

# Sources of natural phenolic antioxidants

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An important field of research today is the control of 'redox' status with the properties of food and food components. Natural antioxidants present in the diet increase the resistance toward oxidative damages and they may have a substantial impact on human health.

Dietary antioxidants such as ascorbates, tocopherols and carotenoids are well known and there is a surplus of publications related to their role in health. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant materials.

Extensively studied sources of natural antioxidants are fruits and vegetables, seeds, cereals, berries, wine, tea, onion bulbs, olive oil and aromatic plants. Attempts are also made to identify and evaluate antioxidants in agricultural by-products, ethnic and traditional products, herbal teas, cold pressed seed oils, exudates resins, hydrolysis products, not evaluated fruits and edible leaves and other raw materials rich in antioxidant phenols that have nutritional importance and/or the potential for applications in the promotion of health and prevention against damages caused by radicals.

## Introduction

Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. The health benefits of fruits and vegetables are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties.

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Sources of tocopherols, carotenoids and ascorbic acid are well known and there is a surplus of publications related to their role in health. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant materials.

The objective of the present work is to provide an overview of the findings related to the presence of antioxidant phenols in plant sources that have not been extensively studied and evaluated such as traditional products, agricultural by-products, herbal teas, cold pressed vegetable oils and other lesser known raw materials that have nutritional importance and/or the potential for applications in the promotion of health and prevention against damages caused by radicals. This objective is in line with current needs to upgrade by-products, to evaluate better traditional products and to develop compositional databases to improve accuracy of antioxidants consumption data, taking into consideration the limited existing compositional data available and the different cultural preferences among populations.

## The antioxidant hypothesis

Reactive oxygen and nitrogen species, ROS/RNS are essential to energy supply, detoxification, chemical signaling and immune function. They are continuously produced in the human body and they are controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase). When there is an over-production of these species, an exposure to external oxidant substances or a failure in the defense mechanisms, damage to valuable biomolecules (DNA, lipids, proteins) may occur (Aruoma, 1998). This damage has been associated with an increased risk of cardiovascular disease, cancer and other chronic diseases. The antioxidant hypothesis says that 'as antioxidants can prevent oxidative damages, increased intakes from the diet will also reduce the risks of chronic diseases' (Stanner Hughes, Kelly, & Buttriss, 2004). This explains the huge volume of research work and the efforts of many researchers to link diets rich in natural antioxidants with degenerative disease.

The great focus on the subject resulted in data that not only support but also challenge the hypothesis. One of the main problems is the fact that intervention studies

with humans have not shown a clear benefit that positively confirms the findings of epidemiological studies. Negative studies in the literature involve also vitamin antioxidants (vitamin E, vitamin C, carotenoids) given at high doses. However, in recent reviews in top nutritional journals it is underlined that the existing studies on humans demonstrate a ‘convincing effect of polyphenols on some aspects of health’ (Kroon & Williamson, 2005). It is also a fact that bioavailability studies are accumulating (Manach, Scalbert, Morand, Remesy, and Jimenez, 2004), while new databases are created for the various classes of polyphenols (Beecher, 2003; USDA, 2003) and estimates of intakes in many countries are discussed more and more seriously. The importance of antioxidant plant phenols is also seen in the efforts of researchers:

- (a) to increase the content of phenolics in plants (Wilhelm, Klaus, & Juergen, 2000)
- (b) to produce less hydrophile derivatives by enzymic modification of their structure with improved pharmacological characteristics (Kontogianni, Skouridou, Sereti, Stamatis, & Kolisis, 2003)
- (c) to explore novel effects
- (d) to elucidate the quantitative structure–activity relationships of various phenol classes (Nenadis, Zhang, & Tsimidou, 2003; Kontogiorgis, Pontiki, & Hadjipavlou-Litina, 2005).

### Research related to plant phenols

Widely distributed in the plant kingdom and abundant in our diet plant phenols are today among the most talked about classes of phytochemicals. In the last decade, much work has been presented by the scientific community, which focuses on:

- The levels and chemical structure of antioxidant phenols in different plant foods, aromatic plants and various plant materials.
- The probable role of plant phenols in the prevention of various diseases associated with oxidative stress such as cardiovascular and neurodegenerative diseases and cancer.
- The ability of plant phenols to modulate the activity of enzymes, a biological action not yet understood.
- The ability of certain classes of plant phenols such as flavonoids (also called polyphenols) to bind to proteins. Flavonol–protein binding, such as binding to cellular receptors and transporters, involves mechanisms of polyphenols which are not related only to their direct activity as antioxidants.
- The stabilization of edible oils, protection from off-flavours formation and stabilization of flavours.
- The preparation of food supplements.

### Top antioxidant sources

Top antioxidant sources are fruits and vegetables. The most important sources and the classes of phenols they contain are briefly presented in Table 1.

### Traditional and ethnic products olive oil

The polar phenolic compounds present in olive oil are a very important class of minor constituents and they are related to the stability of the oil but also to its biological properties (Visioli, Bogani, Grande, & Galli, 2004; Boskou, Blekas, & Tsimidou, 2005). The latter have received much attention and today many phenolic compounds contained in the oil, mainly hydroxytyrosol and its derivatives, are thoroughly investigated with the aim of establishing a relationship between dietary intakes and the risk of cardiovascular disease or cancer. Ongoing and completed studies in this area associate these phenols with the beneficial role of olive oil in human health.

A significant part of the research related to olive oil polar phenolics indicates a scavenging activity of these compounds against superoxide anion and hydrogen peroxide and a capability to prevent the generation of reactive oxygen species. An *in vitro* inhibitory effect on eicosanoids production and on platelet aggregation have also indicated some mechanisms by which olive oil phenols help to protect against various cardiovascular disorders, while their capacity to scavenge nitrogen reactive species such as peroxy-nitrite suggests a protective effect against nitration of tyrosine and DNA damage.

Compounds which often appear in lists of olive oil polyphenols are (in alphabetical order): 4-acetoxy-ethyl-1,2-dihydroxybenzene, 1-acetoxy-pinoresinol, apigenin, caffeic acid, cinnamic acid (not a phenol), *o*- and *p*-coumaric acids, elenolic acid (not a phenol), ferulic acid, gallic acid, homovanillic acid, *p*-hydroxybenzoic acid, hydroxytyrosol and derivatives, luteolin, oleuropein, pinoresinol, protocatechuic acid, sinapic acid, syringic acid, tyrosol and derivatives (Boskou *et al.*, 2005). The dialdehydic forms of elenolic acid linked to hydroxytyrosol and tyrosol, 1-acetoxy-ethyl-1,2-dihydroxybenzene (hydroxytyrosol acetate), 1-acetoxypinoresinol, pinoresinol, oleuropein aglycone, luteolin, and ligstroside aglycone are the phenols with the higher concentration (Fig. 1).

In a recent study Bianco, Caccioli, Guiso, and Marra (2001) found a new class of phenols, hydroxyl-isochromans derivatives. Hydroxy-isochromans are now investigated (Togna *et al.*, 2003) for their antioxidant power and their ability to inhibit platelet aggregation.

The polyphenol content differs from oil to oil. Wide ranges have been reported (50–1000 mg/kg) but values are usually between 100 and 300 mg/kg. The cultivar, the system of extraction, and the conditions of processing and storage are critical factors for the content of polyphenols.

**Table 1. Top sources of antioxidant plant phenols**

	Antioxidants	References
<i>Fruits</i>		
Berries	Flavanols hydroxycinnamic acids, hydroxybenzoic acids, anthocyanins	Hakkinen <i>et al.</i> (1998), Belitz and Grosch (1999), Wang and Lin (2000), Yanishlieva-Maslarova and Heinonen (2001), and Manach <i>et al.</i> (2004)
Cherries	Hydroxycinnamic acids, anthocyanins	Belitz and Grosch (1999), Yanishlieva-Maslarova <i>et al.</i> , (2001), and Manach <i>et al.</i> (2004)
Blackgrapes	Anthocyanins, flavonols	Belitz and Grosch (1999), Yanishlieva-Maslarova <i>et al.</i> (2001), and Manach <i>et al.</i> (2004)
Citrus fruits	Flavanones, flavonols, phenolic acids	Yanishlieva-Maslarova <i>et al.</i> (2001), Beecher (2003), and Manach <i>et al.</i> (2004)
Plums, prunes, apples, pears, kiwi	Hydroxycinnamic acids, catechins	Belitz and Grosch (1999), Yanishlieva-Maslarova <i>et al.</i> (2001), and Manach <i>et al.</i> (2004)
<i>Vegetables</i>		
Aubergin	Anthocyanins, hydroxycinnamic acids	Manach <i>et al.</i> (2004)
Chicory, artichoke	Hydroxycinnamic acids	Manach <i>et al.</i> (2004)
Parsley	Flavones	Manach <i>et al.</i> (2004), and Beecher (2003)
Rhubarb	Anthocyanins	Manach <i>et al.</i> (2004)
Sweet potato leaves	Flavonols, flavones,	Chu <i>et al.</i> (2000)
Yellow onion, curly	Flavonols	Manach <i>et al.</i> (2004)
Kale, leek		
Parsley	Flavones	Manach <i>et al.</i> (2004)
Beans	Flavanols	Manach <i>et al.</i> (2004)
Spinach	Flavonoids, <i>p</i> -coumaric acid	Bergman <i>et al.</i> (2001)
<i>Flours</i>		
Oats, wheat, rice	Caffeic and ferulic acids	Yanishlieva-Maslarova <i>et al.</i> (2001), and Manach <i>et al.</i> (2004)
<i>TEAS</i>		
Black, green	Flava-3-ols, flavonols	Manach <i>et al.</i> (2004), and Beecher (2003)
<i>Alcoholic drinks</i>		
Red wine	Flavan-3-ols, flavonols, anthocyanins	Manach <i>et al.</i> (2004), and Beecher (2003)
Cider	Hydroxycinnamic acids	Manach <i>et al.</i> (2004)
<i>Other drinks</i>		
Orange juice	Flavanols	Manach <i>et al.</i> (2004)
Coffee	Hydroxycinnamic acids	Manach <i>et al.</i> (2004) Sanchez-Gonzales <i>et al.</i> (2005)
Chocolate	Flavanols	Beecher (2003), and Manach <i>et al.</i> (2004)
<i>Herbs and spices</i>		
Rosemary	Carnosic acid, carnosol, Rosmarinic acid rosmanol	Yanishlieva-Maslarova <i>et al.</i> (2001) Ibanez <i>et al.</i> (2003)
Sage	Carnosol, Carnosic acid, lateolin, rosmanol, rosmarinic acid	Yanishlieva-Maslarova <i>et al.</i> (2001)
	Rosmarinic acid,	Zheng and Wang (2001)
Oregano	Rosmarinic acid, phenolic acids, flavonoids	Yanishlieva-Maslarova <i>et al.</i> (2001) Exarchou <i>et al.</i> (2002), and Belhatab <i>et al.</i> (2004)
Thyme	Thymol, carvacrol, Flavonoids, lubeolin	Yanishlieva-Manach <i>et al.</i> (2004) Zheng <i>et al.</i> (2001) and Exarchou <i>et al.</i> (2002)
Summer savory	Rosmarinic, carnosol, carvacrol, flavonoids	Yanishlieva-Maslarova <i>et al.</i> (2001)
Ginger	Gingerd and related companids	Yanishlieva-Maslarova <i>et al.</i> (2001) Moure <i>et al.</i> (2001)

Mean concentrations of polyphenols determined in 10 monovarietal olive oils are given by Aparicio and Luna (2002).

### Table olives

Table olives have a different qualitative and quantitative phenolic composition than the raw olive fruits from which they are prepared. The reason is the diffusion of phenols and other water soluble constituents from the

olive fruit to the surrounding medium (water, brine or lye) and vice versa, the lye treatment and hydrolysis during fermentation. When Californian-type black olives are prepared, hydroxytyrosol and caffeic acid levels decrease markedly during the darkening process. Iron salts, used for colour fixation, catalyze the oxidation of hydroxytyrosol, which disappears.

Commercially available table olive samples, analyzed for individual phenols by RP-HPLC, were found to

**Table 2. Antioxidant plant phenols in herbal teas**

	Phenols	References
<i>Lamiaceae</i>		
Oregano	Phenolic acids, rosmarinic acid Flavonols, flavones	Exarchou <i>et al.</i> (2002) Kosar <i>et al.</i> (2003), Belhatab <i>et al.</i> (2004), and Atoui <i>et al.</i> (2005)
Mountain tea	Various phenols	Triantaphyllou <i>et al.</i> (2001), Koleva <i>et al.</i> (2003)
Sage	Phenolic acids, flavonoids	Kosar <i>et al.</i> (2003), Atoui <i>et al.</i> (2005)
Mint	Various phenolics	Atoui <i>et al.</i> (2005)
Dictamnus	Various phenolics	Triantaphyllou <i>et al.</i> (2001), Atoui <i>et al.</i> (2005)
<i>Other plants</i>		
Roibos	Various phenolics	Kroyer (2005)
Mate	Various phenols	Kroyer (2005)
Mallow	Various phenols	Kroyer (2005)
Chamomile	Various phenolics	Trouillas <i>et al.</i> (2003), Kroyer (2005)
Linden (tilia)	Various phenolics	Atoui <i>et al.</i> (2005)
Eucalyptus	Various phenolics	Atoui <i>et al.</i> (2005)
Yarrow	Phenolic acids, flavonoids, tannins	Trouillas <i>et al.</i> (2003)
Artichoke (cynara)	Phenolic acids, flavonoids	Trouillas <i>et al.</i> (2003)

contain hydroxytyrosol as the prevailing phenolic compound (Blekas, Vasilakis, Harizanis, Tsimidou and Boskou (2002)). Its levels (in flesh) were found to be higher than 100 and 170 mg/kg in Greek-style naturally black olives and Spanish-style green olives in brine, respectively. In Kalamata olives, a special type of Greek-style naturally black olives, this phenol was found at levels ranging from 250 to 760 mg/kg. Remarkable levels of luteolin were determined also in Greek-style naturally black olives (25–75 mg/kg),

It can be concluded from the above that table olives are excellent sources of antioxidants, baring in mind that a few olives may provide approx 10 mg of polyphenols, the quantity obtained from 50–100 g of olive oil

### Sesame seed and products

Sesame seed and its oil have been used for about 6000 years. The plant, *Sesamum indicum*, L. is believed to originate in the savannas of Central Africa. From there, it spread to Egypt, India and China. The high nutritional value of sesame seed is mentioned even by Hippokrates.

Sesame seed has many culinary applications. It is used for many bakery products, for the production of oil (raw or roasted), as well as for the preparation of Tehina and Halva. Tehina is a paste, while halva is a cake-like product.

The antioxidant activity of sesame seed and sesame seed oil and the various healthful properties are attributed to the presence of lignans such as sesamin, sesamol, sesaminol, sesangolin, 2-epicalatin and others (Kamal-Eldin, Appenquist and Yousif, 1994; Shyu and Hwang, 2002) (Fig. 2). Sesame oil is extremely resistant to oxidative rancidity and this is due to the presence of other strong antioxidants (not lignans) but chemically related compounds such as sesamol and sesamol dimer.

There are many claims for the health effects of sesame seed and its lignans. These have been related to the enhancement of vitamin E activity, to a capacity to protect low-density lipoproteins against oxidative damage, to a serum triacylglycerol lowering effect, a hypocholesteremic effect and others (Ide, Kushiro, Takahashi, Shinohara, Fukuda and Sirato-Yasumoto, 2003; Yamashita, Iizuka, Imai and Namiki, 1996; Nakai, Harada, Nakahara, Akimoto Miki *et al.*, 2003; Hirata, Fujita, Ishikura, Hosoda, Ishikawa and Nakamura 1996).

### Cold pressed seed oils

In addition to olive oil, a rich source of natural antioxidants, some cold pressed seed oils have become recently commercially available. The seeds used are agricultural byproducts and some of them are good sources of tocopherols and biologically important carotenoids. Cold pressed marionberry, boysenberry, raspberry, blueberry, black caraway, black currant, carrot, cranberry and hemp seed oils have been reported to contain antioxidants and possess a remarkable radical scavenging activity and oxygen radical absorption capacity, when tested with the DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS cation {2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt} radical-scavenging assays or the oxygen radical absorption capacity (ORAC) assay (Yu, Zhou, & Parry, 2005; Parry *et al.*, 2005). The nature of the antioxidants is not yet known, but due to the mode of preparation these oils retain phenols present in the seed and they may have the potential for applications in the promotion of health and prevention against oxidation damages mediated by radicals.

Other cold pressed seed oil currently investigated are date seed oil, argan oil, cold-pressed onion, cardamom,

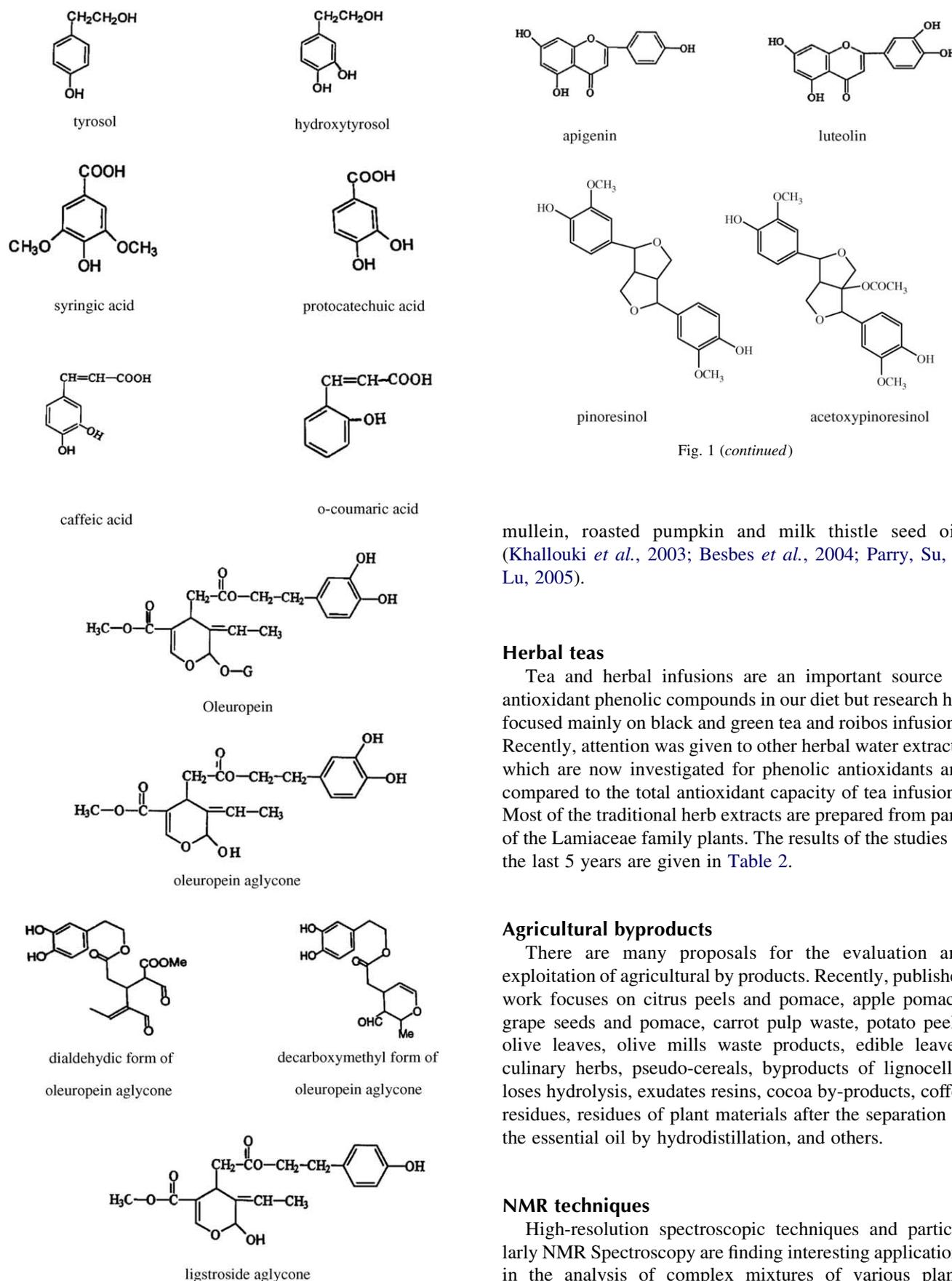


Fig. 1 (continued)

mullein, roasted pumpkin and milk thistle seed oils (Khallouki *et al.*, 2003; Besbes *et al.*, 2004; Parry, Su, & Lu, 2005).

### Herbal teas

Tea and herbal infusions are an important source of antioxidant phenolic compounds in our diet but research has focused mainly on black and green tea and roibos infusions. Recently, attention was given to other herbal water extracts, which are now investigated for phenolic antioxidants and compared to the total antioxidant capacity of tea infusions. Most of the traditional herb extracts are prepared from parts of the Lamiaceae family plants. The results of the studies of the last 5 years are given in Table 2.

### Agricultural byproducts

There are many proposals for the evaluation and exploitation of agricultural by products. Recently, published work focuses on citrus peels and pomace, apple pomace, grape seeds and pomace, carrot pulp waste, potato peels, olive leaves, olive mills waste products, edible leaves, culinary herbs, pseudo-cereals, byproducts of lignocelluloses hydrolysis, exudates resins, cocoa by-products, coffee residues, residues of plant materials after the separation of the essential oil by hydrodistillation, and others.

### NMR techniques

High-resolution spectroscopic techniques and particularly NMR Spectroscopy are finding interesting applications in the analysis of complex mixtures of various plants extracts containing phenols.

Fig. 1. Major olive oil phenols.

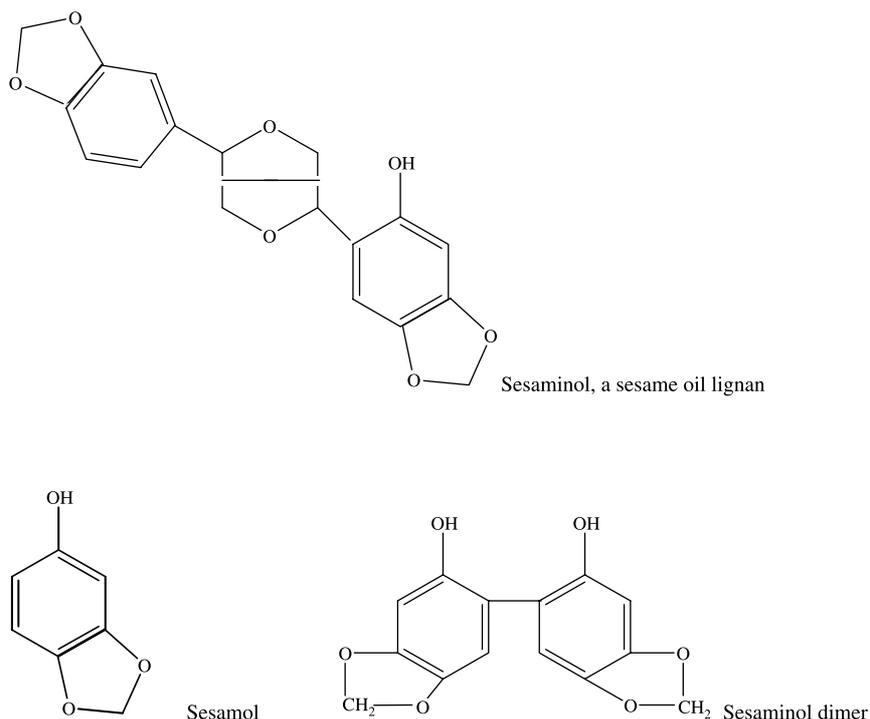


Fig. 2. Important antioxidants in sesame seed.

The NMR spectrum of certain flavonoids indicates a significant deshielded signal in the region 11–13 ppm which is attributed to the hydroxyl proton OH (5), participating in a strong six membered ring intramolecular bond with CO (4). Since, this region in a  $H^1$  NMR spectrum of an extract is not crowded, the identification of flavonols and their differentiation from flavones can be achieved by ID proton NMR spectroscopy.

Figure 3 shows the selected region of the  $H^1$  NMR spectrum of the acetone extract of oregano in  $CD_3CO CD_3$  at 300 K (Belhattab *et al.*, 2004) and the peak of quercetin (a flavonol) which is separated from the peaks of flavones centered at 13 ppm.

Combined advanced NMR methodologies have also been described for the analysis of mixtures of phenolic compounds that occur in natural products. These methodologies may be a combination of variable temperature two-dimensional proton–proton double quantum filter correlation spectroscopy ( $H^1$ – $H^1$ –DQF COSY) and proton – carbon heteronuclear multiple quantum coherence ( $H^1$ – $^{13}C$ –HMQC). (Exarchou *et al.*, 2002) On-line solid phase extraction (SPE) in LC-NMR for peak storage after the liquid chromatography separation prior to NMR analysis or similar techniques have been recently applied. Exarchou, Godejohann, van Beek, Gerathanassis, and Vervoort (2003) used LC-UV-solid-phase extraction-NMR-MS combined with a cryogenic flow to characterize phenols in Greek oregano. The compounds identified were taxifolin,

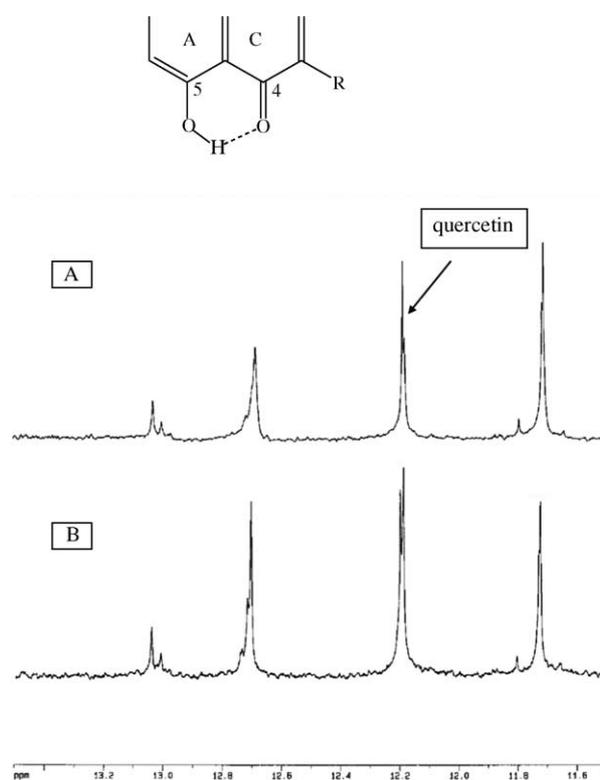
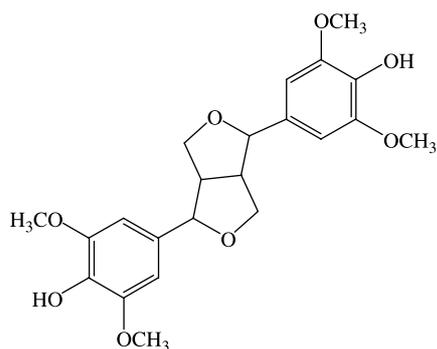


Fig. 3. Selected region of the  $H^1$  NMR spectra of the acetone extract of *Origanum glandulosum* in  $CD_3COCD_3$  (A) at 300 K and (B) after the addition of quercetin (spiking) at 300 K. The arrow denotes the presence of quercetin OH (5) signal. (Belhattab *et al.*, 2004).



Syringaresinol

Fig. 4. Syringaresinol.

aromadendrin, eriodictyol, naringenin, apigenin, rosmarinic acid, carvacrol and thymol.

More recently, Christoforidou, Dais, Tseng, and Spraul (2005) applied hyphenated liquid chromatography–solid phase extraction–nuclear magnetic resonance, to identify new phenols in the polar fraction of olive oil. The addition of a post-column SPE system in replacement of the loop system of the LC-NMR technique, resulted in advanced sensitivity (significant increase of the signal to noise ratio). The spectra recorded were one dimensional (1D)  $^1\text{H}$  NMR and two dimensional (2D) NMR. The presence of phenolics was confirmed from the respective LC-SPE-NMR spectra, which were assigned on the basis of existing  $^1\text{H}$  NMR databases and with total correlation spectroscopy (TOCSY). The most interesting findings of this study was the verification of the presence of the lignan syringaresinol (Fig. 4), the presence of two stereochemical isomers of the aldehydic form of oleuropein and the detection of homovanillyl alcohol.

## Conclusion

In spite of certain discrepancies related to the ‘antioxidant hypothesis’, the message that antioxidants are good for health has a considerable momentum. The nutrition and other scientific societies tend to recognize today the importance of dietary antioxidants as agents promoting health.

Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. The health benefits of fruits and vegetables are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties. Sources of tocopherols, carotenoids and ascorbic acid are well known and there is a surplus of publications related to their role in health. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant materials. Work published so far covers many aspects but it is limited to the best-known

fruits and vegetables. Many plant materials and foods have not yet received much attention as sources of antioxidant phenols due to limited popularity or lack of commercial applications. However, existing research work indicates that utilization of underexploited sources and better evaluation of ethnic and traditional foods can offer many benefits in the promotion of human health.

In order to utilize such sources of antioxidants, to evaluate traditional products, to develop more complete compositional databases and to obtain more accurate antioxidants intake data, further chemical characterization is needed. Identification and quantitation, based mainly on HPLC, GC–MS and LC–MS methods can be aided today by NMR methodology, especially homonuclear two-dimensional correlated spectroscopy,  $^1\text{H}$ – $^{13}\text{C}$  heteronuclear multiple bond correlation spectroscopy and other techniques such as LC-NMR or LC-NMR/MS, which may provide information on the overall composition and enable the identification of individual phenols in complex matrices.

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