compounds [153].

Antioxidant activity from aroma compounds and essential oils

Some volatile compounds from aroma and/or essential oils possess the potential as natural agents for food preservations [183-185]. The antioxidant activities of aroma extracts obtained from spices, herbs, brewed coffee and beans have been investigated in various model systems [186-188]. Some known natural aroma components, such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone, maltol, and 5-hydroxy methylfurfural have been reported to possess appreciable antioxidant activities [189].

There is now evidence of beneficial influence of essential oils on lipid metabolism, ability to stimulate digestion and antioxidant properties. Eugenol (clove), linalool (coriander) and cuminaldehyde (cumin) were found to be effective antioxidants. These compounds inhibited lipid peroxidation by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase. At the same time, there was no alteration in the fatty acid composition of membrane lipids and the levels of endogenous antioxidants, vitamin E, ascorbic acid and glutathione [190-194].

Aroma extract from dried clove buds (*Syzygium aromaticum* (L.) Merr. et Perry, *Myrtaceae*) and its two major aroma chemicals, eugenol and eugenyl acetate, showed antioxidative activity in two different assays: oxidation of hexanal and inhibition malonaldehyde formation from cod liver oil. Their activity is not as strong as α-tocopherol and BHT [187].

Eucalyptus polyanthemos Schauer was examined for its inhibitory effect on the system malonaldehyde formation from horse blood plasma oxidized with Fenton's reagent. Thus, the chemicals from aroma of *Eucalyptus* inhibited malonaldehyde formation by 23%, at level of 400 μg/ml, whereas α-tocopherol and BHT inhibited malonaldehyde formation by 52 and 70% respectively, at the same level [195].

The antioxidant activity of eugenol has been reported several times on various systems [196,197]. As eugenol and thymol have a phenolic group, the phenolic group plays an important role in their antioxidant activities. Benzyl alcohol that does not contain any phenolic group displayed reasonable antioxidant potential. The radical formed on benzyl carbon is stabilized by the adjacent benzene ring. This radical consequently abstracts a hydroxyl radical to form benzaldehyde [195].

The essential oil of *Salvia tomentosa* Miller (Lamiaceae) was particularly found to possess low antioxidant activity. However, the free radical scavenging activity of aqueous methanol extract was superior to all other extracts of *S. tomentosa* (IC_{50} = 18.7 μg/ml) [198].

The glycosidicaly bound volatiles amounted to 20 mg kg^{-1} in dried leaves and flowers of oregano (*Origanum vulgare* ssp. *hirtum*). Thymoquinone was identified as the major component. Other important aglycones were benzyl

alcohol, eugenol, 2-phenyl-ethanol, thymol, 3-hexen-1-ol and carvacrol. It was found that all of the aglycones have an antioxidant effect when tested by measuring peroxide values of lard stored at 60^0C [199].

The free radical scavenging activity of the essential oil of *Thymus sipyleus* subsp. *sipyleus* var. *rosalans* was superior to var. *sipyleus* oil (IC_{50} = 220 and 2670 μg/ml, respectively). However, in the case of linoleic acid system, oxidation of linoleic acid was effectively inhibited (92%) by *T. sipyleus* subsp. *sipyleus* var. *rosalans*, while the var. *sipyleus* oil had no activity [198].

The values of IC_{50} for *S. cryptantha* and *S. multicaulis* were 17.8 and 14.5 μg/ml, whereas BHT exhibited values of 7.8 μg/ml [13].

Among the constituents of essential oils, thymol, carvacrol, 4-allylphenol and eugenol exhibited potent antioxidant activities; those aroma chemicals inhibited hexanal oxidation by 95 – 99 % at 5 μg/ml over 30 days, which is comparable to that of α-tocopherol or BHT. The hexanal oxidation was almost completely inhibited by 4-allylphenol at 5 μg/ml over 30 days. Among other aromatic components tested for their antioxidant activity, benzyl alcohol showed slight antioxidant activity at the level of 50 μg/ml over 30 days. At the lowest level of 1 μg/ml, eugenol displayed the highest antioxidant activity among the essential oil components tested. Linalool, methyl-salicylate, estragol, 1,8-cineole, 4-terpineol and benzylaldehyde showed slight antioxidant activity at a level of 50 μg/ml, they inhibited hexanal oxidation among 23% and 10% [200]. Anethole (10 mM) and related compounds inhibited DPPH among 40 - 90%. The introduction of hydroxyl groups in the double bond of the lateral chain of anethole molecule, increased antioxidant activity [201]. Isoeugenol, eugenol and anethole present a methoxybenzene moiety and a propenyl group. The conjugated double bonds of anethole and isoeugenol are known for stabilizing the reactivity of the phenyl and benzyl groups and contribute for the antioxidant properties of these molecules [202,203].

Lippia alba showed antioxidant protecting effects within of range of 5-20 g/l [204]. γ-Terpinene, a monoterpene hydrocarbon present in essential oils, retards the peroxidation of linoleic acid. The peroxidation of γ-terpinene has been shown to yield p-cymene as the only organic product in a chain reaction in which the chain carrier is the hydroperoxyl radical. The peroxidation of linoleic acid is well known to be a chain reaction in which the chains are carried by linoleyl peroxyl radicals, and the products are linoleyl hydroperoxides. The retardation of linoleic acid peroxidation by γ-terpinene has been found to be due to rapid chain termination via a very fast cross-reaction between hydroperoxyl radical and linoleyl peroxyl radicals [205].

Oxidation of LDL (low-density lipoprotein) is believed to play a key role in atherogenesis. For this reason, a sufficient protection of LDL by antioxidants may provide protection from atherosclerosis. γ-Terpinene showed inhibition of

LDL oxidation [206]. The monoterpene terpinolene from the oil of *Pinus mugo* L. also prevents oxidation of LDL [207]. Inhibition of LDL oxidation by eugenol compounds is due to the suppression of free radical cascade of lipid peroxidation LDL by reducing copper ion [208,209].

Essential oils as antioxidants in foods

Aromatic herbs have a long history of culinary use. Not only are many of the flavors and aromas distinctively pleasant, but they can be used to conceal off-flavors and odors. The high susceptibility to oxidation of the fat and oil polyunsaturated fatty acids used in human foods requires the application of antioxidants [210-212]. Thus, in the last years essential oil research has concentrated on two primary area: the antimicrobial [213,214] and the antioxidant activities. However, Smith et al. [215] have developed a guide to evaluate the safety of essential oils, for their intended use as antioxidants and/or flavor ingredients.

Enzymatic browing in fruit and vegetable tissues can cause undesirable quality changes during handling, processing and storage. This reaction results mostly from polyphenol oxidase and peroxidase. Both enzymes catalyse more than one reaction and act on a number of substrates, not only causing browning of foods but also leading to discoloration, off-flavors and nutritional damage [216]. Essential oils of *Eucalyptus globulus*, *Melaleuca alternifolia*, *Melissa officinalis*, *Rosmarinus officinalis*, *Syzygium aromaticum* and *Citrus limonum* reduced peroxidase activity of organic leafy vegetables extracts. Thus, essential oil of clove was more effective than the other oils [217].

Botsoglou et al. [218-220] examined the effects of dietary oregano essential oil on the susceptibility of raw and cooked turkey breast, thigh meat and chicken breast to lipid oxidation during refrigerated storage for 9 days. Oregano oil at 200 mg kg^{-1} was significantly more effective in delaying lipid oxidation compared to the level of 100 mg kg^{-1}, equivalent to α-tocopheryl acetate at 200 mg kg^{-1}, but inferior to oregano oil plus α-tocopheryl acetate at 100 mg kg^{-1} each, which in turn was superior to all dietary treatments, indicating a synergistic effect. The effect of dietary dried oregano (*Origanum vulgare* subsp. *hirtum*) leaves supplementation on performance and carcass characteristics was determined by Bampidis et al. [221]. The oregano diet for growing of lamb did not affect their performance and carcass characteristics.

Application of antioxidants is one of the technically simplest ways of reducing fat oxidation. Thus, Tomaino et al. [222] studied the effect of heating on antioxidant effectiveness of essential oils. When maintained at room temperature, basil, cinnamom, clove, nutmeg, oregano and thyme essential oils tested appeared endowed with good radical scavenger properties in the DPPH assay, with order effectiveness clove >> cinnamon>nutmeg>basil> oregano >> thyme.

Table 3. Essential oils with antioxidant activity.

Species	Essential oil composition	Antioxidant activity	Ref.
Acantholippia seriphioides	Thymol (55%) carvacrol (10%)	High effect (PV, Conc.EO 0.02 - 0.1%)	11
Achillea millefoium subsp. millefolium	1,8-Cineole (24.6%) camphor (16.7%) α-terpineol (10.2%)	IC_{50} (μg/ml) EO: DPPH 1.56, Hydroxyl, 2.70, LP 13.52. BHT. DPPH 19.3, Hydroxyl 32.0, LP 17.8	158
Artemisia annua.	Camphor (44%), germacrene D (16%), trans-pinocarveol (11%) β-selinene (9%) β-caryophyllene (9%)	Moderate effect	224
Artemisia judaica	Piperitone (45%), trans-ethyl cinnamate (20.8%) ethyl-3-phenyl propionate (11.0%)	Inhibition Peroxide BHT 63.6% EO 56.1%. Reduction DPPH BHT 75% EO 65%	225
Cananga odorata	Linalool (24.5%) benzyl salicylate (12.8%) benzyl benzoate (33.6%)	% Free radical scavenging activity: EO: 65, Trolox: 30 % Antioxidant activity: EO: 75, BHA: 89	226
Clausena anisata	Estragole (66,2%) (E)-anethole (17.6%)	IC_{50} BHT = 42 μg l^{-1} IC_{50} thymol = 333 μg l^{-1} IC_{50} carvacrol = 666 μg l^{-1} IC_{50} EO = 3.4 g l^{-1}	227
Cupressus sempervirens	α-Pinene (19.3%) sabinene (39.6%) γ-terpinene (6.1%)	% Free radical scavenging activity: EO: 20, Trolox: 30 % Antioxidant activity: EO: 58, BHA: 89	226
Curcuma longa	α-Turmerone (19.8%) 4-terpineol (7.3%) zingiberene (6.9%) β-turmerone (7.3%)	% Free radical scavenging activity: EO: 61, Trolox: 30 % Antioxidant activity: EO: 70, BHA: 89	226
Curcuma zedoaria	Epicurzerenone (24.1%) curzerene (10.4%)	Scavenging effect. EO: 12,7 to 90.5% (conc. 0.1 to 15 mg ml^{-1}). Ascorbic acid 87.6%, BHA 93%, α tocopherol 38% (conc. 0.01 mg ml^{-1})	154
Cyclotrichium origanifolium	Pulegone (49.8%) menthone (32.5%) limonene (6.0%)	IC_{50} (μg/ml) EO: DPPH 17.1 β-carotene-linoleic acid (inhibition %) 7.1	164
Cymbopogon citratus	Myrcene (15.4%) neral (32.3%) geranial (41.3%)	% Free radical scavenging activity: EO: 60, Trolox: 30 % Antioxidant activity: EO: 50, BHA: 89	226
Eucalyptus cinerea	1, 8-cineole (70%)	Low effect (PV, Conc. EO 0.02 - 0.1%)	11
Eucalyptus globulus	α-Pinene (20.0%) 1,8-cineole (52.6%)	% Free radical scavenging activity: EO: 15, Trolox: 30 % Antioxidant activity: EO: 58, BHA: 89	226
Eucalyptus globulus	1,8-cineole (80%)	Low effect (PV, Conc. EO 0.02 - 0.1%)	10
Eucalyptus polyanthemos	Thymol 0.74 ppm benzyl alcohol 2.79 ppm	It inhibited malonaldehyde formation by 23% al 400 μg ml^{-1}	195
Foeniculum vulgare sbsp capillaceum var. dulce	Anethole (70%)	High effect (PV, Conc. EO 0.02-0.1%)	10
Laurus nobilis	Eugenol (30%)	High effect (PV, Conc. EO 0.02-0.1%)	10
Lippia alba	Carvone (40-57%), limonene (24-37%)	Low effect	204
Lippia polystachya	α Thujone (69%)	Low effect (PV, Conc. EO 0.02 - 0.1%)	10
Myrcianthes cisplatensis	1,8-cineole (43%)	Low effect (PV, Conc. EO 0.02 - 0.1%)	11
Ocimun basilicum	Linalool (3,94 mg/g), estragole (2.03 mg/g),	High effect	200

Table 3. Continued

	methyl cinnamate (1,28 mg/g) eugenol (0.896 mg/g) 1,8-cineole (0.288 mg/g)		
Origanum majorana	Carvacrol (77%)	High effect (PV, Conc. EO 0.02-0.1%)	11
Origanum vulgare ssp. hirtum	Thymoquinone (40.2%)	Moderate effect	199
Origanum vulgare ssp. hirtum	Thymol, carvacrol, γ-terpinene, p-cymene	Less effective than ascorbic acid, comparable with α-tocopherol and BHT.	228
Origanum vulgare ssp. vulgare	Caryophyllene (14.4%) spathulenol (11.6%), germacrene D (8.1%), α-terpineol (7.5%)	IC_{50} at 8.9 μg/ml. 2.2 mg/ml EO provided 50% inhibition in the linoleic acid oxidation test system	172
Pinus radiata	α-Pinene (21.9%) β-pinene (35.2%) β-phellandrene (12.6%)	% Free radical scavenging activity: EO: 20, Trolox: 30 % Antioxidant activity: EO: 20, BHA: 89	226
Piper crassinervium	α-Pinene (10.0%) β-pinene (15.1%) limonene (26.6%)	% Free radical scavenging activity: EO: 45, Trolox: 30 % Antioxidant activity: EO: 52, BHA: 89	226
Psidium guayava	α-Pinene (29.5%) limonene (33.3%)	% Free radical scavenging activity: EO: 25, Trolox: 30 % Antioxidant activity: EO: 15, BHA: 89	226
Rosmarinus officinalis	Camphor (14.6%) borneol (10.4%) verbenone (21.7%) bornyl acetate (12.3%)	% Free radical scavenging activity: EO: 65, Trolox: 30 % Antioxidant activity: EO: 80, BHA: 89	226
Rosmarinus officinalis	1,8-cineole (57%)	Low effect (PV, Conc. EO 0.02-0.1%)	11
Salvia cryptantha	α-Pinene (18.1%) 1,8-cineole (15.3%), camphor (7,7%)	IC50 μg/ml. DPPH. EO: 3.9, BHT: 19.8. LP EO: 17.8, BHT: 78	13
Salvia multicaulis	α-Pinene (21.9%), 1,8-cineole (20.1%), camphor (11.1%)	IC50 μg/ml. DPPH. EO: 2.4, BHT: 19.8. LP EO: 14.5, BHT: 78	13
Salvia tomentosa	β-Pinene (39.7%) α-pinene (10.9%), camphor (9.7%)	Low effect	198
Satureja parvifolia	Piperitone oxide (31%)	Low effect (PV, Conc. EO 0.02 - 0.1%)	10
Syzygium aromaticum	Eugenol (24.37 mg/g) eugenyl acetate (2,35 mg/g)	It inhibited the oxidation of hexanal by 99% at 500 μgml^{-1}	187
Tagetes filifolia	Anethole (70%) methyl chavicol (25%)	High effect (PV, Conc. EO 0.02- 0.1%)	11
Tagetes minuta	Ocimenone (67%)	Low effect (PV, Conc. EO 0.02- 0.1%)	10
Teucrium marum subsp. marum	Isocaryophyllene (20.24%) β-bisabolene (14.73%) β-sesquiphellandrene (11,27%) α-santalene (10,97%)	IC_{50} (μg/ml). EO: DPPH 13.1, Superoxide, 0.16, LP 12.4. BHT: DPPH 86.6, Superoxide 2.35, LP 3.86	229
Thymus caespititius	α-Terpineol (32%) p-cymene (9.2%) γ-terpinene (6.6%)	Antioxidant activity (%), at 500 mgl^{-1} α tocopherol: 74.2, BHT: 65.6, EO: 76.3	230
Thymus camphoratus	Linalool (17%), linalyl acetate (15%), 1,8-cineole (11%)	Antioxidant activity (%), at 500 mgl^{-1} α tocopherol: 74.2, BHT: 65.6, EO: 51.6	230

Table 3. Continued

Thymus mastichina	1,8-Cineole (58%) limonene (10.7%)	Antioxidant activity (%), at 500 mgl^{-1} α tocopherol: 74.2, BHT: 65.6, EO: 38.9	230
Thymus sipyleus subsp. sipyleus var. rosulans	Carvacrol (58.1%), thymol (20.5%) p-cymene (4.1%)	Free radical scavenging activity. IC50 μg/ml 220. Linoleic acid inhibition rate of EO: 92.0%. BHT: 96.0%	176
Thymus sipyleus subsp. sypyleus var. sipyleus	Borneol (11.2%) α muurolol (9.2%) β-caryophyllene (7.6%)	Free radical scavenging activity. IC_{50} μg ml^{-1}. 2670.	199
Thymus spathulifolius	Thymol (36.5%), carvacrol (29.8%), p-cymene (10.0%)	IC_{50} μg/ml. EO DPPH 243, BHT 19.8. EO β - carotene/linoleic acid 92%, BHT 96%	182
Thymus vulgaris	Thymol (30%), carvacrol (15%)	High effect (PV, Conc. EO 0.02- 0.1%)	10
Thymus vulgaris	p-Cymene (15.3%) geraniol (8.2%) thymol (6.8%) carvacrol (7.9%)	% Free radical scavenging activity: EO: 75, Trolox: 30 % Antioxidant activity: EO: 90, BHA: 89	226
Thymus vulgaris	Thymol (8.55 mg/g), carvacrol (0.681 mg/g)	High effect	200
Thymus x citriodorus	Geraniol (36.4%) geranyl acetate (22.4%)	% Free radical scavenging activity: EO: 25, Trolox: 30 % Antioxidant activity: EO: 50, BHA: 89	226
Zingiber officinale	Camphene (9.9%) β-sesquiphellandrene (10.9%) Ar-curcumene (8.9%) zingiberene (23.9%) β-bisabolene (11.4%)	% Free radical scavenging activity: EO: 55, Trolox: 30 % Antioxidant activity: EO: 68, BHA: 89	226
Ziziphora taurica subsp. cleonioides	(+)-Pulegone (81,8%) limonene (4.4%) piperitenone (2.3%)	β-tocopheryl acetate equivalent mM. 1.145	231

Abbreviature: EO = essential oils, PV = peroxide value, LP = lipid peroxidation. Conc.= concentration.

When heated up to 180°C, nutmeg oil, but not the rest of essential oils, showed a significantly higher free radical - scavenger activity.

Ageing is the progressive accumulation of changes with time, associated with or responsible for the ever-increasing likelihood of diseases and death that accompanies advancing age. There is growing evidence that this processes may be the consequence of free radical reactions. Although, the removal of damaging oxygen species is catalysed by antioxidant enzymes, from foods numerous non-enzymatic defense are also employed to provide protection, these include vitamin E, vitamin C and essential oils [223].

Methods for testing antioxidative activity

Total antioxidant activities of the plant extracts can not be evaluated by any single method, due to the complex nature of phytochemicals. Thus, two or more methods should always be employed in order to evaluate the total antioxidative effects of vegetables [232-235].

The BCB (β-carotene bleaching method) employs an emulsified lipid, which introduces a number of variables influencing antioxidant activity of samples. The BCB method can be helpful especially for investigations of lipophilic antioxidants and it is appropriate for the investigation of the antioxidant activity of essential oils. On the other hand if polar compounds as ascorbic acid, rosmarinic acid, etc, are tested only by the BCB method they would be considered as weak antioxidants [228].

The DPPH method is faster than BCB method and it can be helpful in investigation of novel antioxidants for a rapid estimation and preliminary information of radical scavenging abilities. The method is sensitive and requires small sample amounts [236].

TBA method is also sensitive and achieves reproducible results. This method is preferable in order to obtain useful data in an environment similar to the real-life situation. Both methods, DPPH and TBA, similarly allow testing of both lipophilic and hydrophilic compounds [228,234]. However, the antioxidant power depends on the chosen method, on the concentration and on the nature and physicochemical properties of studied antioxidants [234].

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