Phenolic Compounds: Their Role During Olive Oil Extraction and in Flaxseed – Transfer and Antioxidant Function

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1. Literature Review

1.1. PHENOLIC COMPOUNDS

Phenolics belong to a group of compounds identified in plants that appears to have no intrinsic role in the physiological processes of the producer. They are known as secondary metabolites and have their own specific complement of specialized enzymes and different energetic and metabolic requirements. They are the most studied group of secondary metabolites.

The term phenolic is used to define substances that possess one or more hydroxyl (OH) substituents bonded onto an aromatic ring. The name derives from the simple parent substance phenol and compounds with several phenolic hydroxyl substituents are commonly referred to as polyphenols. Althought, not all hydroxyl groups are phenolics since they are equally likely to occur bonded to non-aromatic cyclic or to non-cyclic structures in which case they do not have the properties of a phenol (Waterman & Mole, 1994).

Distinguished properties that are of consequence in the biosynthesis of phenolic compounds include:

- The ability of the phenoxide ion to delocalize, the negative charge can move into the aromatic ring system to form hemiquinone anions in which the charge resides on a carbon rather than on the oxygen.

- The potential for the phenoxide ion to form the corresponding radical which can also delocalize. Two such radicals can undergo a process known as oxidative coupling in which covalent carbon-carbon or carbon-oxygen bonds are formed.

- The capacity of the phenols to form hydrogen bonds with other molecules through interaction between the acidic (positively charged) phenolic hydrogen and basic (negatively charged) centres in others molecules.

1.1.1 Classification

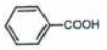
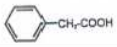
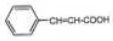
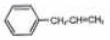
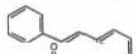
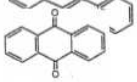
The versatility of organisms, especially plants, in the production of secondary metabolites is quite confounding, particularly when the limited range of building blocks that they employ are considered. Many structural classes of secondary metabolites such as alkaloids, flavonoids and triterpenes, include some compounds that can be classified as phenolics.

The classification of the phenolic ompounds is very complex. Phenolics can be classified in several groups according to their structural skeleton which can range from simple molecule to highly polymerized compounds. They happen primarily in conjugated form with one or more sugar residues linked to hydroxyl groups, direct linkages of the sugar unit to an aromatic carbon atom also exist. Associations with other compounds, such as carboxylic and organic acids, amines and lipids, and linkages with other phenols are also frequent (Bravo, 1998). Harborne (1989) reviewed all the phenolic compounds and their classification. A summary, the main classes of phenolic compounds in plants is provided in Table 1. The structure of the various phenolic influences their diverse roles in the plant. They could be a structural barrier (lignin), a colour (anthocyanin), a toxic agent against microorganism (pinoresinol) or a molecule involved in plant defense (salycilic acid).

1.1.2 Biosynthetic Origin of Phenolic Compounds

The vast majority of phenolic molecules owe their origin to one or more of the three following building blocks, erythrose-4-phosphate, phosphoenol pyruvate and/or acetyl co-enzyme A.

Table 1. Main Classes of Plant Phenolics

Number of C atoms	Basic skeleton	Class	Basic Structure
6	C_6	Simple phenols, benzoquinones	
7	$C_6 - C_1$	Phenolic acids	
8	$C_6 - C_2$	Phenylacetic acids	
9	$C_6 - C_3$	Hydroxycinnamic acids	
		Polypropene	
		Isocoumarins	
10	$C_6 - C_4$	Naphtoquinones	
13	$C_6 - C_1 - C_6$	Xanthones	
14	$C_6 - C_2 - C_6$	Stilbenes	
		Anthrachinones	
15	$C_6 - C_3 - C_6$	Flavonoids, isoflavonoids	
18	$(C_6 - C_3)_2$	Lignans, neolignans	
30	$(C_6 - C_3 - C_6)_2$	Biflavonoids	
N	$(C_6 - C_3)_n$ $(C_6)_n$ $(C_6 - C_3 - C_6)_n$	Lignins catecholmelanine (condensed tannins)	

Source: Harborne, 1989

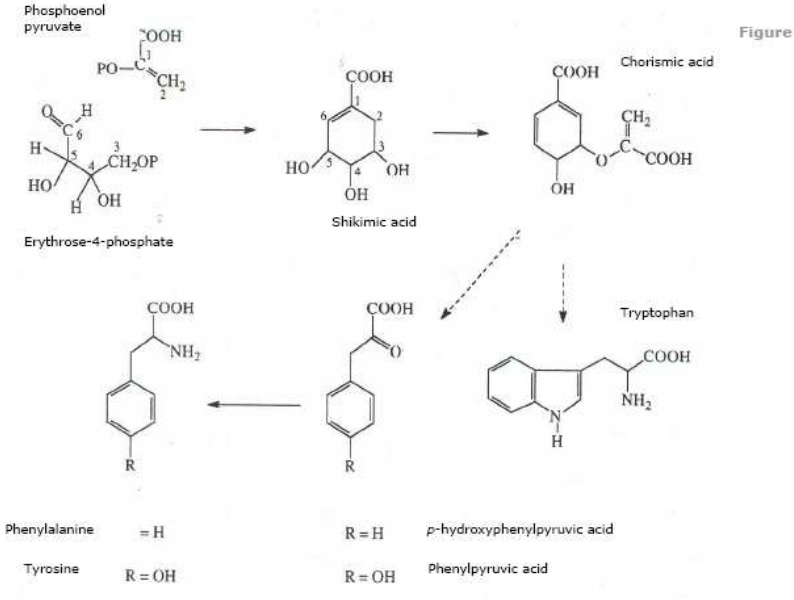
Erythrose-4-phosphate and phosphoenol pyruvate are both responsible for the known shikimic acid pathway, whereas acetyl co-enzyme A and its activated form (malonyl co-enzyme A) are the central point of the polyketide or acetate pathway. Acetyl co-enzyme A may also be converted into mevalonic acid which is the beginning for a wide range of terpene compounds (Waterman & Mole, 1994).

Shikimic Acid Pathway. The shikimic acid route is more complex than the acetate pathway. Gallic acid is the fully aromatized form of shikimic acid and its formation is complex. In the shikimic acid pathway products are not generate until structural developments beyond shikimic acid happened. Addition of a second molecule of phosphoenol pyruvate to shikimic acid leads to the formation of chorismic acid; at this point, the route to aminoacid tryptophan separates. From the formation of the typical shikimate C_6C_3 skeleton, the amino acids phenylalanine and tyrosine are generated from phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid, respectively by the addition of nitrogen. These three amino acids (tryptophan, phenylalanine and tyrosine) are the most important precursors of the alkaloid synthesis (Figure 1) (Waterman & Mole, 1994).

Shikimate-derived non-nitrogenous phenolic synthesis occurs mainly through phenylalanine. Phenylalanine ammonia lyase (PAL) catalyzes the release of ammonia from phenyl-alanine and leading to the formations of a carbon-carbon double bond to yield *trans*-cinnamic acid. In some plants and grasses tyrosine is converted into-4-hydroxy-cinnamic acid via the action of tyrosine ammonia lyase (TAL). Introduction of a hydroxyl group into the *para* position of the phenyl ring of cinnamic acid proceeds via catalysis by monooxygenase utilizing cytochrome P_{450} as the oxygen binding site. The formed *p*-coumaric acid could be hydroxylated in position 3 and 5 by hydroxylases, possibly methylated via an O-methyl transferase with S-adenos-ylmethionine as methyl donor. This result in the synthesis of caffeic, ferulic and sinapic acids. All possess a C6 phenyl ring and a C3 side chain, they belong to the phenylpropanoid group. These compounds act as precursors of the synthesis of lignins and many other compounds (Shahidi & Naczk, 2004).

Benzoic acids and derivates are produced via removal of a two-carbon moiety from phenylpropanoids. Similar to phenylpropanoid series, hydroxylation and possibly methylation of hydroxybenzoic acids lead to the formation of dihydroxybenzoic acid (protocatechuic acid), vanillic acid, syringic acid and gallic acid. Some evidence shows that gallic acid may also be formed, in some plants, directly from shikimic acid (Waterman & Mole, 1994).

Source: Waterman & Mole, 1994



Figure

Biosynthesis of Aromatic Acids Via the Shikimic Acid Route

Hydroxybenzoic acids are commonly present in a bound form in foods. They are often part of a complex structure like lignins or hydrolysable tannins. They are also found in the form of organic acids and sugar derivatives. Normally, phenylpropanoids (cinnamic acid family) and benzoic acid derivatives are conventionally referred as phenolic acids.

Simple phenols are formed by decarboxylation of benzoic acid and phenylpropanoid derivatives (Figure 2). Thermal degradation or microbial transformation of lignin may also produce simple phenols in food. In addition, reduction products of phenylpropanoids also yield various phenolic compounds such as sinapyl, coniferyl, and coumaryl alcohols. Lignin is found covalently bound to cellulose in cell walls (Shahidi & Naczk, 2004).

In almost all naturally occurring coumarins, the precursor appears to be *p*-coumaric acid which has to undergo two critical modifications. The double-bond must change from the *trans* to the *cis* configuration and the aromatic nucleus must undergo oxidation in the side-chain to give 2,4-hydroxy-*cis*-cinnamic acid. The main compounds are the simple coumarins, furanocoumarins and pyranocoumarines, present in free and glycosidic forms in foods.

Another group of simple shikimate-derived secondary metabolites are the lignans from NDGA (nordihydriguaiaretic acid) which is probably a derivative of *p*-coumaric acid of the corresponding alcohol. From *p*-coumaric acid a series of radicals can be formed culminating in that in which the β-carbon of the 3-C side-chain is the activated position and return to the fully phenolic form by regaining the hydrogen lost in the initial radical formation to give NDGA. The biosynthesis of pinoresinol is more complex. From coniferyl alcohol, the initial formation of the β-β bond needs to be followed by two further cyclizations involving, in each case, an alcohol or one monomer for the first one and α-C for the second one (Waterman & Mole, 1994).

Combination of Shikimate and Acetate Pathways. The combination of shikimate and acetate, in the form $C_6C_3 + (C_2)_n$, where n is usually three ($C_6C_3C_6$) generate several groups of phenolics which the flavonoids are by the far the most important (Figure 3).

Flavonoids are formed via condensation of a phenylpropane (C_6-C_3) compound with the participation of three molecules of malonyl coenzyme A. This results in the formation of chalcones, that subsequently cyclize under acidic conditions. Once formed, the flavanone nuclei can undergon modifications involving the

pyran-4-one ring. Oxidation (either adding oxygen or removing hydrogen to form a new double-bond) or reduction (addition of hydrogen), give a wide variety of flavonoid classes such flavonoids as flavonols, flavones, and flavonols.

Source: Shahidi & Nackz, 2004

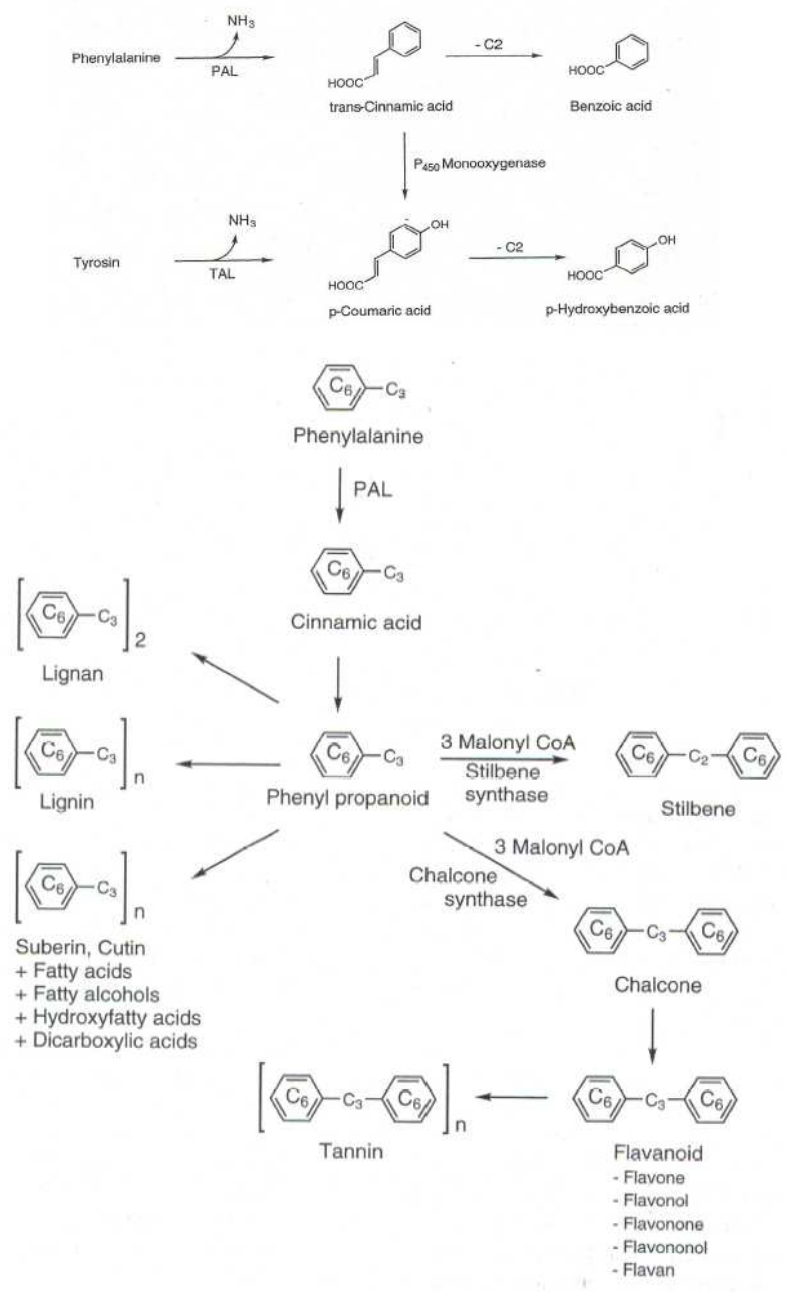


Figure 3. Biosynthesis of Stilbenes, Lignans, Lignins, Suberins, Cutins, Flavonoids, and Tannins

PAL = Phenylalanine Ammonia Lyase

Individual flavonoids may also vary with the number and distribution of hydroxyl groups as well as in their degree of alkylation or glycosylation. The formation of flavonol and flavone glycosides depends on the action of light. The monoglycosides occur greatly as 3-O-glycosides whereas glycosylation in positions 5, 7, 3´ and 4´ is rarely reported for flavonols in fruits and vegetables.

In the case of diglycosides, the two sugar moieties may be linked to the same or two different carbons. Rutin is an example of a diglycoside found in a number of fruits and vegetables. Dimerization and polymerization could also be found in the flavonoid family. Biflavonoids can be explained by oxidative coupling reactions. The condensed tannis seem to be formed from flavan-3,4-diols, the precursors of the catechins. Among flavonoids, anthocyanis and catechins are known as flavans due to the lack of a carbonyl group in the 3-position. Anthocyanins are glycosidically bound anthocyanidins present in many flowers and fruits. Chalcones and flavones are yellow, while anthocyanins are water-soluble pigments responsible for the bright read, blue and violet colors of fruits and other foods (Shahidi & Nackz, 2004).

Phenolic Compounds Originating from Mevalonic Acid. Mevalonate or terpene pathway is formerly regarded as the universal route to terpenoid, and steroid biosynthesis. It is less prominent in secondary metabolism than the more recently discovered mevalonate-independent pathway via deoxyxylulose phosphate. Meroterpenoids contain a terpenoid unit as part of a more complex structure and iridoids represent a large group of monoterpenoid compounds that apparently seem to be formed in plants by an alternative cyclization of geranyl diphosphate. The structures of these compounds based on a cyclopentan-[C]-pyran skeleton, carbocyclic iridoids, and oxidative cleavage at the 7,8-bond of the cyclopentane moiety give the called secoiridoids (Dewick, 2002).

1.2. FUNCTION OF PHENOLICS

1.2.1 Antioxidant Function

One of the main roles of phenolic compounds is that they act as antioxidants. An antioxidant could be defined as any substance that, when present at low concentration compared to those of an oxidisable substrate, significantly delays or inhibits oxidation of that substrate. An effective antioxidant in one system is not necessarily an effective agent in another (Halliwel & Gutteridge, 1995). The activity of antioxidant can be estimated by quantitatively determining primary or secondary products of the autoxidation of lipids or by monitoring other variables such as oxygen consumption (Shahidi & Wanasundara, 1997). Furthermore,

antioxidants are hypothesized to play an important role in chronic disease prevention, because they might be able to prevent oxidative damage caused by reactive oxidant species to vital biomolecules, such as lipids and proteins (Halliwel & Aruoma, 1997).

Autoxidation is a natural process that takes place between molecular oxygen and unsaturated fatty acids (Figure 4). The autoxidation of unsaturated fatty acids occurs via a free radical process involving three steps (1) initiation, (2) propagation and (3) termination. In a fatty acid (RH), the initation starts with the abstraction of a hydrogen atom adjacent to a double bond. It could be catalized by light, heat, or metal ions. The formed unstable peroxy free radical (R•) reacts with atmospheric oxygen to form another free radical. The new alkyl free radical initiates further oxidation and contributes to the chain reaction or propagation. The propagation may be ended by formation of non-radical products resulting from the combination of two radical species (Shahidi & Wanasundara, 1997).

Phenolics act as antioxidants because of their hydroxyl groups attached to the phenyl ring and interfer with lipid oxidation by rapid donation of a hydrogen atom to form lipid radicals. The antioxidant activity of plant phenolics is similar to or even higher than the antioxidant capacity of well-known dietary antioxidant vitamin E. Some phenolic compounds are classified as free radical terminators. Peroxidation can also be reduced by antioxidants which may be natural (phenolic compounds and α-tocopherol) or synthetic (butylated hydroxytoluene). Lipid peroxidation reduces the content of essential fatty acids and vitamins and can give rise to products with significant negative health effects (cancer or arteriovascular disease) (Harwood & Yaqoob, 2002).

Mechanism of Action of Phenolic Antioxidants. Phenolics are able to act as antioxidants following several mechanisms. Assessing their antioxidant effect implies evaluating the mechanism of action. According to their type of action, antioxidants may be classified as free radical terminators, metal chelators or oxygen scavengers that react with oxygen in closed systems (Shahidi & Wanasundara, 1997).

Source: Shahidi & Wanasundara, 1997

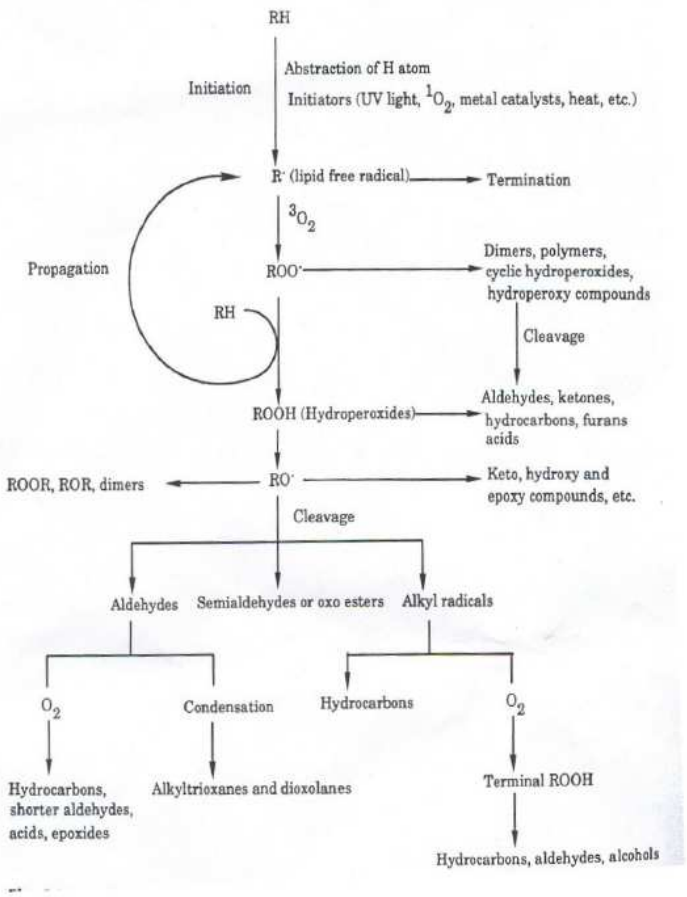


Figure 4. Autoxidation of Polyunsaturated Fatty Acids of Lipids

Primary antioxidants react with high energy lipid radicals to convert them to thermodynamically more stable products. Secondary antioxidants, also called preventive antioxidants, function by retarding the rate of chain initiation by breaking down hydroperoxides (Hall III & Cuppett, 1997).

Boland and ten-Have (1947) were the first researchers to report an exhaustive kinetic study on antioxidant activity. They postulated reactions for free radical terminators (1) and (2). Phenolic antioxidants (AH) interfere with lipid oxidation by rapid donation of a hydrogen atom to lipid radicals (3) and (4). The latter reactions compete with chain propagation reactions (5).

$$ROO^{\circ} + AH \rightarrow ROOH + A^{\circ} \quad (1)$$

$$RO^{\circ} + AH \rightarrow ROH + A^{\circ} \quad (2)$$

$$ROO^{\circ} + A \rightarrow ROOA \quad (3)$$

$$RO^{\circ} + A \rightarrow ROA \quad (4)$$

$$RO^{\circ} + RH \rightarrow ROOH + R^{\circ} \quad (5)$$

The above reactions are exothermic in nature. The activation energy increases with increasing A-H and R-H bond dissociation energy. Consequently, the efficiency of the antioxidants (AH) increases with the decrease of A-H bond strength. The resulting radical itself must not initiate a new free radical reaction or be subject to rapid oxidation by a chain reaction. Phenolic antioxidants are excellent hydrogen or electron donors and, moreover, their radical intermediates are relatively stable due to resonance delocalization and lack of suitable sites for attack by molecular oxygen (Belitz & Grosch, 2004; Nawar, 1985). The phenoxy radical formed by reaction of a phenol with a lipid radical is stabilized by delocalization of unpaired electrons around the aromatic ring. However, phenol itself is inactive as an antioxidant. Substitution of the hydrogen atoms in the *ortho* and *para* positions with alkyl groups increases the electron density of the OH moiety by an inductive effect and therefore enhance its reactivity towards lipid radicals. Substitution at the *para* position with and ethyl or *n*-butyl group rather than a methyl group increases the antioxidant activity of the phenolic whereas, the presence of chain or branched alkyl groups in this position decreases the antioxidant activity.

The stability of the phenoxy radical is improved by bulky groups at the *ortho* positions. The substituents increase the steric hindrance in the region of the radicals reducing the rate of possible propagation reactions that may occur (6), (7) and (8) involving antioxidant free radicals (Gordon, 1990).

$$A^{\circ} + O_2 \rightarrow AOO^{\circ} \quad (6)$$

$$AOO^{\circ} + RH \rightarrow AOHH + R^{\circ} \quad (7)$$

$$A^{\circ} + RH \rightarrow AH + R^{\circ} \quad (8)$$

The effect of antioxidant concentration on autoxidation rates depends on many variables including structure of antioxidant, oxidation conditions, and nature of the sample being oxidized (Hall III & Cuppett, 1997). Normally, phenolic antioxidants lose their activity at high concentrations and act as pro-oxidants by involvement in initiation reactions such as those in reactions (6), (7), and (8). The antioxidant activity by donation of a hydrogen atom is unlikely to be limited to phenolics. Some authors have suggested that the antioxidant effect of chlorophyll in the dark occurs by the same mechanism as phenolic antioxidants (Cillard *et al*, 1980).

Antioxidant Activity Determination. The activity of antioxidants can be estimated by quantitatively determining primary and secondary products of lipid auto-oxidation or by monitoring other variables such oxygen comsumption. The delay in hydroperoxide or secondary product syntheses by chemical or sensory methods are also used. These procedures can be applied to either intact food matrices, their extracts or to model systems. Studies on food have been performed under normal storage conditions or under accelerated oxidation by using the active oxygen method (AOM), oxygen uptake/absorption, oxygen bomb calorimetry, Schaal oven test or Rancimat test. The increase of the induction time by adding an antioxidant has been related to the antioxidant efficacy and expressed as a protection factor or antioxidant index. Phenolic antioxidants appear to be more effective in extending the induction period when added to an oil which has not deteriorated to any great extent. They are ineffective, however, in retarding decomposition of already deteriorated lipids. In consequence, antioxidants should be added to food matrices as early as possible to achieve maximum protection against oxidation (Shahidi & Naczk, 1995).

The Trolox equivalent antioxidant capacity (TEAC) assay is based on the scavenging of the 2,2′-azinobis-(3-ethyl-benzothiazoline-6sulphonic acid (ABTS) radical and can be used for aqueous and lipophilic systems. The biological approach involves the generation of free radicals of pathological significance,

such as peroxyl radicals. The most common assay in antioxidant analysis is the oxygen radical antioxidant activity (ORAC) assay (Hollman, 2001).

According to the method selected for the assay to measure the radical different results will be obtained. The relative ranking of phenols depends on the type of radical used and on whether the assay is performed in aqueous or lipid systems. This creates limitations for the *in vivo* relevance of the methods since they only reflect a trend in the antioxidant capacity of phenolic compounds (Warner, 1997).

The formation of hydroperoxides can also be measured using an iodometric tritation of released iodine hydroperoxides, this number is called peroxide value (PV). Aldehydic compounds, products of the decomposition of hydroperoxides, can be determined by measuring malonaldehyde with the 2-thiobarbituric acid (TBA) test or aldehydes with the para-anisidine test. Model systems for testing the antioxidant activity of food compounds and some additives have been used extensively. Peroxidation and AOM methods may be performed on linoleic acid instead of food to evaluate antioxidant activity. Although a great disadvantage is present in model systems in comparison with the intact food matrix since foods also contain natural compounds which may possess antioxidant or synergistic properties (Shahidi & Wanasundara, 1997).

1.2.2 Nutritional and Sensorial Functions

Many properties of plant products are associated with the presence, type and content of phenolic compounds. Potential antinutritional properties, beneficial health effects or bitterness when phenolic compounds are present in large quantities, are important to both processors and consumers.

For example, astringency is frequently a desirable sensorial attribute for some beverages, e.g. black tea and red wine. Oxidation of polyphenols during processing in food during processing or storage lead to beneficial (cocoa browning or the oxidative polymerization of tea polyphenols during black tea

processing) or underisable (banana or mango browning) results (Shahidi & Nackz, 2004).

Plant phenolics could be used as phytochemicals and nutraceuticals in the dried and powderized form from fruits or fruits by-product sources (Ferrari, 2004).

1.2.3 Biological Functions.

Functions in plant. The biological functions of phenolic compounds in plants are related to their role as structural polymers, UV screens, attractants, defense agents and signal compounds.

UV Screens. The phenolics naturally occurring within the plant cuticle, but especially flavones and flavonols, act as evolved barriers to absorb this UV radiation before the photochemical degradation produced by UV light appears. Therefore, they have the right absorption spectrum to be functional in this aspect (Parr & Bolwell, 2000). Flavonoids are habitually concentrated in or around epidermal tissues where their screening potential would be the highest. It appears that plants respond to the increase of UV light by increasing their accumulation of flavonoids (Cuadra *et al*, 1997; Halbrock & Scheel, 1989).

Structural Polymers. It is suggested that the evolution of a phenylalanine/PAL based phenolic biosynthetic pathway was a major early event in the development of land plants due to the ubiquitous nature of phenolic compounds in plants and ferns and their relative absence in lower organisms and animals. This has been associated with the lignin effect on the mechanical support for plants and their ability, with other compound such as cutin and suberin, for providing water transport systems. Lignin and some recent identified compounds may reflect some of the biochemistry involved in the initial period and control of the lignification process (Parr & Bolwell, 2000).

Defense response. Another role of phenolics, even questionably, is the defense process by which plants attempt to protect themselves from pathogens and

herbivorous predators. Lignans and their higher oligomers act as defense substances (Davin & Lewis, 1992). The most frequently occurring defense response of the plant is the hypersensitive response (HR), which occurs early during the infection process. The HR involves the death of a few cells in the local region of pathogen attack to form a necrotic lesion that may contain antimicrobial compounds. Other biochemical changes include the generation of reactive oxygen species (the oxygen burst) and the production of phenolics (Parr & Bolwell, 2000).

Another essential aspect of the defense role of phenolics is related to the unpalatability of plants to herbivores. Tannins are capable of interacting with proteins to precipitate them. They have an astringent taste and an ability to inhibit digestive enzymes. Foods containing a large variety of phenolic derivates (phenols, phenylpropanoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, and lignans) are well recognized for their contribution as antifeedants (e.g. the intense bitter taste of the flavanone glycoside and naringin in grapefruits) (Harborne, 2001; Butler, 1992). More specific actions based on discrete phenolic/host receptor interactions of individual compounds have recently been studied (Robins *et* al, 1990).

Attractant agents. Phenolic compounds play an important role in making flowers attractive to pollinating animals. There is a close relationship between flower color and the nature of the pollination process. Red flowers, owing to anthocyanins, are generally pollinated by birds whereas insect-pollinated flowers are often yellow as a result of carotenoid accumulation (Harborne, 2001; Parr & Bolwell, 2000).

Signal compounds. The transmission of signals to certain parts of the plants can be a response mechanism to localized pathogen attack and induce the defense synthesis. This event is known as systemic acquired resistance (SAR), and one of the signals involved is salicylic acid, which is synthesized locally to the infection and transported around the plant in the phloem. Dehydrodiconiferyl alcohol glucoside (DCG) is another phenolic compound signal, acting as a secondary messenger involved in aspects of cytokinin action via compounds such as gallic acid glucoside-6′-sulphate and *cis-p*-coumarroyl-agmatine. Some

plants produce signal compounds to act in an external way. Bacteria from the general *Rhizobium* and *Agrobacterium* have a close association with plants in which symbiotic or pathogenic relationships are established (Raskin, 1992; Teutonico *et al*, 1991).

In summary, based on their biological functions, phenolic compound can be classified as Table 2 shows.

Functions in man. Many plant phenolics appear to have evolved as part of the plant´s antipathogen and antipredator strategies, several of their actions in man can be expected to relate to induced effects on human physiology. Interest in phenolic compounds has been increased over the past years due to their potential antioxidant activities and potential effects against degenerative illness (Parr and Bolwell P, 2000).

Hydrogen donation/radical scavenging. Free radicals originated wthin the body can produce extensive damage to macromolecules, including DNA and lipids. This phenomenon is assisted by the autocatalytic nature of many of the processes involved. Radicals abstract a proton from such macromolecules generating highly reactive macromolecular radical forms. These can then decay by interactions with neighboring molecules to produce a degraded product and a further cycle of degradation. Plant polyphenols are multifunctional and can act as hydrogen-donating antioxidants reacting with radicals in a termination reaction breaking the generation of new radicals (Halliwell & Auroma, 1997; Rice-Evans *et* al, 1996).

Inhibition of radical generation. Enzymes such as various cytochrome P450 isoforms, lipoxygenases, cyclo-oxygenases and xanthine oxidase, are likely pro-oxidant and can generate radicals. Particularly, flavonoids and phenylpropanoids are effective inhibitors of these enzymes. For example, 3,4-dihydroxyphenylethanol from olives inhibits arachidonic acid lipoxygenase (Manach *et al*, 2004).

Table 2. Biological Effects of Some Plant Phenolics

Function	Group	Example(s) and Plant Species Where the Effect Was Studied
Flower pigments	Anthocyanes	Cyaniding-3,5-diglucosid in Rosa
	Chalcons	Coreopsin in Coreopsis tinstoria
	Aurones	Areusin in Anthirrhinum majus
	Yellow flavonoids	Gossypetine-7-glucoside in Gossypium
	Flavones	Apigenin-7-glucoside in Bellis perennis
Fruit pigments	Anthocyanines	Petunidin glucoside in Atropa belladonna
	Isoflavones	Osajin in Maclura pomifera
	Chalcons	Ocanin in Kyllingi brevifolia
Allelopathic substances	Quinines	Juglon in Juglans regia
	Phenols	Hydroquinone in Arctostaphylos
	Phenolcarboxylic acids	Sialic acid in Quercus falcate
	Hydrocinnamic acid	Ferulic acid in Adenostoma
Protection against pests	Quinines	Fuglon in Carya ovata
	Tannines	Gallotannine in Quercus robur
	Flavonols	Quercitine-glycosids in Gossypium
Fungicide	Isoflavones	Luteon in Lupinus
Phytoalexines	Stilbens	Reservatrol in Arachis hypogaea

Source: Harborne, 1989

Enzyme Inhibition. Phenolics have the potential to interact strongly with proteins mediated by their hydrophobic benzenoid rings and hydrogen-bonding potential

of the phenolic hydroxyl groups. In the case of some tannins and other polyhydroxy-substituted phenolics this interaction can be largely non-specific, and therefore be considered as a relatively general inhibitory effect. On the contrary, discrete interactions can occur between individual phenolics and the active sites of enzymes resulting in a more specific type of inhibition. This capacity to modify selected enzymes is presumed to have a role in the physiological action of these phenolics, even the multiple interrelated actions of phenolic compounds make difficult to obtain a detailed understanding of their mode of action (Parr & Bolwell, 2000).

Anticarcinogenic Action. This is another biological function to be considered in the human system. Anticarcinogens can be classified as blocking and suppressor depending on the point of action with some compounds having both activities. The most important function of blocking agents is to stimulate the carcinogen-detoxyfying enzymes and to inhibite enzymes which have the portential to activate precarcinogens into carcinogens. Suppressing agents can act by different methods. For example, curcumin inhibits phorbol ester-mediated cell proliferation, possibly by interfering with the induction of ornithine decarboxylase activity and by producing subsequent changes in polyamine metabolism (Scalbert *et al,* 2005; Manach *et al*, 2004; Hollman, 2001).

Inhibition of tyrosine kinase (TK) activity is an indication for compounds which can potentially modulate signal transduction pathways, and this may have relevance as potential anti-carcinogenetic compounds (Manach *et al*, 2004).

1.3 FACTORS AFFECTING PHENOLIC COMPOSITION

Factors affecting the phenolic composition of food such as genetic (internal) factors, agronomic (environmental) conditions, germination, ripening degree, variety, processing and storage have been studied. (Tomás-Barberán & Espín, 2001).

1.3.1 Genetic Factors

The plant species, subspecies and cultivars produce particular phenolics whose analysis may be used to establish taxonomic affinities and/or differences. For example, some of lettuce cultivars ("iceberg" and "butter leaf" type) are very poor in flavonoids, caffeic and acid derivates, whereas others species ("lollo rosso" and "oak leaf") contain large amounts of flavonols, caffeic acid and derivates and anthocyanins (DuPont *et al*, 2000; Tomás-Barberán *et al*, 1997). Some apples cultivars are intensely red because of the accumulation of anthocyanins whereas some show a complete lack of these phenolic compounds (Amiot *et al*, 1992).

The susceptibility to browning associated with PPO (polyphenooxidase) activity varies widely in some plants. Interest has been focused on the cloning of genomic DNA encoding both polyphenol oxidase and peroxidase to explain different PPO and POD activities depending on the cultivar and plant tissue. Induction of PAL (phenylalanine ammonia-lyase) is essential for the accumulation of different phenolic compounds in plants. It is considered the key enzyme in phenolic biosynthesis as a catalyser of the synthesis of phenylpropanoid compounds (lignin, flavonoids and hydrocinnamic acids) (Morelló *et al*, 2005). PAL is encoded by a small multigene family in several plant species such as pea (Kawamata *et al* 1992), tomate (Lee *et al*, 1992), parsley (Lois *et al*, 1989) and rice (Minami *et al*, 1989).

1.3.2 Agronomic Factors

The environment also plays an important role in the phenolic compounds occurring in fruits and vegetables. Irrigation factor (water availability) and soil composition (mineral and organic nutrients) have a remarkable effect on the plant phenolic profile and content. These external conditions may limit the ability of plant products to suffer browning and other phenolic physiological disorders that appear during plant growth and postharvest stages. Data have been reported about the effect of the irrigation on the quantity of polyphenols in the olive oil (Patumi *et al*, 2002; Romero *et al*, 2002). Water stress during a specific period of the olive cycle, pit hardening and fruit growth, could influence the total amount of phenolic content as well as its profile. It is generally accepted that the level of phenolic compounds is higher in oils obtained from drought stressed crops than in those from irrigated crops, and that phenolic compounds in the oil are significantly affected by the irrigation regime (Tovar *et al*, 2001).

1.3.3 Postharvest Factors.

Fruits and vegetables handling during harvest and postharvest storage may have a significant effect on phenolic compounds and enzymes related to phenolic metabolites, potentially resulting in the decrease of quality. PPO can be found in both soluble and membrane-bound forms in chloroplasts, mitochondria, microsomes, peroxisomes and cytoplasm. POD can be found either soluble in the cytoplasm or cell wall-bound. Mechanical damages produce the synthesis of PAL in plant tissues, probably via signaling pathways including compounds such as salicylic and jasmonic acids. In some plant tissues (lettuce and tomato) the activation of latent POO and the induction of PAL and POD after cutting process and storage at refrigerated conditions have been reported. The wound induction of PAL was concurrent with that of the induction of the first enzyme of the shikimate pathway (Cantos *et al*, 2001).

Some authors have demonstrated the positive and negative impact of storage at low temperature on fruit and vegetable phenolics. An increase in anthocyanins was observed when red plant tissues were stored in these conditions. Low temperatures can also increase the content of hydroxycinnamic acid derivatives in some vegetables, e.g. artichokes, however, some cold storage can produce undesirable effects when the phenolic compounds play an important rolev such as scald in apples. Some fruits such as berries presented higher antioxidant activity when they were stored at ambient temperatures. In some cases, an increase in PAL activity produced peel damage of mandarins and oranges (Martínez-Tellez & Lafuente, 1997).

1.3.4 Technological Aspects–Processing

The phenolic content may be affected by food-processing technologies. Industrial treatments such as minimal processing (fresh-cut fruits or vegetables), pressing (single strength or concentrated juices), mixing (olive oil), fermentation (wine, cider, table olives), drying (raisins, prunes), thermal treatments, and radiation greatly determine the composition of phenolic compounds and therefore food quality. During processing, phenolic biosynthesis is interrupted by the destruction of enzyme and or cell structure degradation. In

other cases, processing increases the release of phenolic compounds from plant tissues producing biochemical changes that could affect quality characteristics.

1.4 FOOD PHENOLICS.

Phenolic compounds are ubiquitous in the plant kingdom being found in cereals, legumes and nuts, oilseeds and plant oils, fruits and vegetables. They are in virtually all parts of the plant but with quantitative distributions that vary between different tissues of the plant and within different populations of the same plant species (Robards, 2003). Beverages, herbal and nutraceutical products are also an important sources of phenolics. As in plants, phenolics could be found free, esterified, etherified or in an insoluble bound form (Shahidi & Naczk, 2004).

Phenolic compounds may be responsible of the dark color, bitter taste and astringency of seed meals (Naczk *et al*, 1998; Matsuura *et al*, 1989). They can form complexes with essential aminoacids, enzymes and other proteins. Fruits (grapefruits, oranges, berries, black and red currants, dark grapes and apples), green leafed, yellow, and red vegetables (onion, cabbage, broccoli, cauliflower, Brussels sprouts, pulse seeds, tomatoes and peppers) are abundant in flavonoid compounds. Data related to the quality and quantity of phenolics in fruits and vegetables have been reported. There are variations between the total phenolic content of the different fruits or vegetables or even for the same fruits or vegetables due mainly to the extraction methodologies and analysis (Balasundram *et al*, 2006; Clifford, 2000; Parr & Bolwell, 2000; Robards *et al*, 1999).

Some studies have been performed in order to determine not only the content of polyphenols in fruits and vegetables but also their influence in the quality (Cieślik *et al*, 2006; Tomás-Barberán & Espín, 2001). Phenolic acids can occur in the free, esterified and insoluble bound forms. Some of them may be linked convalently to amine functionalities through a "pseudo peptide" bond. Wet processing (alkaline hydrolysis), cooking or baking may release of some bound phenolics increasing their contribution to the sensory characteristics of the products.

Many of the essential oils contain phenolic compounds, such as eucalyptol from Eucalytus *globules* or clove oil from Syzygium *aromaticum*, although these volatile chemicals are often classified as terpenes. As the terpenes, many phenolic compounds are attached to sugar molecules. Beverages, including fruit juices, tea and wines are important sources of phenolic compounds. Reductions or losses of phenolics reported in commercial juices have been attributed to technological variables. Some studies have showed an increase in the level of phenolics during processing, e.g. compounds of apple mash and juice. In general, the storage does not seem to influence directly the phenolic content (Balasundram *et al*, 2006). Analytical strategies dealing with the determination of bioactive phenolics have appeared during the last years; analyses of total phenolic content (Folin assay) or individual quantification of phenolics (HPLC analysis) have been performed by several research groups (Naczk & Shahidi, 2004; Robards, 2003; Antolovich *et al* 2000; Makkar, 1989).

Tables 3a, 3b and 3c illustrate the phenolic content of the main food groups.

1.5 OLIVE FRUIT AND VIRGIN OLIVE OIL

1.5.1 Olive Fruit (*Olea europaea* L.)

The olive, *Olea europaea* L., belongs to the Oleaceae family. This family contains about 29 genera and 500 species, most of which are placed in the Oleoideae subfamily. Olive is by far the most economically important member of the family. Several important ornamentals also belong to this family: *Fraxinus* (Ash), *Syringa* (Lilac), *Ligustrum* (Privet), *Jasminum* (Jasmine), *Forsythia, Osmanthus* (Fragrant olive), and *Chionanthus* (Fringe tree). The genus *Olea* contains about 35 species (Boskou, 1996).

Table 3a. Content of Main Phenolic Compounds in Different Foods: Vegetables and Fruits

Food	Total Phenolic Compounds	Food	Total Phenolic Compounds	Food	Total Phenolic Compounds
Vegetables		**Fruits**		**Fruits**	
Broccoli	101.6 ± 1.24[a] 87.5 ± 8.1[b]	Apple	296.3 ± 6.4[a]	Papaya	57.6 ± 4.1[a]
Brussels sprouts	68.8 ± 1.3[b]	Banana	90.4 ± 3.2[a] 11.8 ± 0.4[a]	Pineapple	94.3 ± 1.5[a]
Cabbage	54.6 ± 7.0[a] 92.5 ± 2.4[b]	Black plum	143.5 ± 40.6[b]	Plums	174 – 375
Carrot	56.4 ± 7.0[a]	Black Berry	417 ± 555[a]	Prunes	184.0 ± 85.5[a]
Cucumber	19.5 ± 1.6[a] 48.0 ± 0.9[b]	Blue Berry	270 – 930[a]	Raisins	399.4 ± 57.6[b]
Mint	399.8 ± 3.2[b]	Cherry	105.4 ± 27.0[b]	Rambutan	1.64 ± 0.04[d]
Spinach	91.0 ± 8.5[a]	Cram Berry	527.2 ± 21.5[a]	Raspberry	114.0 – 178.0[a]
Tomato	25.9 – 50.0[c]	Guava	126.4 ± 6.0[a] (pink) 247.3 ± 4.5[a] (white)	Red Grape	220.6 ± 61.2[d]
Onion varieties	73.3 – 180.8[a]	Litchi	3.35 ± 0.05[d]	Starfruit (acidic)	142.9 ± 7.1[a]
Yellow Onion	76.3 ± 1.9[a]	Mango	6.25 ± 0.05[a]	Starfruit (sweet)	209.9 ± 10.4[a]
---	---	Peach	84.6 ± 0.7[a]	Straw Berry	160.0 ± 1.2[a]

Source: Balasundram *et al* (2006)
[a] Gallic acid equivalents/100 g fresh weight , [b] Catechin equivalents/100 g fresh weight, [c] Ferulic acid equivalents/100 g fresh weight, [d] Chlorogenic acid equivalents/100 g fresh weigh

Table 3b. Content of Main Phenolic Compounds in Different Beverages: Fruit Juices, Tea and Coffee.

Food	Total Phenolic Compounds	Food	Total Phenolic Compounds [c]	Food	Total Phenolic Compounds [c]
Fruit Juice		**Tea**		**Coffee**	
Commercial Juices		Black Tea	80.5 ± 134.9	Instant Coffee	146-151
Apple	339 ± 43[a]	Black Tea	154.9 ± 162.9	Ground Coffee	52.5 – 57.0
Grape Fruit	535 ± 11[a]	Black Tea	62 – 107	---	
Orange	755 ± 18[a]	Green Tea	65.8 – 106.2	---	
Pineapple	358 ± 3[a]	Green Tea	117.3	---	
Prune	441 ± 59[b]	Green Tea	61 – 200	---	
Fresh Juices				---	
Grape (red)	1728[a]	---		---	
Grape (white)	519[a]	---		---	
Orange	382 – 1147[b]	---		---	

Source: Balasundram *et al* (2006)
[a] Gallic acid equivalents/L, [b] Ferulic acid equivalents/L, [c] mg gallic acid equivalents/g dry matter

Table 3c. Content of Main Phenolic Compounds in Different Beverages: Wines

Beverage	Total Phenolic Compounds[a]	Beverage	Total Phenolic Compounds[a]	Beverage	Total Phenolic Compounds[a]
Red Wines		**White Wines**		**Rose Wines**	
Argentine	1593 – 1637	Argentine	216	Italian	1304
Brazilian	1947 – 1984	Brazilian	256 – 353	Japanese	340
Californian	1800 - 4059	Californian	165 – 331	---	
Chilean	2133	Californian	220 – 306	---	
French	1847 - 2600	French	245	---	
French	1018 – 2151	French	262 – 1425	---	
Greek	1217 – 3722	Italian	439 – 854	---	
Italian	3314 – 4177	Italian	191 – 296	---	
Japanese	1810 – 2151	Japanese	295 – 556	---	
Portuguese	1615	Spanish	292	---	
Spanish	1869	---		---	

Source: Balasundram *et al* (2006)
[a] mg gallic acid equivalents/ L

Cultivar. Olives are large evergreen shrubs in their native state, but are trained as stout trees on massive trunks, especially in older plantings. Most trees have round, spreading crowns, however, tall, cylindrical trees are also grown in some parts of Europe. Trees in neglected groves grow almost imperceptibly slow. Leaves are small, linear, with entire margins and acute tips, silver-green in

color, and fairly thick. As for all members of the Oleaceae, leaf arrangement is opposite.

Flower. Small, off-white flowers are borne in racemose panicles of 15-30 flowers in axils of one year old wood. Most flowers are staminate by pistil abortion, leaving only 1-2 perfect flowers per inflorescence, which may set fruit. The ovary is superior, and there are 4 sepals and petals, and 2 stamens. Flowering occurs rather late relative to other tree crops.

Fruit. The fruit of the *Olea europaea* is an oval-shaped drupe consisting of a pericarp and endocarp (pit, kernel). The pericarp has two parts: the epicarp (skin), and the mesocarp (pulp, flesh) that accounts for about 65-83% of the total weight. The endocarp may vary form 13% to 30% or the total fruit weight. The fruit type is a drupe oblong with smooth, waxy surfaces. Color is green when immature, turning yellow-green in autumn, with red, purple, or black coloration at full maturity. Dark coloration results from anthocyanin production in the exocarp and mesocarp. Olives require 6-8 months for full maturation, but table olives are harvested earlier when firm, and oil olives are left on trees until oil reaches 20-30% (early winter) (Boskou, 1996; Raina, 1995).

There are approximately 2500 known varieties of olives of which 250 are classified as commercial cultivars by the International Olive Oil Council (IOOC). These cultivars are used for the production of either virgin olive oil or table olives depending on the oil content and size of the fruit. Different cultivars are generally used for oil (e.g., Arbequina, Hojiblanca, Picual, Leccino, Frantoio) and table olives (Manzanillo, Sevillano, Ascolano, Calamata) (Ryan & Robards, 1998).

Table 4 shows the olive oil fruit composition.

Table 4. Olive Fruit Composition

Constituent (%)	Flesh	Stone	Seed
Water	50-60	9.3	30.0

Oil	15-30	0.7	27.3
Proteins	2-5	3.4	10.2
Sugars	3-7.5	41.0	26.6
Cellulose	3-6	38.0	1.9
Ash	1-2	4.1	1.5
Phenolics	2-2.5	0.1	0.5 – 1.0

Source: Raina *et al*, 1995

Harvest, Postharvest Handling. Most table olives are harvested when they change from green to yellowish-green in color and are firm usually mid-autumn. Oil olives are harvested in late autumn or in winter when they have turned black and reach their maximum oil content (20-30%). Delaying harvest results in poor oil quality due to loss of essential oils and aromas and an increase in acidity. Delaying harvest also results in increased alternate bearing; trees used for table olives often fluctuate and yield less than trees used for oil.

Olives are traditionally hand harvested a process that is not only tedious and laborious, but represents the major proportion of the costs of production. Hand harvest is accomplished by three techniques: 1) collection of fallen fruit from the ground, 2) "milking", or the stripping of fruit with half open hands from limbs which falls into picking bags or onto nets below the tree, 3) beating limbs with large sticks to dislodge fruits, which are also collected on nets. Collecting fruits which fall naturally to the ground is inexpensive, but seriously compromises oil quality. Milking or beating are most commonly used.

Mechanical harvest of olives has been studied and attempted in various forms for years. It is used to a limited extent in more intensive orchards. Compared to other tree fruits that are mechanically harvested, olive trees are problematic. Olives require about 5 times more shaking energy than other fruits such as prunes and almonds, due to the willowy nature of the tree and the resistance to detachment of fruit. Using mechanical shakers, only 65-80% of the fruit can be

removed from the tree at best. The remaining fruits are either lost or must be hand harvested (Di Giovacchino, 1996a).

1.5.2 Virgin Olive Oil Process

Production of Olive Oil. Conventional discontinuous pressing cycle, the continuous centrifugation and the percolation-centrifugation systems are in use to process olive fruits. The olive oil extraction process includes crushing, malaxation (kneading) and separation of must into oil and water by centrifugations. The resulting products are oil, husk (solid waste) and vegetable water (wastewater).

Discontinuous extraction is an ancestral procedure that only distinguishes two phases by pressing or centrifugation. The liquid phase is later filtered in order to obtain oil. In this case, the by-product is a plastic paste which has the advantage of avoiding the production of vegetable waters. However, although it is more ecological, this technique provides a lower yield and is not always seen as an advantage for the main producing countries. In the traditional cycle, millstone (hammer stone) and hydraulic presses are used for milling and pressing of olives, respectively. Continuous systems use metal crushers (hammer, disc and roller types) to grind olives, and a horizontal centrifugal decanter for centrifugation of the olive paste. A vertical centrifuge is used for separation of the oily must into oil and water (Figure 5) (Soler-Rivas *et al*, 2000).

The extra virgin olive oil is obtained by the first physical, cold pressing of the olive paste. Phenolic content in the oils is the result of a complex interaction between several factors such as cultivar, ripening index and climate (Morelló, *et* al, 2004; Criado *et* al, 2004; Rotondi *et al*, 2004; Motilva *et al*, 2000). Phenolic profile and concentration are also influenced by processing factors, the most important being pressing and centrifugation operations (Salvador *et al*, 2003; Di Giovacchino *et al*, 2002, Di Giovacchino *et al,* 2001). The oil extraction method has a significant effect on the content of total phenols and 1,2-diphenols. Various extraction systems differ in two essential aspects, the physical forces used to recover the oil, and the amount of water added to the olive paste during extraction. Oil extraction is more effective with olives of low water content. Phenolic compounds are water soluble and consequently addition of water to the

olive paste reduces significantly phenolic content and quality of the oil produced. This has been been supported by the findings of Di Giovacchino *et al* (1996b), who studied the effect of the three different types of extractions systems on virgin olive oil quality. Table 5 shows selected results from this study. Total phenolic and *o*-diphenol contents of oils obtained by pressing were significantly higher than that of centrifugally extracted oils. However, sensorial evaluations of the oils obtained by the two processes showed the same results. It is therefore, possible to choose an extraction system to ensure the highest oil quality.

Source: Olive Oil Industry

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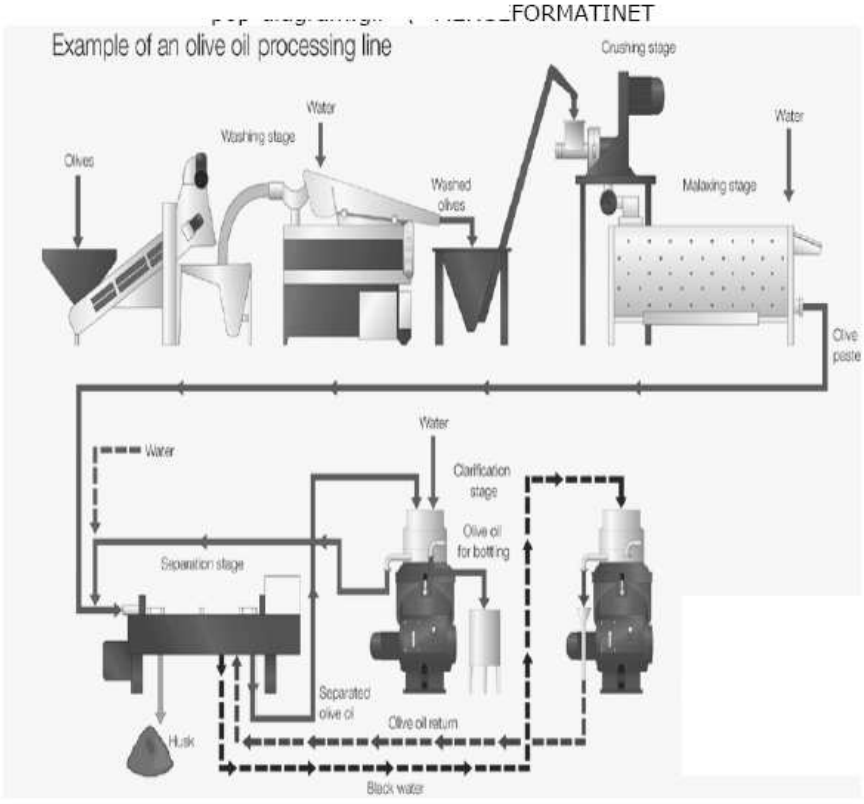


Figure 5. Olive Oil Processing Line

Table 5. Quality Parameters of Virgin Olive Oil Obtained by Pressing and Centrifugation

Parameter	Experimental System[a]		Industrial System[a]	
	Pressing	Centrifugation	Pressing	Centrifugation
Free Fatty Acids	0.23	0.22	0.35	0.29
Peroxide Value (meq O_2/kg)	4.0	4.9	4.8	5.7
Total Phenolic Content (mg/L as gallic acid)	158	121	155	114
o-Diphenols (mg/L as caffeic acid)	100	61	106	62
Oxidative Stability (induction time, h)	11.7	8.9	12.0	9.5
Chlorophyll pigments (mg/kg)	5.0	9.1	6.6	9.9
K_{232}	1.93	2.01	1.91	1.96
K_{270}	0.120	0.127	0.108	0.122

Source: Di Giovacchino *et al*, 1996
[a] Mean Values

Products of Processing.

Virgin Oil. This is the leat processed oil. It has three subcategories (extra, fine, and ordinary) based largely on acidity and flavor. Extra virgin olive oil has less than 0.8% acidity (by weight from oleic acid) and excellent flavor. Fine oil is often just termed "virgin" and has acidity of <2%. Ordinary virgin oil has acidity of up to 3.3%. Virgin oils with acidity greater than 3.3% are not used for human consumption, and are designated "lampante" meaning lamp oil.

Refined Oil. This virgin olive oil has been refined to remove off-flavors and odors by lye or other treatments, which do not alter the glyceridic structure of the oil. It has an acidity of < 0.3%.

Blended Oil. This is a virgin oil blended with a refined oil with an acidity <1%. It is labeled "pure".

Olive-Pomace Oil or Residue Oil. Oil recovered from pressed olive paste by solvents falls into this category. It cannot be submitted to re-esterification processes or mixed with other plant oils. Olive-pomace oils are classified as "crude" or "refined". Refined oils have a lower acidity than crude olive oil. A third

category, called "olive pomace oil", is a blend of refined pomace oil and virgin olive oil with acidity <1%.

By-products. Pomace is the solid residual derived after first pressing or centrifugation (a little of olive, pieces of nut, etc.). It may be used for livestock feeding or going through a chemical extraction to produce olive-pomace oil.

Vegetable water is the liquid phase obtained as a result of centrifugation. They are abundant in the three phases extraction method due to the injection of water made to the paste before centrifugation. As vegetable waters still contain oil, they are re-treated to recover the maximum amount of oil. However, since this is a combination of water and fat, it is difficult to recycle them. Vegetable waters are highly polluting and negatively affect underground waters. The most serious ecological problem in olive oil production is the recycling of vegetable waters.

Technological Development. The three phase system is the most widely used in intensive production areas. It dates back to the seventies and eighties. The main disadvantage of this process is the huge amount of water needed and therefore the production of vegetable waters and their resulting pollution. Since 1991 new industrial techniques of continuous extraction have been used. The production of vegetable water is reduced and there is an increase in the pomace obtained. This process does not need much water and is being more and more widely used.

Traditional olive oil extraction using a pan crusher and hydraulic press is not a continuous process and transformation costs are high. In 1965, olive oil production began with the extraction by means of a three-phase centrifuge, with horizontal axle, which separates oil, water, and husk from the olive paste. The centrifuge system reduces processing time and therefore excessive storage period of olives; the obtained oils have usually a higher quality. Even though, this extraction system requires the addition of lukewarm water which produces a decrease in phenolic compounds due to their high water solubility. The production of vegetable water generated by milling plants is considered an important technological problem due to the increase in waste disposal as well as costs. In 1992, a new olive oil extraction system was introduced in processing plants consisting in a decanter faciliting the separation of the oil phase from the olive paste without the addition of warm water. Moreover, the amounts of

vegetable water generated are not significant (Angerosa & Di Giovacchino, 1996). Oils obtained from a two-phase system possess higher concentration level of phenolics and tocopherols and show higher antioxidant stability in comparison with oils extracted by three-phase decanter. The continuous two-phase process is the most accepted extraction process throughout the olive oil industry in Spain, as shown by its use in 90% of the olive mills. It is achieving a very wet pomace with a water content varying from 65% and 70%. In Italy the centrifugal decanters employed rather have three exits (oil, pomace and vegetable wastewater) and are able to separate the oil requiring the addition of a small quantity of water to dilute the olive paste if needed (Di Giovacchino *et al*, 2001).

1.5.3 Phenolic Fraction

Phenolic compounds in olive pulp and oil constitute a complex mix that has not been elucidated yet. The nature of many phenolics occurring in low concentrations remains unidentified and their significance and action still unknown.

Olive Fruit Phenolics. The main classes of phenolics occurring in olive fruit are phenolic alcohols, phenolic acids, secoiridoids and flavonoids (Servilli & Montedoro, 2002). Considerable differences in the content of hydroxytyrosol (3,4-DHPEA), tyrosol (*p*-HPEA) and tyrosol glucoside have been found in the fruits during growth and ripening of the drupe. The increase in their levels is correlated to the hydrolysis of the compounds with higher molecular weight. Hydroxytyrosol or elenolic acid contents are considered an indicator of the maturation of olives (Ryan *et al*, 2002a).

In the fruit, a wide range of phenolic structures has been reported including simple phenolic acids such as the isomers of coumaric acid (Romani *et al*, 1999). Caffeic acid occurs in pulp, olive leaf, (Baldi *et al* 1995) and peel (Servilli *et al* 1999a; Brenes *et al*, 1995). Seed and husk possess caffeic acid in a relative high quantity (Ryan *et al*, 2002b), whereas its presence has been reported in oil by several authors (Akasbi *et al*, 1993; Montedoro *et al* 1992; Tsimidou *et al* 1992). Mousa *et al* (1996) reported that ferulic and gallic acids occur in pulp tissue. In some Spanish cultivars hydroxytyrosol, tyrosol, vanillic acid, *p*-coumaric and ferulic acid have also been found (Montedoro *et al*, 1993; Pirisi *et al* 1997).

Biochemically, members of the Oleaceae family can be characterized by the presence of a number of coumarin like compounds known as secoiridoids which are related to the iridoids. Secoiridoids, characterized by an exocyclic 8,9-olefinic function, are known as oleosides. These compounds are restricted to the oleaceous plant. They are not considered as phenolics but may include a phenolic moiety as a consequence of esterification via a branching in the mevalonic acid pathway in which terpene and phenolic synthesis fuse (Damtoft *et al*, 1993) (Figure 6). Oleuropein, demethyloleuropein, ligstroside and oleoside are the main phenolic oleosides in Olea *europaea* (Servilli *et al*, 1999b). In the development of the olive fruit, three phases are usually distinguished: accumulation of oleuropein during a growth phase, reduction in the levels of chlorophyll and oleuropein (green maturation), and the appearance of anthocyanins and continuous falling of oleuropein, a phase coinciding with the black maturation. Oleuropein is found in higher amounts in the early stages of maturity reaching 14% on a dry matter basis (Soler-Rivas *et al,* 2000). Oleuropein is easily extracted as part of the phenolic fraction of leaves and seeds but it has not been reported in olive oil. Elenolic acid glucoside and demethyloleuropein appear at the beginning of the green maturation while the oleuropein decrease. They reach their maximum level during black maturation. Because of its interaction with a diphenol oxidase, oleuropein is also involved in the browning that occurs in green table olives either after impact and wounding during harvesting or during the consequent industrial treatments.

Some studies resulted in the detection of oleuropein, demethyloleuropein, oleuroside, and ligstroside in the olive leaves, pulp, peel and husk (Ryan *et al,* 1999; Rovellini *et al* 1997; Brenes *et al*, 1995; Baldi *et al*, 1995; Movsumov *et al* 1987; Gariboldi *et al* 1986). Very few studies have been performed on the phenolic compounds of olive seeds. Compounds such as salidroside, nüzhenide, and nüzhenide oleoside were recently found in seed at all ripening stages (Maestro-Durán *et al*, 1994; Servili *et al* 1999a).

Color change during olive fruit maturation is associated with the decline in chlorophyll and oleuropein levels and the appearance of anthocyanins. Cyaniding and delphinidin glucoside are the most common anthocyanins found in olive (Servilli *et al*, 1999a). However, information concerning this latest is more scarced than that on cyanidin glycosides. The distribution of these compounds is extremely restricted, being limited to the maturation stage where the first appearance is in the fruit skin at either the proximal or distal end of the fruit and

spread from there to the rest of the skin and then to the mesocarp. Aglycones have not been reported and are hardly ever found in fresh plant matter other than other materials. Cyanidin-3-glucoside and cyaniding-3-rutinoside have reported only in pulp (Vlahov, 1992; Romani *et al*, 1999) whereas cyanidin-3-glycosides have been detected in pulp and leaf matrices.

Source: Damtoft *et al*, 1993

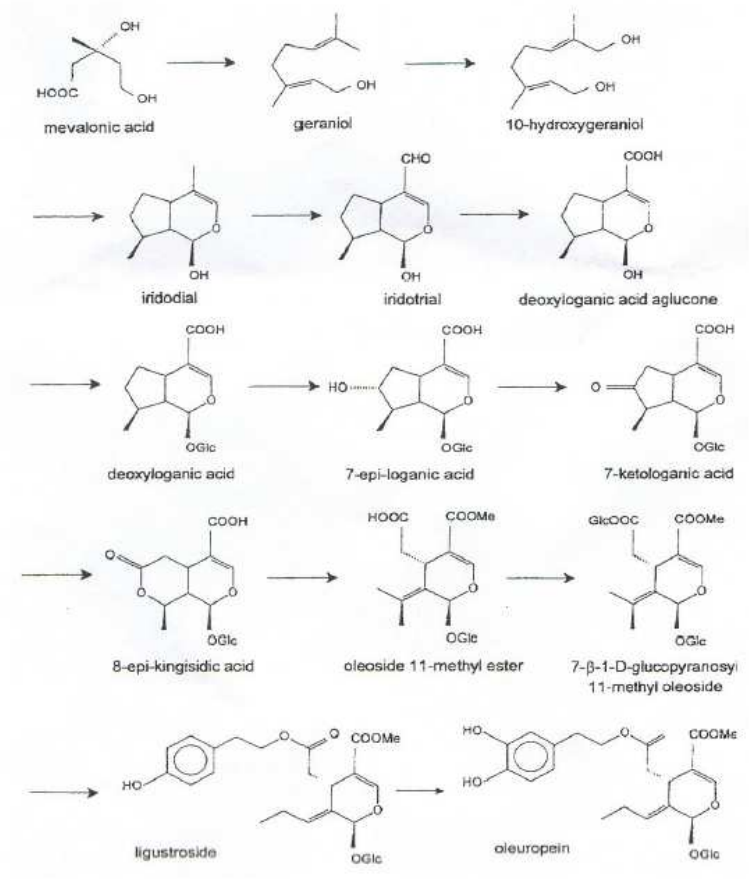


Figure 6. Proposed Biosynthetic Pathway for Oleuropein in Oleaceae

In young small olives, verbascoside was present only in traces, although verbascoside is the predominant hydroxycinnamic derivative of olive fruit at maturation stage (Ryan *et al*, 2002a). Its chemical structure was assigned by

Andary *et* al (1982) and confirmed by Servilli *et* al (1999b). Verbascoside content has been related to an inverse oleuropein concentration in olive fruit. Heimler *et al* (1992) detected and isolated flavonoids glucosides and biflavonoids in olive leaves. Some of them were identified as quercitrin (quercitrin-3-rhamnoside), rutin (quercetin-3-rutinoside), luteolin and chlorogenic acid (5-caffeoylquinic acid). In addition, olive leaf hairs contained UV-screening pigments, which have been characterized as phenolics with a considerable flavonoid contribution (Karabourniotis *et al*, 1992). Rovellini *et al* (1997) reported the presence of luteolin-glucoside (the commonest of all flavone glycosides) in leaf and luteolin-7-glycoside has been detected in the same olive matrix (Heimler *et al*, 1992). The pulp and leaf matrix has been characterized by the occurrence of apigenin, apigenin-7-glucoside, apigenin-7-glycosides and apigenin-7-rutinoside (Ryan *et al*, 2002a).

The detection of lignans presumable in fruits of Olea *europaea* has been reported since the beginning of this decade (Owen *et al*, 2000a). 1-acetoxypinoresinol and 1-hydroxypinoresinol glucosides have been isolated from bark of olive trees (Tsukamoto, 1984; Tsukamoto, 1985). Pinoresinol glucoside linked to oleoside 11-methyl ester, which is a secoiridoid as oleuropein, has been isolated form oleaceous plants (Tanahashi *et al*, 1987). Therefore, the lipid affinity of the lignans detected in olive oils could be originated via hydrolysis of compounds similar to lignan linked to secoiridoid glucoside. Figures 7a, 7b and 7c show the main phenolic compounds occurring in olive fruit.

Virgin Olive Oil Phenolics. Some of the major phenolic compounds identified in virgin olive oil (VOO) also occur in olive fruit. VOO contains differtent classes of phenolic compounds such as phenolics alcohols, phenolic acids, secoiridoids derivarives, flavonoids and lignans. Hydroxytyrosol, tyrosol, caffeic, *p*-coumaric and vanillic acids have been reported by several authors (Brenes-Balbuena *et al*, 1992; Nergiz & Unal, 1991). Similarly, ferulic, homovanillic *p*-hydroxybenzoic, syringic and gallic acids have been identified by Poiana *et al*, (1997), Mannino *et al* (1995), and Nergiz & Unal (1991). Flavonoids such as apigenin and luteolin have been reported in olive oil by Artajo *et al* (2006), Rovellini *et al* (1997) and Criado *et al* (2004).

Source: Ryan & Robards, 1998

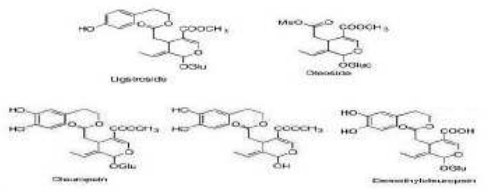


Figure 7a. Chemical Structures of Secoiridoid Compounds Occurring in Olive Fruit

Source: Obied *et al*, 2005

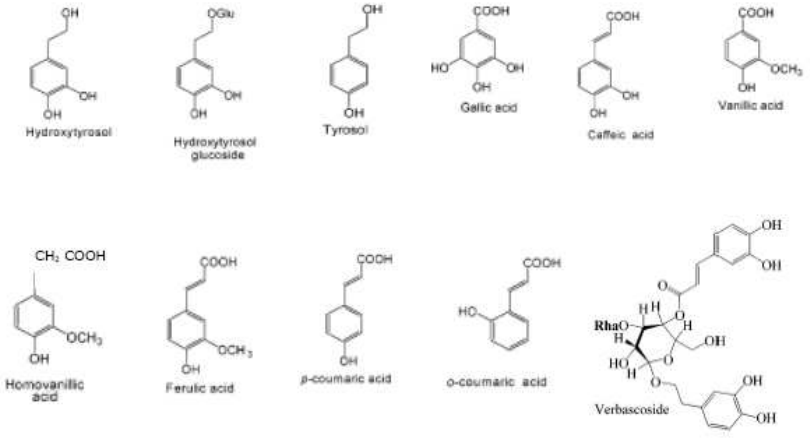


Figure7b. Chemical Structures of Phenolic Alcohols, Phenolics Acids and Derivates Ocurring in Olive Fruit

Source: Ryan *et al*, 2002a

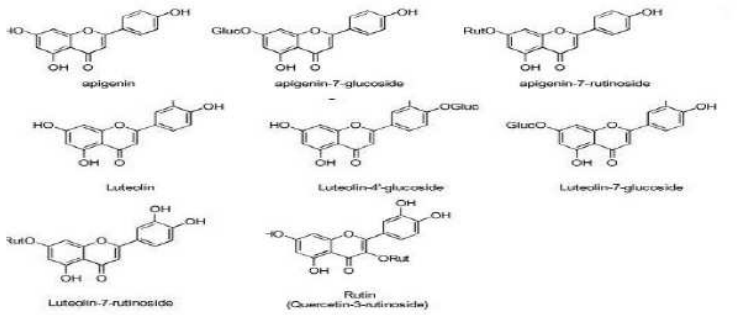
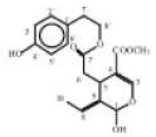


Figure 7c. Chemical Structures of Flavonoids Occurring in Olive Fruit

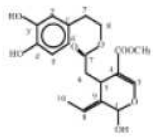
Recently, Brenes *et al* (2000) identified two new phenolic compounds in olive oil, 1-acetoxy-pinoresinol and pinoresinol. Pinoresinol is formed biosynthetically by stereospecific reductive coupling of two molecules of coniferyl alcohol (Katayama & Davin, 1992).

The prevalent phenolic compounds, however, of virgin olive oil are secoiridoid derivatives, the dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA) and the aldehydic form of elenolic acid linked to 3,4-DHPEA and *p*-HPEA (3,4-DHPEA-EA or *p*-HPEA-EA) (Servilli & Montedoro, 2002) (Figure 8).

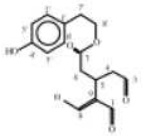
Source: Servilli & Montedoro, 2002



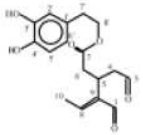
***p*-HPEA-EA**



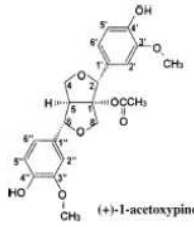
3,4-DHPEA-EA



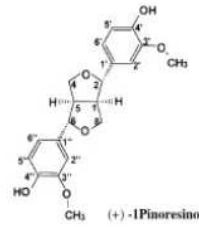
***p*-HPEA-EDA**



3,4-DHPEA.EDA



(+)-1-acetoxypinoresinol



(+)-1Pinoresinol

Figure 8. Chemical Structures of the Main Secoiridoid Derivatives and Lignans Ocurring in Olive Oil

Wastewater Phenolics. Some phenolic compounds have been reported in the wastewater phase from olive oil obtained by laboratory scale press (Servilli *et al*, 1999b) and processing plants (Mulinacci *et al*, 2001; Visioli *et al*, 1999a). A method was developed for the measurement of simple phenolics such hydroxytyrosol, tyrosol and caffeic acid by liquid chromatographic (Ceccon *et al*, 2001). Other studies have reported the characterization of the biophenolic extract from wastewaters (Obied *et al*, 2005). Hydroxytyrosol and verbascoside were found in an important concentration in wastewater, but caffeic acid was not quantified.

1.5.4 Transformation of Biophenolic Compounds During Processing

The iridoids are of terpenoid origin and their biosynthesis has been fairly well investigated (Jensen, 1991; Inouye & Uesato, 1986). It is known that two main routes exist. One route is leading to deoxyloganic acid via iridotrial. Deoxyloganic acid is the known precursor of many carbocyclic iridoids with the b-stereochemistry such as loganin and loganic acid, secologanin and secologanic acid as well as the derived secoiridoids and complex indole alkaloids. Most of the iridoids reported from Oleaceae formally belong to the secoiridoid group, but it has been shown that their biosynthesis is different from that of the ordinary secoiridoids from the Gentianales, which are usually derived from secologanin or secologanic acid.

Metabolites isolated from natural sources are not necessarily metabolites found in living tissues. Extraction process and purification must perturb the *status quo* of the organisms. Chemical changes produced by exposure to oxygen, solvents,

and change of pH are especially common with phenolic metabolites. Moreover, fruits are in a dynamic state with the level of metabolites representing a combination of both catabolic and anabolic processes.

The leaf hairs play an important role in plant protection serving to ward off biotic attack and reducing the level of UV radiation able to reach the leaf interior. Flavonoids (luteolin, apigenin and quercetin) in their glucoside and aglycone forms were detected; it appears that these compounds play also an important role in UV-B radiation. The high UV-B absorptive capacity of the hairs of young leaves shows a metabolic priority for flavonoid production during the early stages of leaf development (Ryan *et al*, 2002a).
Manifest routes are described for the degradation of oleuropein and related compounds (Ryan *et al*, 2002a). Cleavage by specific esterases gives rise to either elenolic acid glucoside or demethyloleuropein, which both occur in ripe olives. Even though, the source of these compounds either from catabolic or anabolic processes has not been completely established. The mechanism that could make clear the quantitative modification of secoiridoids in the oil matrix during malaxation is far from being elucidated (Vierhuis *et al*, 2001); very complicated isomerisations and equilibrium between different functionalities have been shown. Different mechanisms have been reported for the formation of the derivatives compounds (De Nino *et al*, 2000; Bianco *et al*, 1999a; Limiroli *et al*, 1995).

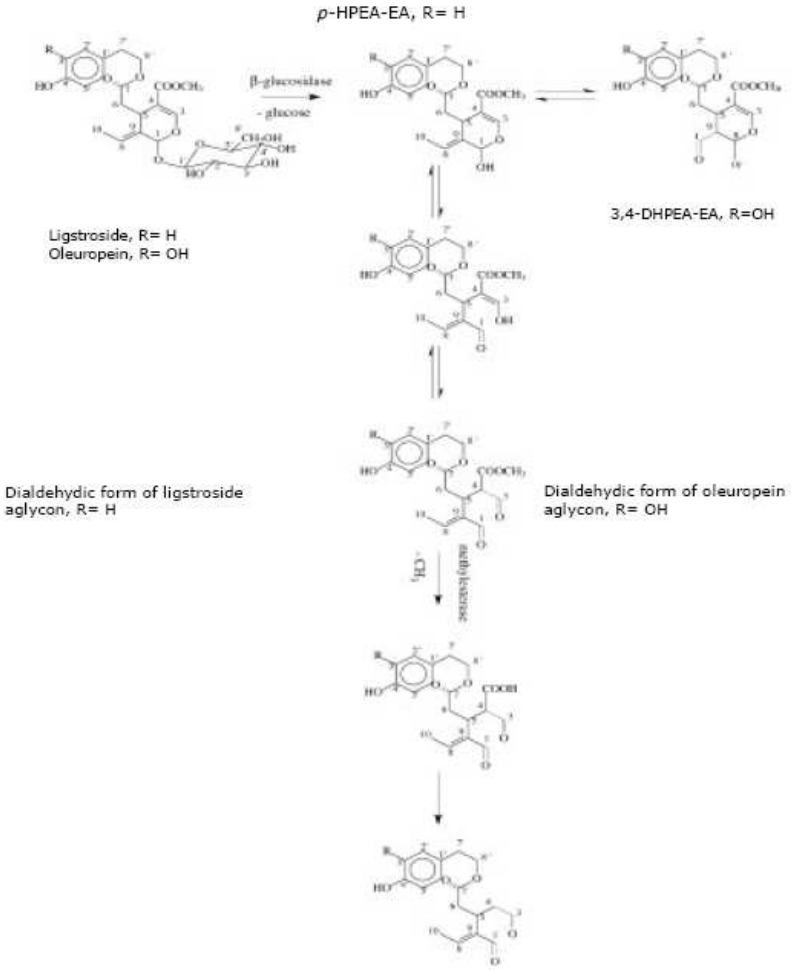
On the opposite, secoiridoid aglycons such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA and 3,4-DHPEA-EA are originated, during crushing, by the hydrolysis of oleuropein, demethyloleuropein and ligstroside; the reaction is catalysed by the endogenous β-glucosidases, according to the proposed mechanism reported by Servili *et al* (2004) (Figure 9). Servili & Montedoro (2002) reported that the concentration of oleuropein and demethyloleuropein was not significantly modified in olives blanched before crushing, to inactivate endogenous glycosidases; as a consequence of the enzymatic inhibition; furthermore the aglycon derivatives such as 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA were not found in the olive pastes and in the corresponding VOO. However, while the production of 3,4-DHPEA-EDA as final product of the demethyloleuropein enzymatic hydrolysis is well known (Lo Scalzo & Scarpati, 1993), the formation mechanism of 3,4-DHPEA-EDA and *p*-HPEA-EDA from oleuropein and ligstroside, respectively, are still unknown. Bianco *et al* (1999b) studied the hydrolysis of oleuropein glucoside by β-glucosidase in a model

system and showed the formation of the dialdehydic form of oleuropein aglycon as final product of the enzymatic reaction; the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), on the contrary, was not found.

1.5.5 Phenolics and Fruit Quality

Phenolics, especially oleuropein, may contribute to fruit quality in a number of ways, e.g. by contributing to sensory attributes such as color, flavor and bitterness intensity of the olive fruit, particulary oleuropein. Other bitter phenolics happening in the olive fruit encompass salidroside, nuezhenide and nuezhenide oleoside, in conjunction with two secoiridoid glucosides of uncertain structure containing tyrosol, elenolic acid and glucose moieties.

Source: Servilli *et al*, 2004



igure 10. Proposed Biochemical Transformations of Secoiridoid into Derivatives

p-HPEA-EDA, R = H

3,4-DHPEA-EDA, R = OH

Figure 9. Proposed Biochemical Transformations of Secoiridoids into Derivatives

Phenolics also contribute to olive quality in relation to browning reactions. Oxidation products of oleuropein, together with those of other native phenolics are known to be responsible for the black color of mature olive fruits. Enzymatic oxidation of endogenous *o*-diphenols into *o*-quinones, which can then polymerise into brown compounds results in the formation of the discoloration and softening of olive fruits. This reaction is catalyzed by polyphenols oxidases, large group of enzymes characterized by their ability to use molecular oxygen in the oxidation of phenolic substrates. The susceptibility of olive fruits to browning can be established using model solutions since browning reactions are mediated by metal ions, iron (III) and manganese. These reactions correspond to complex interactions between polyphenol oxidase activity and phenolic content. Some studies have shown a positive correlation between the content of oleuropein, the mayor substrate for the reaction, and the polyphenol oxidase activity (Ryan & Robards, 1998).

Similarly, the phenolic profile of olive oil depends on the olive variety (Stefanoudaki *et al*, 2000; Brenes *et* al, 1999). Variation in phenolic concentration with harvesting period and its effect on oil quality has been reported (Cimato *et al*, 1990). Several studies showed that phenolic levels were higher during the first harvesting period irrespectively of environment and cultivar and then gradually decreased with the olive ripening (García *et al,* 1996, Deidda *et al,* 1994; Alessandri *et al*, 1999).

The phenolic composition of olive fruit and olive oil reveal significant differences which are attributed to a series of chemical and enzymatic modifications of some compounds during oil processing. These changes include hydrolysis of glycerides by lipases, with the formation of free fatty acids, hydrolysis of glycosides and oligosaccharides by glucosidases, oxidation of phenolic compounds by phenoloxidases and polimerisation of free phenols.

1.5.6 Biological and Nutritional Properties

Olives and olive oil are a natural part of Mediterranean diet and culture (Willett *et al*, 1995). The antioxidant potential of phenolic compounds in olive oil has also been the subject of a great interest due to its chemoprotective effect in human beings; their beneficial health properties have been studied for many years (Wahrburg *et* al, 2002; Visioli & Galli, 1995).

The high content, over 70%, of the monounsaturated fatty acid (MFA), oleic acid, is also important because it is far less susceptible to oxidation than the polyunsaturated fatty acid, linoleic acid. A significant prevention against particular diseases due to the olive olive oil is also recognized because of the occurrence of squalene, and natural antioxidants in the oil (α-tocopherol, and phenolic compounds) (Owen *et al*, 2000b, Owen *et al,* 2000c).

Establishing a biological function for phenolic compounds is very difficult because their metabolic pathways and their alternative metabolic fates vary markedly from tissue to tissue, from one growth condition to another, and in response to environmental stimuli. Alll of the phenolic compounds possess several common biological and chemical properties and therefore, they are able to inhibit lipid peroxidation and exhibit various physiological activities. Extra virgin olive oil contains phenolic compounds with antioxidant activity (Vissers *et al*, 2002). As a result of their fundamental chemical properties, Oleuropein, responsible for the bitterness of raw olives, is one of the phenolics. Other simple phenols (e.g., tyrosol) and lignans (pinoresinol) also function as antioxidants (Visioli & Galli, 1995; Visioli & Galli, 1998).

Those eating the Mediterranean diet (rich in olive oil, fruits, vegetables, and fish) are known to have lower rates of colon, breast, and skin cancer, and coronary heart disease (Visioli & Galli *et* al, 2002; Harwood & Yaqoob, 2002; Tuck & Hayball, 2002; Owen *et al*, 2000c). Extra virgin oils are higher in these protective compounds that processed oils. Olive oil may act by reducing the LDL and raising the HDL forms of cholesterol in the blood (Vissers *et al*, 2002). Olive extracts have been shown to have hypoglycemic activity, and oil reduces gallstone formation by activating the secretion of bile from the pancreas. In addition, antioxidant and anti-inflammatory activities were measured in a complete investigation of recovery and bioactivity of biophenols from wastewaters. Extracts containing elenolic acid, hydroxytyrosol and tyrosol showed good antioxidant and anti-inflammatory activities (Visioli *et al*, 1999b).

Hydroxytyrosol also had a remarkable effect as an antibacterial activity (Capasso *et al*, 1995).

The antioxidant potential of phenolic compounds is also considered the major factor in the high stability (shelf life) of olive oils. Papadoupoulos & Boskou (1991) have studied the antioxidant effect of natural phenolic compounds on olive oil. The addition of extracts from virgin olive oil containing 200 mg/kg of polyphenols to a refined, bleached, and deodorized oil not containing phenolics, demonstrated significant inhibition of autoxidation (peroxide value) over time compared with control samples without phenolics addition. A study of the effect of individual compounds naturally occurring in olive oil showed that this effect was more pronounced in the presence of hydroxytyrosol than caffeic acid, and protocatechuic acid, while other simple phenolics were only marginally effective (Owen *et al*, 2000d).

Total hydrophilic phenols and the oleosidic forms of 3,4-dihydroxyphenylethanol (hydroxytyrosol) were high correlated (r = 0.97) with the oxidative stability of virgin olive oil while tocopherols showed low to no correlation (r=0.05) (Baldioli *et al*, 1996). Other authors have also reported a positive relation between 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA concentrations and the oxidative stability of the olive oils measured by Rancimat test at 120 ºC and 20 L/h air flow (Tovar *et al*, 2001).

1.6 FLAXSEED

1.6.1 *Linum usitatissimum*

Cultivar. Flax (*Linum usitatissimum*), also known as Common Flax or Linseed is a member of the genus *Linum* in the family Linaceae. It is an erect annual plant growing to 120 cm tall, with slender stems. The leaves are glaucous green, slender lanceolate, 2-4 cm long and 3 mm broad. The flowers are usually pure pale blue, 1.5-2.5 cm diameter, with five petals. The fruit is a round, dry capsule 5-9 mm diameter, containing several glossy brown seeds shaped like an apple pit, 4-7 mm long.

Flaxseed is used for both its fiber and its edible seed. It is said to have originated in the Mediterranean region of Europe. Stone Age dwellings in Switzerland contained remnants of flax, ancient Egyptians made some of their finer linens (often used to wrap mummies in tombs) from flax fiber. In the United States, early colonists grew flax for home use; commercial flax production for fiber began in 1753 (Wescott & Muir, 2000).

Flax is an annual oilseed that only accounts for approximately one percent of the world's oilseed supplies. It is used in industrial applications, for human consumption, and as a component in livestock feed. Two main types of flax grown throughout the world: seed flax and fiber flax. Seed flax is grown for the oil in its seed. Linseed or flax oil, a primary product of seed flax, is obtained by compressing the flaxseed and the application of a petroleum solvent. It is a non-edible drying oil used for manufacturing paints, varnishes linoleum, printing ink, oil cloth, putty, and plastics. Linseed oil production results in residual products such as linseed meal for livestock feed, and flaxseed stems that are used to make paper products such as cigarette paper (Daun *et al*, 2003).

Harvesting. Flax is more difficult to harvest than other small grain oilseeds, however, flax does not shatter or lodge as easily. Because of green weeds and uneven ripening, flax is usually windrowed and allowed to dry before combining. If swathed, a stubble height of 4-6 inches should be maintained to hold the windrow off the ground and aid in drying. Flax may be direct combined if it is relatively weed free and appears to be uniform in maturity. Flax is ready to harvest when 75 to 90% of the bolls have turned brown and the seeds contain less than 12% moisture.

In wet summers, the stems may remain green and the plants could continue to flower long after the early bolls are ripe. Under such conditions, flax should be harvested when all but the late bolls are ripe. Delaying harvest too long can lead to weathering of seeds in fall rains and blackening of seed from frost. It is also important to harvest soon after flax is mature because weeds could become an important problem. If left standing for a long period of time, the seed quality for oil purposes could be seriously reduced. Sharp, well-adjusted cutter bars are essential when combining, because the seed coat of flax is easily broken during combining (especially that of yellow-seeded varieties). Flax straw should be chopped and spread to speed decomposition and avoid problems with subsequent field operations.

Composition. Flaxseed is recognized as having about 35-40% oil, 35-50% dietary fiber, 20-30% protein and 4-6% ash. Values of proximate composition vary significantly according to the samples and the methodology employed to measure each of the components. Oilseed flax is characterized as being made up of about 45% oil and 55% meal on a dry basis. Flax has been utilized for production of linseed oil. Linseed oil was not used for human consumption because it contains high amounts of fatty acids and goes rancid faster than several other vegetable oils. Linseed oil was strictly used in industrial processes. Human consumption of flaxseed and flax oil has recently increased. An edible vegetable oil-type flaxseed called "Linola" or Solin has been developed to give heat stable oil. These new lines of flaxseed contain less α-linolenic acid (less than 3%) and can be used in food processing (Daun *et al*, 2003; Babu & Wiesenfeld).

Flaxseed has been used as food for centuries in Asia, Europe, and Africa. Flax is beneficial to humans and animals alike because it has a very high content of α-linolenic acid, a high percentage of dietary fiber (both soluble and insoluble), and the highest content of plant "lignans" of all plant or seed products used for human food (Daun *et al*, 2003).

Table 6 shows the proximate analysis of flaxseed reported from different sources.

Table 6. Proximate Analysis of Flaxseed

	Fat	Fiber[a]	Fiber[b]	Protein	Moisture	Ash	Energy
	(g/100g)						(cal)
Source							
University of Saskatchewan	41.0	28.0	---	26.0	0	4.0	ND
USDA	34.0	27.9	---	19.5	8.74	3.5	492
Flax Council of Canada	41.0	28.0	---	20.0	7.00	4.0	450
Canadian Grain Commission	39.8 - 45.6	---	30.5 – 36.8	17.4 – 24.1	4.2-4.9	ND	ND

Source: J. Daun *et al* (2003)

[a] Total dietary fiber
[b] Sum of soluble and insoluble fiber
Abbreviation: ND, no data

1.6.2 Processing of Flaxseed

Commercial processing of flaxseed and other oilseeds is performed to extract the oil and to produce a residual meal. The processing operations include seed cleaning, flaking, cooking, pressing, solvent extraction, and solvent removal. Recent data on modern oilseed processing have been reported by Eskin *et al*, 1996, Kolodziejcy & Fedec, 1995 and Unger, 1990. Before flaking, the seeds are passed through shaker screens and aspirators to remove foreign material (weed seeds, stones and soil). The clean seeds which may be conditioned by heating treatment, are rolled into flakes and passed to the cooker where the seed temperature is maintained at 65ºC for 20 min. The flakes are then transported from the cooker to a press where 60-70% of the oil is expelled. The remaining oil in the residual cake is extracted with hexane at 70ºC. Hexane is removed under vacuum with the addition of steam in a desolventizer toaster. The extracted cake generally enters the desolventizer toaster at 75ºC and is discharged at 105ºC for 30 min and then ground to obtain meal. Cold-pressed flaxseed oil is considered unsuitable for frying at high temperatures because of its high poly unsaturated fatty acid (PUFA) content, but a market has developed for it among health conscious consumers, who either consume it directly or take it in an encapsulated form. An oil suitable for use in cooking is obtained from solin, the yellow-seeded flax varieties low in linolenic acid. Brown-seeded flax and golden flax, a yellow-seeded flax variety called Omega have the same nutritional properties and human health potential benefits whereas solin offers different properties because of its low omega-3 fatty acid content (α-linolenic acid) and high linoleic acid content (Oomah & Mazza, 1998).

Some uses of flax for human consumption include ready-to-eat breakfast cereals, breakfast drinks, salad dressings, salad toppings, biscuits, crackers, soups, bagels, energy bars, and cakes. Flaxseed flour is used commercially in breads and cookes in the United States (Babu & Wiesenfeld, 2003; Daun *et al,* 2003; Westcott & Muir, 2000).

1.6.3. Phenolic Fraction

Flaxseed is a rich source of phenolic acids, containing 800 to 1000 mg of these compounds per 100 g of seed. Esterified phenolic acids can be up to 500 mg/100 g and etherified phenolic acids can reach 300 to 500 mg/100 g of seed

(Shahidi & Naczk, 2004). Esterified phenolic acids represent 48 to 66% of the total phenolic compounds independently of the cultivar (Oomah *et al*, 1996) Some studies have shown that soluble and insoluble phenolic acids constitute 54 and 26 to 29% of total phenolic acids of total phenolic acids in flaxseed flour, respectively (Varga & Diosady, 1994). *Trans*- ferulic and *trans*–cinnamic acids were reported as the major phenolic aids and *trans*- caffeic, *p*-coumaric and *p*-hydroxybenzoic acid the minor compounds found in dehulled and defatted flaxseed meal (Dabrowski & Sosulski, 1984). However, further data showed that the content of ferulic and chlorogenic acids accounted for 84% of total phenolic acids in methanolic extracts of defatted flaxseed meal (Harris & Haggerty, 1993).

Flaxseed also contains phenolic acids such as *p*-coumaric and ferulic in the glucosilated forms and phenylpropanoids such as gentisic, vanillic, and sinapic acids (Eliasson *et al*, 2003; Johnson *et al*, 2002). Flavonoids, coumarins and lignans have been reported as an important part of the phenolic compounds occurring in flaxseed. Total flavonoid content of flaxseeds ranges from 35 to 71 mg/100 g (Oomah *et al*, 1996); flavone C- and O-glycosides are the main flavonoids found in flaxseed cotyledons. An important phytochemical that has both phytoestrogenic and antioxidant properties is the secoisolariciresinol (SECO), the major lignan identified in flaxseed and the main precursor of the mammalian lignans (Charlet *et al,* 2002; Meagher *et al*, 1999; Mazur *et al*, 1996), whereas isolariciresinol, pinoresinol, and matairesinol have been reported as minor lignan components (Meagher *et al*, 1999).

The particular phenolic findings in flaxseed depend on the extraction performed to analyze them. Various organic solvents followed by hydrolysis treatments have been used in several studies to lead the release of phenolic compounds. For the analysis of SDG, alkaline hydrolysis with sodium hydroxide have been reported as effective method (Cacace *et al*, 2006; Coran *et al*, 2004; Eliasson *et al,* 2003; Johsson *et al*, 2002; Fritsche *et al*, 2002; Madhusudhan *et al*, 2000; Muir *et al*, 2000) (Figure 10). However, research on the identification of some compounds is so far from being concluded since phenolics such as pinoresinol, matairesinol have been found after solvent extraction followed by acid and enzymatic hydrolysis (Milder *et al*, 2004; Charlet *et al*, 2002; Meagher *et al*, 1999). Additionally, some studies have shown that the extraction of flaxseed meal with methanol-ammonia results in a decreasing of the content of soluble esterified phenolic acids and insoluble bound phenolic acids by 20 and 29%, respectively (Varga & Diosady, 1994).

Different techniques have also been used to identifie the lignans in flaxseed and other food sources. Various chromatography (HPLC) and gas chromatography (GC) with mass spectrometry (MS) have been found as valuable for the separation of these compounds (Slanina & Glatz, 2004). Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry have been reported by Peñalvo *et al* (2005). Milder *et al* (2004) reported a method for the quantification of the four major enterolignan precursors (secoisolariciresinol, matairesinol, lariciresinol and pinoresinol) based on a liquid chromatography-tandem mass spectrometry (LC-MS/MS). High-performarce thin-layer chromatographic-densitometric (HPTLC) determination of seicolariciresinol diglucoside in flaxseed was also reported by Coran *et al* (2004). The method showed precision and accuracy and could be used for the direct quantitative determination of SDG both in simple and in complex matrices using an external standard approach. In addition, isolation of SDG from flaxseed have been performed by high-speed counter-current chromatography (HSCCC) (Degenhardt *et al*, 2002) with advantages when compared to conventional chromatographic methods avoiding adsorption losses and the formation of artifacts due to the lack of active surfaces. Fritsche *et al* (2002) used liquid-chromatography-nuclear magnetic resonance spectroscopy-mass spectrometry (LC-NMR-MS) coupling for the separation and characterization of secoisolariciresinol diglucoside isomers in flaxseed.

1.6.4 Effect of Processing

Compositional changes occurring during processing of flaxseed are of primary importance in adding value to flaxseed products. Cold-pressed flaxseed oil is considered unsuitable for frying at high temperatures, but a market has developed for it among health conscious consumers, who either consume it directly or take it in an encapsulated form.

The physicochemical characteristics of flaxseed products at four stages of commercial processing, cleaning, flaking, pressing and solvent extraction have been studied by Oomah and Mazza, 1998. The processes of flaking, heating, solvent extraction and solvent extraction and solvent removal significantly increased the contents of protein, ash and soluble carbohydrate, and decreased oil content, total phenolics and protein solubility. In particular, total phenolic

acid decreases in a significant way on processing flaxseed from flakes to meal maybe because of the the strictness of heat treatment during these processing stages.

Oomah & Mazza (1997) reported that abrasive dehulling of the flaxseed concentrated phenolic acids in the dehulled flaxseed and observed that those changes in phenolics are highly cultivar dependent ($p < 0.0001$). At the same time, SDG content of flaxseed is significantly affected by cultivar, year of harvest, and, to lesser extent, growing location. Additionally, upon the dehulling, the total content of phenolic acids of Mc Greegor seeds changes from 700 to 1980 mg/100 g, but only marginal changes are observer for cultivars such as NorMan, Omega, and Vimy (Westcott and Muir, 1996).

Source: Johsson *et al*, 2002

(+)-secoisolariciresinol diglucoside, SDG

4-O-β-D-glucopyranosyl-*p*-coumaric acid, R= H
4-O-β-D-glucopyranosyl ferulic acid, R=OCH_3

Figure 10. Chemical Structures of Phenolic Compounds Ocurring in Flaxseed

1.6.5 Biological and Nutritional Properties

Throughout history, flaxseed has been primarily used as a mild laxative. It is high in fiber and a gummy material called mucilage. These substances expand when in contact with water; they add bulk to stool and help it move more quickly through the gastrointestinal tract, thereby acting as a laxative.

The seeds and oil of the flax plant also contain substances that promote good health. α-linolenic acid (ALA) contributes to moren than 50% of the total fatty acids of the flaxseed lipids. In addition to the important omega-3 fatty acid ALA, flaxseed is the reachest soucoisolariciresinol (SECO) and matairesinol (Milder *et*

al, 2005). In addition, pinoresinol, isolariciresinol are reported to exist in flaxseed. A few lignans have phytoestrogen activity they are able to mimic the action of the hormone estrogen in mamals. Secoisolariciresinol and matairesinol are converted into into metabolites, enterodiol (ED) and enterolactone (EL), known as enterolignans (mammalian lignans) once hydrolyzed by intestinal microfloral enzyme (Milder *et al*, 2005, Rafaelli *et al*, 2002; Heinonen *et al*, 2001). Lignans possess several biological activities including antioxidant and anti-oestrogenic properties. They may play a role in the reduction the risk of certain cancers and cardiovascular deseases (Arts & Hollman, 2005; Rafaelli *et al*, 2002; Oomah, 2001; Harris & Haggerty, 1993).

Antioxidant activity of SDG is expected to be strong because its chemical structure is very similar to nordihydroguaiaretic acid (NDGA) which is known to be a strong antioxidant (Shukla *et al*, 1997). Recent studies have shown that when flaxseed is eaten regularly and in moderate amounts, flaxseed improves regulation of the intestinal system, moderately reduces low-density lipoprotein (LDL) cholesterol, and increases urinary lignan formation and excretion (Horn-Ross *et al*, 2000)

In addition, aqueous ethanolic (95%) extracts of flaxseed meal exhibited moderate antioxidant properties as evaluated in β-carotene-linoleate model system. Chromatographic profile of ethanolic extract of flaxseed gave four fractions in which the highest antioxidant activity was detected in fractions containing lignans (Shukla *et al*, 1997).

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