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Aromatization of olive oil

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1. Definition and labelling of “flavoured oil”

A “flavoured olive oil” can be defined as an olive oil (generally an extra-virgin olive oil) that has been processed with vegetables, herbs, spices or other fruits in order to improve the nutritional value, enrich the sensory characteristics and increase shelf-life (Lagouri and Boskou 1996, Nouhad and Tsimidou 1998, Tsimidou and Boskou 1994). Gourmet Olive Oil is a fancy name meaning that it has an organoleptic superior quality.

According to EU Regulation 1989 (2003), an Extra Virgin Olive Oil is a liquid fat that conforms to a series of chemical parameters (free fatty acid percentage $\leq 0.8\%$, peroxide value ≤ 20 meq O₂ /kg, K₂₃₂ ≤ 2.50 , K₂₇₀ ≤ 0.22), is free of defects and possesses an impeccable aroma and flavour. In order to be Extra Virgin Olive Oil, it must be extracted “only from olives with a superior quality”, and can not undergo any treatment other than washing the fruits, and decanting, centrifuging and filtering the extracted olive oil. It excludes oils obtained from seeds by chemical or mechanical methods or the use of solvent extraction or re-esterification methods, and those mixed with oils from other sources. Thus, by definition, flavoured olive oils are not Extra Virgin ones.

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Concerning the United States, although there are no federal laws against it, the members of the California Olive Oil Council (COOC) signed an agreement in order to avoid the labelling of flavoured oil as Extra Virgin. Because of the great success of these oils, the COOC is trying to come up with a meaningful labelling standard for flavoured oils.

According to the International Olive Oil Council standards, flavoured oils are considered seasonings and thus they couldn't named "olive oil or virgin or extra virgin olive oil flavoured with...". Instead, such products may be named "seasoning made from olive oil and flavourings, spices or herbs" and the list of ingredients may state the category of olive oil and flavouring according to the Codex general standard for labelling of pre-packaged foods (CODEX STAN 1-1985, Rev. 1-1991, amended in 2001). In reality, few producers comply with this label their infused or flavoured olive oil.

Since flavoured oils are new to many customers, a number of olive oil producers are adding pictures highlighting the ingredients to the labels applied on bottles. Fig. 1 reports some examples of labels found on the market.



Figure 1. Examples of flavoured olive oil labels present on the market.

The Food and Drug Administration (FDA) has recognized the nutritional and health quality of olive oil allowing, on November 2004, the labelling of olive oil and products including it according to the so called “Qualified Health Claim”: “Limited and not conclusive scientific evidence suggests that eating about 2 tablespoon (23g) of olive oil daily may reduce the risk of coronary heart disease”.

2. Brief history and applications of flavoured olive oils

Olive oil is a product widely used throughout the ages in the Mediterranean cuisine and is appreciated for its delicious taste and aroma, as well as for its nutritional benefits (Jacotot 1994, Morales *et al.* 2000, Visioli *et al.* 2004) primarily related to its balanced fatty acid composition and the presence of considerable amounts of natural antioxidants (Moldão-Martins *et al.* 2004). Although flavoured oils could be considered a simple fad, the infusion of herbs or flowers in oils is an ancient practice born in the Mediterranean area when gardeners preserved vegetables and herbs by drying and immersing them in the locally pressed olive oil in order to prevent degradation reactions. The resulted oils acquired a vegetable flavour and were used for pasta and salad dressing sauces or as a dip for bread. Each Mediterranean Country has its proper traditional flavoured oils voted to specific uses. For example, in Italy, herb-infused olive oil is particularly used on crusty bread; in Portugal, olive oil is infused with tiny hot red peppers, black peppercorns, garlic and, sometimes, brandy; in Spain it is infused with hot red peppers and herbs.

The flavoured oils also represented an ancient practice performed during traditional olive oil extraction in order to clean the press and to make less unpleasant the oils obtained from olives over-ripened or stored in a wrong way.

Consumers coming from North Europe, U.S.A., and Canada have slowly been introduced to flavoured oils when they began to travel more and ventured beyond the Mediterranean area. Among these non-traditional consumers, the consumption of flavoured olive oils is gaining interest since they pay particularly attention to the possibility to prevent diseases through a healthy diet. These potential consumers are not familiar with the application of the pure olive oil and, for this reason, they may be willing to use preparations of olive oil enriched with other ingredients of the Mediterranean diet such herbs, spices and fruit essential oils (Antoun and Tsimidou 1997).

For all these reasons, flavoured oils have become some of the most popular dressings used by both gourmet chefs and common people. Their versatility, ease of use, and wide range of tastes have made them staples of traditional and non traditional consumers across many Countries in the world.

The largely diffused applications for this kind of product are salad dress, roasting vegetables and pasta, dips and marinades, although they are also gaining an increasing popularity in meat and poultry applications beyond that in fried potatoes. Other applications include nuts and snacks, which can be sprayed with the oils before being cooked, as well as pastes, sauces and crumb coatings.

3. Spices, herbs, fruits and essential oils used for oil aromatization

Lots of different types of flavoured oil are available on the market. The assortment is very wide since it is possible to choose among oils flavoured with:

- vegetables (garlic, onion, peppercorns, chilli, sun dried tomatoes);
- herbs (rosemary, oregano, basil, sage, thyme, fennel, juniper, estragon);
- spices (clove, nutmeg, ginger);
- mushrooms (truffles);
- fruits (lemon, orange, mandarin, apple, banana);
- nuts (almond, hazelnut, pine nuts);
- aromas (for example, oils aromatized with vanilla are used to season shellfish, poultry and salads with vegetables, fruit and shellfish).

A further flavouring ingredient is grass (U.S. Patent 20020164413).

Tables 1 and 2 (USDA 2007) respectively report the lists of antioxidants and compounds having a flavouring activity found in some of the vegetables, spices and fruits most commonly used in oil flavouring. It's plain that some compounds are endowed with both antioxidant and flavouring activity.

Table 1. Non exhaustive lists of antioxidant active compounds isolated from some of the most commonly used spices, herbs, and fruits (USDA 2007).

Product (common and scientific name)	Antioxidant compounds
Apple (<i>Malus domestica</i>)	Alanine, α -tocopherol, ascorbic acid, β -carotene, caffeic acid, chlorogenic acid, chlorophyll, ferulic acid, histidine, hyperoside, isoquercitrin, lauric acid, lutein, myristic acid, <i>p</i> -coumaric acid, <i>p</i> -hydroxy-benzoic acid, palmitic acid, protocatechuic acid, quercitrin, selenium, sinapic acid, sucrose, tryptophan

Table 1. Continued

Black pepper (<i>Piper nigrum</i>)	Ascorbic acid, β -carotene, camphene, carvacrol, eugenol, γ -terpinene, lauric acid, linalyl-acetate, methyl-eugenol, myrcene, myristic acid, myristicin, palmitic acid, piperine, terpinen-4-ol, ubiquinone
Chilli pepper (<i>Capsicum frutescens</i>)	Alanine, ascorbic acid, β -carotene, caffeic acid, campesterol, capsaicin, capsanthin, chlorogenic acid, hesperidin, histidine, kaempferol, lauric acid, lutein, methionine, myrcene, myristic acid, myristic acid, <i>p</i> -coumaric acid, palmitic acid, palmitic acid, pentadecanoic acid, quercetin, scopoletin, stigmasterol, terpinen-4-ol, tocopherol, tryptophan
Dill (<i>Anethum graveolens</i>)	α -Tocopherol, anethole, ascorbic acid, β -sitosterol, caffeic acid, camphene, carvacrol, chlorogenic acid, eugenol, ferulic acid, γ -terpinene, histidine, isoeugenol, isorhamnetin, kaempferol, lauric acid, methionine, myrcene, myristic acid, myristicin, myristicin, palmitic acid, quercetin, scopoletin, selenium, stigmasterol, terpinen-4-ol, <i>trans</i> -anethole, vicenin
Fennel (<i>Foeniculum vulgare</i>)	Alanine, ascorbic acid, β -carotene, β -sitosterol, caffeic acid, camphene, ferulic acid, fumaric acid, γ -terpinene, γ -tocotrienol, gentisic-acid, histidine, isoquercitrin, methionine, myrcene, myristicin, <i>p</i> -coumaric acid, <i>p</i> -hydroxy-benzoic acid, <i>p</i> -hydroxycinnamic acid, palmitic acid, protocatechuic acid, quercetin, rutin, scopoletin, selenium, shikimic-acid, sinapic acid, stigmasterol, syringic acid, terpinen-4-ol, terpinolen tocopherol, <i>trans</i> -anethole, tryptophan, vanillic acid, vanillin
Garlic (<i>Allium sativum</i> var. <i>sativum</i> L.)	Alanine, allicin, alliin, allixin, α -tocopherol, ascorbic acid, β -carotene, caffeic acid, diallyl-disulfide, diallyl-sulfide, diallyl-tetrasulfide, diallyl-trisulfide, ferulic acid, glutathione, histidine, methionine, <i>p</i> -coumaric acid, quercetin, <i>s</i> -allyl-cysteine-sulfoxide, selenium, sucrose, tryptophan
Marjoram (<i>Origanum majorana</i>)	Ascorbic acid, β -carotene, β -sitosterol, caffeic acid, carvacrol, eugenol, hydroquinone, linalyl-acetate, myrcene, oleanolic acid, phenol, rosmarinic acid, tannin, terpinen-4-ol, <i>trans</i> -anethole, ursolic acid

Table 1. Continued

Onion (<i>Allium cepa</i>)	Alanine, allicin, alliin, α -tocopherol, ascorbic acid, β -carotene, β -sitosterol, caffeic acid, campesterol, catechol, cholesterol, cysteine, ferulic acid, fumaric acid, histidine, kaempferol, methionine, myristic acid, oleanolic acid, <i>p</i> -coumaric acid, palmitic acid, protocatechuic acid, pyrocatechol, quercetin, rutin, selenium, sinapic acid, spiraeoside, stigmasterol, sucrose, tryptophan, vanillic acid
Orange (<i>Citrus sinensis</i>)	Alanine, α -tocopherol, ascorbic acid, β -carotene, β -sitosterol, caffeic acid, campesterol, cholesterol, delphinidin-3-glucoside, ferulic acid, γ -terpinene, histidine, lutein, methionine, myrcene, naringin, <i>p</i> -coumaric acid, palmitic acid, scutellarein, selenium, sinapic acid, stigmasterol, sucrose, tangeretin, terpinen-4-ol, terpinolene, tryptophan
Oregano (<i>Origanum vulgare</i>)	Camphene, carvacrol, γ -terpinene, linalyl-acetate, myrcene, terpinen-4-ol, thymol
Red (sweet) pepper (<i>Capsicum annuum</i>)	Alanine, α -tocopherol, ascorbic acid, β -carotene, β -sitosterol, caffeic acid, campesterol, camphene, capsaicin, capsanthin, chlorogenic acid, eugenol, γ -terpinene, hesperidin, histidine, lupeol, lutein, methionine, myrcene, myristic acid, <i>p</i> -coumaric acid, palmitic acid, palmitic acid, pentadecanoic acid, scopoletin, selenium, stigmasterol, terpinen-4-ol, tocopherol, tryptophan
Rosemary (<i>Rosmarinus officinalis</i>)	Apigenin, ascorbic acid, β -carotene, β -sitosterol, caffeic acid, camphene, carnosic acid, carnosol, carvacrol, chlorogenic acid, γ -terpinene, hesperidin, hispidulin, isorosmanol, labiatic acid, luteolin, luteolin-3'- <i>o</i> -(3''- <i>o</i> -acetyl)- β -D-glucuronide, luteolin-3'- <i>o</i> -(4''- <i>o</i> -acetyl)- β -D-glucuronide, methyl eugenol, myrcene, oleanolic acid, rosmadial, rosmanol, rosmaridiphenol, rosmarinic acid, rosmariquinone, squalene, tannin, terpinen-4-ol, thymol, trans-anethole, ursolic acid
Sage (<i>Salvia officinalis</i>)	Alanine, apigenin, ascorbic acid, β -carotene, β -sitosterol, β -sitosterol, caffeic acid, campesterol, camphene, carnosic acid, carnosol, carnosol, carnosolic acid, catechin, chlorogenic acid, cholesterol, chrysoeriol, ferulic acid, fumaric acid, gallic acid, γ -terpinene, hispidulin, labiatic acid, linalyl acetate, luteolin, myrcene, oleanolic acid, oleanolic acid, <i>p</i> -coumaric acid, palmitic acid, rosmano, rosmarinic acid, rosmarinic acid, salicylic acid, selenium, stigmasterol, tannin, terpinen-4-ol, thymol essential oil, ursolic acid, uvaol, vanillic acid

Table 1. Continued

Thyme (<i>Thymus vulgaris</i>)	4-terpineol, alanine, anethole essential oil, apigenin, ascorbic acid, β -carotene, caffeic acid, camphene, carvacrol, chlorogenic acid, chrysoeriol, eriodictyol, eugenol, ferulic acid, gallic acid, γ -terpinene, isochlorogenic acid, isoeugenol, isothymonin, kaempferol, labiatic acid, lauric acid, linalyl-acetate, luteolin, methionine, myrcene, myristic acid, naringenin, oleanolic acid, <i>p</i> -coumaric acid, <i>p</i> -hydroxy-benzoic acid, palmitic acid, rosmarinic acid, rosmarinic acid, selenium, tannin, thymol plant, tryptophan, ursolic acid, vanillic acid
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Table 2. Non-exhaustive list of compounds having flavour activity isolated from some of the most commonly used spices, herbs, and fruits (USDA 2007).

Product (common and scientific name)	Compounds with flavouring activity
Basil (<i>Ocimum basilicum</i> L.)	1,8-cineole, α -pinene, α -terpinene, α -terpineol, β -pinene, borneol, camphene, carvone, caryophyllene, <i>cis</i> -3-hexenol, citral, citronellol, estragole, eugenol, farnesol, fenchone, fenchyl-alcohol, furfural, γ -terpinene, geraniol, limonene, linalyl-acetate, menthone, methyl-cinnamate, methyl-eugenol, nerol, <i>p</i> -cymene, terpinolene, <i>trans</i> -anethole
Bay leaf (<i>Laurus nobilis</i>)	1,8-cineole, α -phellandrene, α -pinene, α -terpinene, α -terpineol, benzaldehyde, β -pinene, borneol, camphene, carvone, caryophyllene, cinnamyl-acetate, <i>cis</i> -3-hexenol, δ -3-carene, eugenol, formic acid, γ -terpinene, geraniol, geranyl-acetate, hexanal, hexanol, limonene, methyl-eugenol, myrcene, nerol, neryl-acetate, <i>p</i> -cymene, propionic acid, terpinolene, <i>trans</i> -2-hexenal
Chilli pepper (<i>Capsicum frutescens</i>)	1,8-cineole, α -phellandrene, α -terpineol, benzaldehyde, β -pinene, capsaicin, carvone, caryophyllene, citric-acid, δ -3-carene, hexanoic acid, limonene, methyl-nonanoate, methyl-phenylacetate, myrcene, nonanoic acid, octanoic acid, oleic acid, <i>p</i> -methyl-acetophenone, palmitic acid, pulegone, stearic acid, threonine, valine
Fennel (<i>Foeniculum vulgare</i>)	1,8-cineole, α -phellandrene, α -pinene, α -terpinene, α -terpineol, benzoic acid, β -pinene, camphene, cinnamic acid, citric acid, d-limonene, estragole, fenchone, fumaric acid, γ -terpinene, limonene, myrcene, myristicin, oleic acid, <i>p</i> -cymene, palmitic acid, tartaric acid, terpinolene, threonine, <i>trans</i> -anethole, valine, vanillin

Table 2. Continued

Ginger rhizome (<i>Zingiber officinale</i>)	1,8-cineole, α -phellandrene, α -pinene, α -terpinene, α -terpineol, β -pinene, bornyl-acetate, camphene, citral, citronellal, citronellol, citronellyl-acetate, dodecanoic acid, ethyl-myristate, farnesol, γ -terpinene, geraniol, geranyl-acetate, hexanol, limonene, methyl-nonyl-ketone, MUFA, <i>n</i> -heptane, nerol, oleic acid, <i>p</i> -cymene, palmitic acid, stearic acid, terpinolene, threonine, valine, zingerone
Hazelnut (<i>Corylus avellana</i>)	Oleic acid, palmitic acid, stearic acid
Lemon (<i>Citrus limon</i>) pericarp essential oil	α -pinene, α -terpineol, γ -terpinene, limonene, thymol
Mandarin (<i>Citrus reticulata</i>)	1,8-cineole, α -phellandrene, α -pinene, α -terpinene, α -terpineol, benzyl-alcohol, β -pinene, camphene, carvone, caryophyllene, cis-3-hexenol, citric acid, citronellal, citronellol, citronellyl-acetate, decanoic acid, decyl-acetate, δ -3-carene, dodecanal, dodecanoic acid, dodecanol, γ -terpinene, geraniol, geranyl-acetate, isopulegol, limonene, myrcene, nerol, neryl-acetate, nonanoic acid, nootkatone, octanoic acid, oleic-acid, <i>p</i> -cymene, palmitic acid, stearic acid, threonine, thymol, valine
Marjoram (<i>Origanum majorana</i>)	α -phellandrene, α -pinene, α -terpinene, β -pinene, cadinene, carvacrol, carvone, caryophyllene, citral, δ -3-carene, estragole, ethyl-laurate, ethyl-myristate, ethyl-oleate, ethyl-palmitate, eugenol, geraniol, geranyl-acetate, hydroquinone, limonene, linalyl-acetate, myrcene, neryl-acetate, <i>p</i> -cymene, terpinolene, <i>trans</i> -anethole
Oregano (<i>Origanum vulgare</i>)	1,8-cineole, 3-octanol, α -phellandrene, α -pinene, α -terpinene, α -terpineol, β -pinene, borneol, bornyl-acetate, camphene, carvacrol, carvone, caryophyllene, cinnamic acid, decanoic acid, δ -3-carene, γ -terpinene, geraniol, geranyl-acetate, hexanal, limonene, linalyl-acetate, myrcene, naringin, nerol, neryl-acetate, nonanoic acid, oleic acid, <i>p</i> -cymene, palmitic acid, stearic acid, terpinolene, terpinyl-acetate, thymol, <i>trans</i> -anethole
Pepper (<i>Piper nigrum</i>)	1,8-cineole, α -pinene, α -terpinene, α -terpineol, benzoic acid, β -pinene, borneol, camphene, carvacrol, carvone, cinnamic acid, citral, citronellal, citronellyl-acetate, <i>d</i> -limonene, δ -3-carene, dihydrocarveol, eugenol, γ -terpinene, geranyl-acetate, hexanoic acid, isopulegol, linalyl-acetate, methyl-cinnamate, methyl-eugenol, myrcene, myristicin, nerolidol,, oleic acid <i>p</i> -cymene, <i>p</i> -methyl-acetophenone, palmitic acid, phenylacetic acid, piperidine, piperine, terpinolene, terpinyl-acetate

Table 2. Continued

Savory (<i>Satureja montana</i>)	1,8-cineole, α -phellandrene, α -pinene, α -terpinene, α -terpineol, β -pinene, borneol, bornyl-acetate
Spearmint leaf (<i>Mentha spicata</i>)	α -pinene, α -terpineol, benzaldehyde, benzyl-alcohol, benzyl-isobutyrate, β -pinene, borneol, camphene, carvacrol, carvone, caryophyllene, diacetyl, dihydrocarveol, dihydrocarvyl-acetate, dimethyl-sulfide, ethyl-isobutyrate, eugenol, farnesol, furfural, γ -terpinene, geraniol, menthone, <i>n</i> -butyraldehyde, <i>n</i> -valeraldehyde, neryl-acetate, <i>p</i> -cymene, phenethyl-alcohol, phenyl-acetaldehyde, piperitone, propionaldehyde, pulegone, terpinolene, threonine, thymol, valine, vanillin
Sweet pepper (<i>Capsicum annuum</i>)	α -phellandrene, α -pinene, α -terpineol, β -pinene, camphene, capsaicin, caryophyllene, citric acid, δ -3-carene, eugenol, γ -terpinene, hexanal, hexanoic acid, limonene, myrcene, octanoic acid, oleic acid, <i>p</i> -cymene, palmitic acid, piperidine, pulegone, stearic acid, terpinolene, threonine, valine
Thyme (<i>Thymus vulgaris</i>)	4-terpineol, α -phellandrene, α -pinene, α -terpinene, α -terpineol, β -pinene, camphene, carvacrol, carvone, cinnamic acid, citral, δ -3-carene, eugenol, γ -terpinene, geraniol, geranyl-acetate, limonene, linalyl-acetate, menthone, myrcene, nerolidol, oleic acid, <i>p</i> -cymene, palmitic acid, threonine, thymol, valine

Among the products mentioned in Table 1, the one showing the highest antioxidant concentration is the onion with about 843 174ppm total and 32 antioxidant species (USDA 2007). Among the plants reported in Table 2, the highest concentration of compounds having flavour activity is founded in lemon (2426 840ppm on a total of 11 flavour compounds) (USDA 2007).

4. Advantages of the aromatization of olive oil

Aromatic plants and fruits have been used throughout the ages in many fields, from food flavouring to pharmaceutical, cosmetic and perfumery due to their content of essential oils and other compounds (Moldão-Martins *et al.* 2004) to which antimicrobial and antioxidant properties are usually recognized (Prakash 1990, Loo and Richard 1992).

As already said at the beginning of this chapter, the aims of the olive oil aromatization including the improvement of its sensory and nutritional properties and the prolonging of the oil shelf life (Gambacorta *et al.* 2007).

The sensory aspects of flavoured oils are strongly affected by the compounds reported in Table 1 and 2. Antoun and Tsimidou (1997) prepared

oregano, rosemary, and garlic gourmet olive oils at several percentages. They found that consumers were able to differentiate between levels of addition and preferred the samples with a low to moderate odour and flavour. Furthermore, all the considered flavourings were organoleptically accepted by consumers. The acceptability is not only dependent on the incorporation level but also on the essential oil composition. Moldão-Martins *et al.* (2002) report the results of a panel test performed on sunflower oil flavoured with different aromatic extracts from *Thymus zygis*. The flavoured oils were well accepted when the levels of geraniol and geranyl acetate were high. Samples in which p-cymene and γ -terpinene were present at high levels were not accepted or negatively evaluated. Moldão-Martins *et al.* (2004) evaluated the sensory response to flavoured oils by direct incorporation of the *Mentha x piperita* and *Thymus mastichina* L. essential oils mixtures. The consumer tests showed that in the flavoured olive oil the presence of thyme essential oil was always desirable whereas mentha flavoured olive oil was accepted only at low levels of addition. The oregano and rosemary flavoured olive oils resulted well accepted by consumers for flavour and taste (Antoun and Tsimidou 1998) and it seemed that the infusion time did not significantly affect their sensorial characteristics (Damiechki *et al.* 2001). Gambacorta *et al.* (2007) evaluated the sensory acceptability of extra-virgin olive oils flavoured with hot pepper, garlic, oregano and rosemary. The addition of herbs and spices enhanced the sensorial characteristics of the original extra virgin olive oil. Tasters were able to distinguish among the levels of addition. All the flavoured oils were judged as acceptable, with the exception of that flavoured with garlic at the highest concentration, but judges preferred the oils flavoured at intermediate levels of addition.

Extra virgin olive oil is strongly endowed with substances having healthy effects: oleic acid, other unsaturated fatty acids, and natural antioxidants including chlorophyll, carotenoids, α -tocopherol and phenolic compounds such as pinosresinol, oleuropein derivatives (hydrotyrosol, 3,4-DHPEA-EDA, 3,4-DHPEA-EA) and ligstroside derivatives (tyrosol, *p*-HPEA-EDA, *p*-HPEA-EA). Interesting antioxidant properties are usually assigned to the compounds (Table 1) extracted from aromatic plants. As a consequence, the addition of parts of these plants to oil enhances its nutritional properties and healthy effects particularly in terms of oxidation prevention. Reactive oxygen and nitrogen species are continuously produced in the human body since they are essential to energy supply, detoxification, chemical signalling and immune function. They are controlled by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, catalase but in case of a failure in the defence mechanisms or an exposure to external oxidant substances, damages to biomolecules (DNA, lipids, proteins) may occur (Aruoma 1998,

Boskou 2006). This damage has been associated with an increased risk of cancer (resulting from DNA modifications), cardiovascular pathologies (correlated to lipid peroxidation) and other chronic diseases. The antioxidant diet intakes could prevent or reduce the risk of such diseases (Stanner *et al.* 2004). The results of in vivo-epidemiological studies are not very clear though, recently, some researches performed on humans demonstrate a ‘convincing effect of polyphenols on some aspects of health’ (Kroon and Williamson 2005). The positive effects of antioxidants are determined by their ability to terminate radical reaction chains, scavenge active oxygen species, and trap electrophiles (Offord *et al.* 1997). Among antioxidants, polyphenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions (Middleton *et al.* 2000); many of these biological functions have been attributed to their free radical scavenging and antioxidant activity. Molecular studies have revealed that phenolics can exert modulatory actions in cell by interacting with molecular targets central to the cell signalling machinery (Soobrattee *et al.* 2005). Thus, their protective effect is also due to the ability of selectively inhibiting or stimulating key proteins in the cell signalling cascades (Mandel and Youdim 2004). Traditionally, several aromatic plants have been used as medicinal plants. This is the case of basil, used in the treatment of headaches, coughs, diarrhoea, constipation, warts, worms, and kidney malfunction (Simon *et al.* 1999) and of the thyme applied for its antiseptic, and carminative properties (Baranauskiene *et al.* 2003). Mühlbauer *et al.* (2003) investigated several common herbs rich in essential oils (sage, rosemary, and thyme) and essential oils extracted from these herbs and other plants (sage, rosemary, juniper, pine, dwarf pine, turpentine, and eucalyptus) as well as their monoterpene components (thujone, eucalyptol, camphor, borneol, thymol, α -pinene, β -pinene, bornyl acetate as well as menthol) and found that they inhibit bone resorption when added to the food of rats. These compounds active on bone are therefore candidates for a dietary approach to osteoporosis.

“Natural” does not mean “safe”. Asekum *et al.* (2007) investigated the effects of drying methods on quality and quantity of the essential oils of *Mentha longifolia* L. subsp. *Capensis*, a wild mint largely used in food preparation in South Africa. The results showed that only oven drying brought about significant losses of the major compounds (menthone, pulegone and 1,8-cineole) in the essential oil when compared to the fresh plant material. This might be due to some chemical transformations during the process of drying. Pulegone is reported to be a potent hepatotoxin, even at low concentrations. It is metabolised in the liver to menthofuran, a highly reactive metabolite which binds irreversibly to the components of liver cells

in which metabolism takes place. It quickly destroys the liver (Chen *et al.* 2001, Gordon *et al.* 1987) and can also destroy cytochrome P450 in rats (Moorthy 1991). Due to the significant reduction of pulegone and menthone by oven-drying, it is suggested that this herb should be oven-dried or cooked before consumption in order to reduce toxicity. Eating of the raw plant should be discouraged, especially in patients with a history of liver disease.

The oil flavouring has an impact also on the product shelf life. In fact, antioxidants have been widely used in fats and oils in order to prevent their oxidation and the consequent production of undesirable flavours (Frankel 1993, Karpinska *et al.* 2001, Nakatani 1997). In addition, oxidized lipids may lower the food nutritional value (Addis and Warner 1991) and have undesirable effects on the human organism (Benzie 1996). The problem is particularly important for industries with a view to increasing the oxidative stability of fats and oils. Recently, there has been a certain reluctance to use synthetic additives, including antioxidants. In particular, some synthetic antioxidants, such as BHT and BHA, might be dangerous for organism (Attmann *et al.* 1986, Powell *et al.* 1986) whereas natural antioxidants contained in vegetables used for oil flavouring answer to the characteristics individuated by Pokorny (1991): safety, consumer's acceptance, ability to stabilize oil but also to add nutraceutical value.

Thus, there is an increasing interest in herbs and spices as sources of harmless natural antioxidants (Dorman *et al.* 1995, Marinova and Yanishlieva 1997, Baratta *et al.* 1998, Lis-Balchin *et al.* 1998). Phenolics and other compounds can act as antioxidants by many potential pathways such as free radical-scavenging, oxygen radical absorbance, and chelating of the metal ions (Halliwell *et al.* 1995). Damiechki *et al.* (2001) performed a study aimed to examine the presence of antioxidants and pro-oxidants in oregano and rosemary gourmet oils. They found that the total polar phenolic content in the flavoured oils increased in comparison with that of the control. These data confirm that total phenols correlate well with oxidative stability of olive oil (Tsimidou 1998): in fact, the oxidative stability of gourmet oils was greater than that of the control using the Rancimat test (an apparatus used to predict the shelf life of oils). The flavouring also determined an increase of the total chlorophyll content that resulted negatively correlated with the stability to photo-oxidation. As expected for primary antioxidants, total phenolic content was not affected by light whereas total chlorophylls quickly degraded with the disappearance of the green colour. Thus, chlorophylls content is a critical factor for the shelf life of these preparations and labelling suggesting avoidance of light could be useful. Baiano *et al.* (2005) analyzed the effects of adding a mixture of garlic, laurel, and marjoram on selected chemical indices of olive oil from canned dried tomatoes. The addition of the herbs

slowed polymerization reactions but did not inhibit triglyceride oxidation. On the contrary, it determined an increase in the kinetic constants of acidity, peroxide, and *p*-anisidine values. Antoun and Tsimidou (1997) found that oregano and rosemary were able to slow the primary oxidation whereas the addition of garlic did not improve the oil stability. When added to sunflower oil, the extracts from *Ocimum basilicum* and *Origanum vulgare* L. did not improve the oxidation stability whereas the ethanol extracts of *Satureia hortensis* L., *Mentha piperita* L., *Melissa officinalis* L., and *Mentha spicata* L. appeared strongly active in retarding the oxidation process (Marinova and Yanishlieva 1997). The limit of these results is that experiments were performed at 100°C, a temperature usually used to accelerate the tests of oxidative stability. The same authors confirmed the greater interest of studies on the antioxidant activity carried out at room temperature as well as at frying temperatures. Tomaino *et al.* (2005) studied the effect of heating (80, 100, 120, and 180°C) on antioxidant effectiveness and the chemical composition of basil, cinnamon, clove, nutmeg, oregano and thyme essential oils. At room temperature, all the essential oils showed good radical-scavenger properties with the following DPPH assay (effectiveness order: clove>>cinnamon>nutmeg>basil≥oregano>>thyme). Up to 180°C, only nutmeg oil showed a significantly free radical-scavenger activity and evident changes in its chemical composition. In addition, all the essential oils tested appeared able to prevent the α -tocopherol loss following the virgin olive oil heating at 180°C for 10min (efficiency order: clove> thyme≥cinnamon>basil>> oregano>nutmeg). Rosemary and sage are two plant-derived antioxidants that have been studied intensively and found to be effective for stabilizing frying oils (Che Man and Tan 1999, Jaswir *et al.* 2000) thanks to their good thermal resistance and (Houlian and Ho 1985). When added into palm olein, rosemary and sage are effective to retard oil deterioration during repeated deep-fat frying of potato chips and are able to increase the acceptability of the product (Irwandi and Che Man 1999, Che Man and Irwandi 2000).

5. Aromatization methods

Several methods of oil aromatization are available and the choice is very important since the extraction method affects both acceptability and stability oxidation of the flavoured oil.

Infusion is a traditional method of oil aromatization. Natural materials containing antioxidants and flavour compounds such as herbs, spices and fruits, are finely ground and mixed with oil and the mixture is left at a room temperature for a defined time and with periodic shaking (Riva *et al.* 1993). The mixture is then filtered to remove the solid parts and ready to use. When

performed at room temperature, infusion can take a long time (hours but also days and months). In order to speed up the process, possible changes of this aromatization method include the infusion in vacuum conditions or nitrogen atmosphere and the heating at moderate temperature or the microwave assisted extraction. Di Cesare *et al.* (1993) produced garlic aromatized oils by infusion (25°C, 16h), heated-assisted extraction, and microwave-assisted extraction. They found that the extraction of diallyl-disulphide was 5 times quicker with the microwave technique than with the traditional method in the first 5 minutes of treatment. In addition, the flavoured oils produced by microwave showed oxidation induction times longer than the conventionally heated and the untreated ones. Obviously, the effectiveness of these extractions depends on the ratio vegetable-oil, and the size of vegetable pieces (vegetables must be finely ground and sieved if necessary). Furthermore, vegetables must be previously dried to avoid the release of water that negatively affects oil quality and safety. An alternative to the moderate heating is represented by the method described in the U.S. Patent 5320862. According to this method, a vegetable oil is put in contact with a flavouring agent in a particulate form at a temperature between 100 and 200°C. A negative aspect is the possibility of change in oil taste and flavour and a reduction of oil quality.

To avoid the presence of turbidity and dosage troubles, an alternative to classical infusion is reported by Gambacorta *et al.* (2007). They first prepared a concentrated extract obtained by infusion of vegetables in extra-virgin olive oil. Then, the flavoured olive oils were prepared by adding different aliquots of the filtered extract to the pure extra-virgin olive oil.

The safest method to obtain a flavoured an oil is represented by the cold pressing of olives together with the herb, spice or vegetable. The mixture is then subjected to a crushing and malaxation treatment obtaining a malaxation mash. At the end, the flavoured olive oil is separated from the malaxation mash by pressure or centrifugation and collected. In this manner the flavours from the flavouring agents are very well absorbed into the oil, the residue of the flavouring ingredient is separated from the oil together with the olive residue and the watery part of the spices are removed along with the olive vegetable water. Thus, the process results in a clear olive oil.

Using infusion, heating, and microwave assisted extraction, flavour compounds are co-extracted with undesirable ones, such as waxes and bitters, modifying, in this way, the sensory characteristics and stability during shelf life (Moldão-Martins *et al.* 2004). To avoid these problems, a possible approach is the use of the essential oils or vegetable extracts as flavouring agents. The essential oils can be obtained by solvent extraction (Suhaj 2006), steam distillation (Moldão-Martins *et al.* 2000), and supercritical CO₂

extraction (Esquivel *et al.* 1999). Hawthorne *et al.* (1993) compared supercritical fluid extraction and hydro-distillation in order to extract essential oils from savoury, peppermint and dragonhead founding the greater effectiveness of the former. The same results were obtained by Esquivel *et al.* (1999). The extraction with solvents commonly uses petrol ether, toluene, acetone, ethanol, methanol, ethyl acetate, cyclohexane. Methanol is the most commonly employed solvent. Due to its polarity, aqueous methanol effectively extracts polyphenols linked to polar fibrous matrices whereas acetone/water mixtures are more useful for extracting polyphenols from proteic matrices, since they degrade the polyphenol– protein complexes. The limitation of this extraction method is the difficulty to obtain solvent-free extracts (Daood *et al.* 2002) since these solvents are toxic and flammable. Ultrasound-assisted extraction is often used for the extraction of plant material using liquid solvents. The solvent extraction can be also performed in a Soxhlet apparatus, thus combining percolation and immersion techniques (Suhaj 2006). Ultrasound was used to increase the efficiency of essential oils solvent extraction. Sonication improved the yields and shortened the extraction times (Albu *et al.* 2004).

Recently, the pressurized liquid extraction has been introduced for the phenolic extraction. In this technique high temperature and high pressure are used to accelerate the extraction. The pressurized hot water extraction of phenolics from sage was found to be the most effective extraction procedure, followed by maceration with 70% ethanol, hydrodistillation, and ultrasonication-assisted methanol extraction (Ollanketo *et al.* 2002).

In the last years (Chemat *et al.* 2003, Lucchesi *et al.* 2004), a patent describing a new method for extracting natural products without added any solvent or water by using microwave has been set up. The solvent free microwave extraction (SFME) apparatus is a combination of microwave heating and dry distillation at atmospheric pressure. This method involves placing plant material in a microwave reactor, without any added solvent or water. The internal heating of the in situ water within the plant material distends the plant cells and leads to rupture of the glands and oleiferous receptacles. The free essential oils evaporate by the in situ water of the plant material. A cooling system outside the microwave oven condensed the distillate continuously. The excess of water is refluxed to the extraction vessel in order to restore the in situ water to the plant material.

6. Potential hazard related to flavoured olive oils production

The flavoured olive oils available on sale often have a long shelf life guaranteed by the way of production. Unacquainted with the potential hazard,

some consumers try to infuse flavoured oils by application of home-made procedures and store them also for long time at room temperature. The production of flavoured olive oils requires the addition of fresh vegetables to the oil itself. Some of the methods frequently suggested to consumers for infusing flavour from fresh vegetables into oil allow the potential development of botulism toxin. This is because plants, including herbs and spices, can carry spores produced by *Clostridium botulinum* (Topp *et al.* 2003). The presence of water in the vegetable additives and the absence of oxygen provide a hospitable growth medium and can create the right conditions for the bacteria to multiply within the oil. In 1985, more than three dozen people in Vancouver, (Canada) became ill after consuming restaurant-prepared garlic in soybean oil. The cause of the sickness was just identified as botulism. All unrefrigerated garlic-in-oil products throughout Canada were subsequently recalled by the Health Protection Branch (HPB) of Health and Welfare Canada.

Topp and Howard (1997, 1999), together with Health Canada, developed a method for the production of safe flavouring oils using a fixed amount of vegetable and then determining the time required to reach 140°C in a domestic oven. Topp *et al.* (2003) analysed the principal factors affecting the heating of an oil–vegetable mixture and developed a theoretical model to represent the heating process. They considered that the heating of oil with varying amounts of fresh vegetable is different from that of an oil. Furthermore, the water contained in the vegetable increases the heat capacity of the oil–vegetable mixture and slows the heating rate (Hallström *et al.* 1988). In their model, Topp *et al.* (2003) took into account three variables which consumers are able to control: type of heating container, amount of vegetable added, and size of vegetable pieces.

In any case, the most important rule to make a safe flavoured oil is the completely removal of the water from the vegetables.

Ciafardini *et al.* (2004) performed a study on the presence of microorganisms in lemon, oregano, garlic, and red chilli pepper flavoured extra virgin olive oils and found the presence of a different type of microflora according to the flavouring ingredient used. Moulds were present in all types of commercial flavoured olive oils, yeasts were only found in the oil enriched with oregano, while bacteria were only occasionally observed. The results obtained from the tests demonstrated that microorganisms are capable of surviving in flavoured extra virgin olive oils, but the ingredients affect the life of the microbes in the oil in a way depending to their chemical characteristics.

7. Techniques and methods for the evaluation of acceptability, shelf life, and quality of flavoured olive oils

A flavoured olive oil must satisfy the consumer sensory requirements. Thus, the study of the consumer acceptability is one of the most suitable techniques for the evaluation of a flavoured oil. The instrument used for the sensory analysis is a panel of trained or untrained people. In the case of acceptability evaluation, the aim of the study is to check if the product could meet the sensory requirements of a large number of consumers and thus a panel of a number of untrained people as great as possible should be used. Antoun and Tsimidou (1997) and Damiechki *et al.* (2001) used a panel of 32 and 18 untrained people, respectively. Samples were evaluated in triplicates and presented on small bread slices. The panellists were asked to smell, taste and then rank them in order of their degree of odour and flavour acceptability. The tasters used a six-point (from 0 to 5) acceptability scale with the following designations: no odour and flavour, very weak, weak, medium strong, strong and very strong. The overall grading for each sample was the mean of all the repeated scores. All panel scores were converted to ranked data prior to statistical analysis. The statistical analysis of these data can be performed through the analysis of variance (a Friedman two-way ANOVA model to analyse all data and the significance of the differences among samples can be evaluated according to a number of tests (Duncan's Multiple Range test, T-test and Tukey's Studentized Range (HSD) at a 0.05 p-level. Moldão-Martins *et al.* (2004) used both a trained and untrained panel, the first made of 12 panelists with experience of assessing olive oil, the second (suitable to simulate a consumer test) made of 46 subjects. The trained panel evaluated the samples under the COI regulation conditions (COI 1992). Samples were presented in blue glasses to allow the aroma and taste evaluation without the potential interference of light and the influence of the oil colour (a descriptor not usually applied in the assessment of the organoleptic characteristics of virgin olive oils) on the panellist judgement. Line scales (10 cm) anchored with extremely low and extremely high related to the perception of the stimuli were used to characterize the flavoured olive oils. The assessors' responses were converted to numerical values using a 0.1 cm accuracy for the analysis of the results. The panellists were briefed on the use of the sensory evaluation forms. The following descriptors were considered: aromatic plant aroma intensity, cooling, pungency, aromatic plant taste intensity and bitterness. The predicted equation for each sensory attribute was obtained using stepwise multiple regression analysis by fitting to a second-order polynomial equation based on predicted model equations,

surface plots were generated. For the consumer test, fresh bread was used as a carrier of the flavoured olive oil and the descriptors to be evaluated in a rank preference test (ISO 8587, 1988) were cooling, plant aroma intensity and pungency. The Friedman test was performed in order to check assessor recognition of the differences between samples. When the flavouring is used to stabilize an oil during repeated deep frying, the fried products have to be sensorially evaluated. Jaswir *et al.* (2000) studied the sensory attributes and the overall acceptability of potato chips fried in rosemary and sage flavoured palm olein. In this study, appearance, taste, odour, crispness, and overall acceptance of the potato chips were evaluated using a 7-point scale (1=dislike extremely, 4=moderate, 7=like extremely) by 20 panelists that regularly participated in sensory evaluation and are also regular consumers of potato chips.

Another important element to be evaluated is the oxidative stability of the flavoured oils since it strongly affects the product shelf life. In order to accelerate the oxidation process, oil is submitted to high temperature (up to 100°C) and oxygenation through a great airflow. Traditionally, a Rancimat apparatus is used (AOCS 1993) in order to evaluate the so-called “oil stability index” and the “protection factor” (ratio between sample induction time and control induction time) (Damiechki *et al.* 2001). The induction time can be determined by the methods of the tangents to the two parts of the kinetic curve representing the peroxide accumulation (Le Tutour and Guedon 1992). The oxidative stability is strictly related to the change in phenolic and pigment content induced by the flavouring of the oil. Flavoured oil can also be submitted to the evaluation of its photo-oxidation susceptibility by putting oil in a light chamber under fluorescent lamps (Damiechki *et al.* 2001).

Concerning the effect of the flavouring agents on the antioxidant activity of the oil, a variety of tests are available. They can be divided into two groups: a) assay of the radical scavenging ability and b) assay of the ability to inhibit the oxidation of a lipidic substrate (Schwarz *et al.* 2001). The elements involved in an oxidation reaction are a substrate, an oxidant, an initiator, intermediates and final products. Measurement of any of one of these can be used to assess antioxidant activity (Antolovich *et al.* 2002). An important limitation of these tests is that the reducing capacity does not necessarily reflect antioxidant activity (Wong *et al.* 2006, Katalinic *et al.* 2006). The radical scavenging tests measure either the reduction of stable radicals or radicals generated by radiolysis, photolysis, or other reactions. The DPPH (2,2-diphenyl-1-picrylhydrazil) test is considered an easy assay to evaluate the antioxidant scavenging activity since the radical is stable and doesn't have to be generated during analysis. Another common method for the evaluation of spice and herb antioxidant activity is that of the ABTS^{•+}

(2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)) but it needs to be generated by enzymatic or chemical reactions (Arnao 2000). Another important difference is that ABTS^{•+} can be dissolved in aqueous and organic media. On opposite, DPPH can only be dissolved in organic media, especially in ethanol and this represents an important limitation when interpreting the role of hydrophilic antioxidants. Both radicals show similar bi-phase kinetic reactions with many antioxidants (Wojdylo *et al.* 2007). The total antioxidant capacity can also be determined using the ferric reducing ability of plasma FRAP assay based on the reduction of a ferrioxal analogue, the Fe³⁺ complex of tripyridyltriazine Fe(TPTZ)³⁺ to the intensely blue-coloured Fe²⁺ complex Fe(TPTZ)²⁺ by the antioxidants dissolved in an acidic medium. Wojdylo *et al.* (2007) compared the antioxidant activity of 32 selected herbs from 21 botanical families by the ABTS, DPPH, and FRAP methods and expressed as total equivalent antioxidant capacity (TEAC). They found a significant linear relationship between TEAC and total phenolic content (measured by HPLC) only in family groups with many representative herbs within Labiatae and Compositae. The values correlation coefficients showed a dependence on the specific antioxidant test (higher for ABTS and FRAP; lower for DPPH). Among the radical scavenging ability tests used for the evaluation of the spice antioxidant activity, an important role is played by the measure of the hydrogen peroxide-scavenging (a common free radical found in biological substrates) activity using the peroxidase-based assay and the deoxyribose assay. For example, the first has been used for the evaluation of Mediterranean spices, garlic and oregano extracts (Martínez-Tomé *et al.* 2001, Ryu *et al.* 201, Jun *et al.* 2001). The second one found application for the evaluation of Mediterranean spices but also of onion and garlic extracts (Martínez-Tomé *et al.* 2001, Suh *et al.* 1999, Zang *et al.* 1994). Another suitable method available for the evaluation of antioxidant activity is the so-called ORAC (Oxygen-Radical Absorbance Capacity). This spectrofluorimetric method is extensively described in literature (Cao *et al.* 1993, 1995). In the presence of free radicals or oxidising species, the protein b-phycoerythrin loses more than 90% of its fluorescence within 30 min. The addition of antioxidant species, which react with the free radicals, inhibits the fluorescence of this protein. To generate peroxide radicals (ROO[•]), ABAP (2,2'-azobis-(2-amidinopropane) dihydrochloride) can be used. The scavenging of hypochlorous acid (HClO) is another method for the antioxidant activity assay. In this method, the sample is subjected to oxidation through HClO: the antioxidant substances contained in the sample react with the acid and can be quantified by measuring the excess of HClO with a spectrophotometer. Bonanni *et al.* (2007) determined the antioxidant capacity of 12 dry spices using ORAC, HClO, and the superoxide dismutase biosensor

method. The superoxide radical is determined using a biosensor obtained by coupling a transducer (an amperometric electrode for hydrogen peroxide) with the superoxide dismutase enzyme immobilised in a kappa-carrageenan gel. The superoxide radical is produced by the oxidation of xanthine in aqueous solution to uric acid in the presence of the xanthine oxidase enzyme free in solution. The disproportion reaction of the superoxide radical, catalysed by the superoxide dismutase immobilised on the electrode, produces oxygen and hydrogen peroxide. The hydrogen peroxide produced is oxidised at the anode, generating an amperometric signal that is proportional to the concentration of the superoxide radical present in solution. The addition of a sample possessing antioxidant properties produces a decrease in the signal. Superoxide anion radical scavenging activities has also been measured by a lactate dehydrogenase (LDH-NADH) oxidation system on crude extracts of oregano (marjoram) (Jun *et al.* 2001).

Among the tests that evaluate the ability to inhibit oxidation of lipid or other sensitive components, peroxide value, thiobarbituric acid value, iodine value, polymer content, and formation of conjugated dienes and trienes at 232 and 268 nm have to be considered (Antolovich *et al.* 2002) but the most important test concerns the ability of spice antioxidant extracts to inhibit oxidation of the β -carotene-linoleate emulsion (β -carotene bleaching assay) or the peroxidation of linoleic acid (FTC, Ferric thiocyanate assay). Sahin *et al.* (2004) applied the β -carotene bleaching assay in order to test the antioxidant activity of methanolic extract of *Origanum vulgare* ssp. *vulgare*. Shobana and Akhilender Naidu (2000) measured the antioxidant activity of some Indian spices on lipid peroxidation catalyzed by soybean lipoxygenase enzyme. Another way to measure the antioxidant activity is the ability of spices, herbs, and vegetables to inhibit the low density lipoprotein (LDL) in vitro oxidation. This method has been used by Sánchez-Moreno *et al.* (2000) to measure the antioxidant activity of dietary polyphenols.

Two instrumental methods for the evaluation of the flavoured oil oxidative stability are represented by differential scanning calorimetry (DSC) and the differential electronic paramagnetic resonance (EPR) spectroscopy. Gouveia *et al.* (2006) studied the oxidative stability of oil flavoured by *Capsicum frutescens* extracts obtained by supercritical fluid. They found that DSC represents a useful methodology, which demands smaller oil samples and shorter times in comparison with the methodology using the Rancimat apparatus. EPR spectroscopy has been used for free radical determination of basil, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage (Calucci 2003) and for scavenging of hydroxyl radicals in a mixture of 3 or 4 spices (Schwarz *et al.* 2001).

The quality of a flavoured oil can be evaluated in terms of its antioxidant

content and profile and its richness in compounds responsible of flavour. Wojdylo *et al.* (2007) analyzed the phenolics compounds of 32 selected herbs belonging to several families and determined the total phenolic content using the Folin-Ciocalteu colorimetric method. The identification and quantification of phenolic compounds were performed after enzymatic extraction on the methanolic phase using a HPLC system equipped with a photodiode array. The separation of compounds was carried out using a reversed-phase (RP) column and a mobile phase composed by two solvents (the first represented by an aqueous solution of formic acid 4.5%; the second made of 80% acetonitrile and 20% of the first solvent. Major phenolic acids identified in the analyzed species were caffeic, p-coumaric, ferulic and neochlorogenic, while the predominant flavonoids were quercetin, luteolin, apigenin, kaempferol and isorhamnetin. Demo *et al.* (1998) submitted several Mediterranean herbs and spices to the extraction by percolation of tocopherols and performed a thin layer chromatography to separate the unsaponifiables, a gas-chromatography couplet with mass spectrometry to identify tocopherols and a RP-HPLC-UV (methanol/water 96:4 v/v as a mobile phase) to quantify them. They found that the predominant tocopherol was the α -type. Tomaino *et al.* (2005) evaluated the protective effects of basil, cinnamon, clove, nutmeg, oregano and thyme essential oils on the tocopherol content of extra-virgin olive oils submitted to heating. The tocopherol fraction was extracted with hexane and analyzed by a HPLC equipped with a fluorescence detector using a mixture of hexane and 2-propanol as eluents. All the essential oils tested appeared able to prevent the α -tocopherol loss following oil heating at 180°C for 10 min but with a different efficiency. Spices herbs and fruits are rich in essential oils responsible of antimicrobial and antioxidant effects but also of the sensory properties of flavoured oils. Sahin *et al.* (2004) analyzed the chemical composition of a hydrodistilled essential oil of *Origanum vulgare* ssp. *vulgare* by a gas chromatography-mass spectrometry system. A total of 62 constituents were identified. Caryophyllene and spathulenol were found to be the main constituents, followed by germacrene-D and α -terpineol. Gherman *et al.* (2000) used gas chromatography and gas chromatography-mass spectrometry in order to study the volatile fraction of different types of *Menta piperita* L. The main volatile compounds identified were menthol, menthone, isomenthone, 1,8-cineole, menthyl acetate, limonene, β -myrcene, carvone. The main active principles of *M. piperita* L. oil are: menthol, menthone, isomenthone, menthyl acetate, α -pinene, β -pinene, champhor, limonene, linalool, piperitone. Asekun *et al.* (2007) applied GC-MS to the evaluation of the effects of drying methods on the quality and quantity of the essential oil of

Mentha longifolia L. subsp. *capensis*. They found that the monoterpenoids represented 92.6% of the total oil content in the oil from the fresh plant and that pulegone (35.0%), menthone (31.0%) and 1,8-cineole (13.0%) were the most abundant volatiles. In the air-dried oil, the monoterpenoid content increased to 93.3%, withenthone (47.6%), pulegone (18.4%) and 1,8-cineole (16.4%) as the most prominent components. The sun-dried oil was also dominated by monoterpenoids (91.8%) divided into menthone (38.3%), pulegone (20.2%) and 1,8-cineole (16.6%). The monoterpenoid content of the oven-dried oil was 63.5%, limonene (40.8%) and α -pinene (15.0%), in addition to a sesquiterpenoids content higher than in the other oils. By GC and GC-MS, Lee *et al.* (2005) identified and quantified the volatile components of basil and thyme. The major aroma constituents of basil were 3,7-dimethyl-1,6-octadien-3-ol (linalool), 1-methoxy-4-(2-propenyl) benzene (estragole), methyl cinnamate, 4-allyl-2-methoxy phenol (eugenol), and 1,8-cineole. The major aroma constituents of thyme were 2-isopropyl-5-methylphenol (thymol), 4-isopropyl-2-methylphenol (carvacrol), linalool, α -terpineol, and 1,8-cineole. Calvo-Gómez *et al.* (2004) applied the solid phase micro extraction (SPME) coupled with GC-MS to the analysis of garlic oil obtained by hydrodistillation. The most abundant volatile compound was diallyl disulfide, followed by diallyl trisulfide. Among the 47 totally identified compounds, 18 were linear sulfur-containing volatile compounds (accounted for 94% of the total amount), 6 were of non-sulfur nature, and the other 23 were cyclic compounds.

8. Market of the flavoured oils

The relatively low presence of pure olive oil or extra virgin olive oil in non traditional markets is primarily the result of a lack of knowledge about the product, directly derived from the weak international product marketing. In fact, the international olive oil marketing had traditionally responded more to supply criteria than to demand promotion and new markets capture policies (Mili 1999). Only 2% of total fat consumed in the world today correspond to olive oil (Oil World 2004). Within vegetable oils, olive oil accounts for about 3% of the world consumption (United States Department of Agriculture 2004) whereas the Countries of the Mediterranean area, which account for 95% of the world production, still concentrate 85% of world consumption (IOOC 2004).

Flavoured and infused oils account for only a few per cent of the olive oil market, but even this small number of sales can be extremely profitable for retailers. Furthermore, market studies have demonstrated that consumers are interested in this kind of product (Nouhad and Tsimidou 1998), in particular,

consumers originating from North Europe, America and Japan. These gourmet products are determining an increase of both the use of olive oil among non traditional consumers and the added value of olive oil (Antoun and Tsimidou 1997).

In Great Britain, although starting from a small base (£14 million in 2004) speciality oils, such as sprays, mild or light oils, non-olive/seed based and flavoured oils, having increased their market value by as much as 76% between 2000 and 2004 even if that the mild and light varieties are providing a major boost. According to the information made public by an important company of the field, in the UK, basil oil remains the most popular flavoured oil, making up around 60 percent of its total sales. Chili oil comes next, totalling 15 percent of sales, garlic oil stands at 10 percent and lemon oil at 5 percent. Other infusions, such as coriander, rosemary, thyme and jalapeno remain relatively niche products.

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