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Trends in Edible Vegetable Oils Analysis. Part A. Determination of Different Components of Edible Oils – a Review

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This review presents recent approaches applied to analysis of edible oils. In the last decade increasing attention has been paid to human diet concerning also edible oils and fats as a source of healthy energy. One of the major problems related to fats are oil oxidation reactions which decrease the nutritive value of edible oils. This paper describes methods developed to analyse different components of edible oils. The stress is put on methods used to assess oxidation stability of edible oils, their purity and approaches to determine their geographical origin.

INTRODUCTION

In recent years, increasing attention has been paid to oils consumed by humans. The most popular vegetable oils contain large quantities of unsaturated fatty acids, most of all those that are necessary for a human organism. However, because of the high amount of unsaturated compounds, these oils are susceptible to lipid changes and those of other components, such as sterols, tocopherols, *etc*., occurring during the process of raw-material collection, oil production, or during transport and storage. As a result of the above-mentioned processes, many unnecessary changes occur in food products that deteriorate their sensory properties (colour, taste, odour), and first of all diminish their nutritional properties because they contribute to the loss of the activity of biological precursors, vitamins A and E, as well as increased health risk posed by the emerging free radicals, which display carcinogenic properties.

Another problem related to the edible oil industry is identification of the geographical region of the origin of oils, their purity or their adulteration. In addition, consumers are paying increasing attention to product quality, including food quality. Social knowledge regarding healthy nutrition has an influence on the conscious choice of many food products. This is why the question becomes of control and food analysis, including vegetable oil analysis. The observed advances in analytical chemistry enable the development of new methods which, in turn, allow to reduce time of individual analyses, to decrease detection limits and improve repeatability. On the account of the above and based on a review of the recent literature, this work presents new directions in analytical methods related to analysis of vegetable oils.

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VEGETABLE OIL ANALYSIS

Compounds determined in vegetable oils include a group of substances which naturally appear in oils, which informs of their quality, origin or nutritional value (fatty acid content, phenolic compounds, tocopherols, sterols content), substances purposely added to oil with the aim of changing its properties (*e.g.*, synthetic antioxidants) as well as compounds emerging during unnecessary oil changes (*trans* isomers, saturated compounds, volatile products of the oxidation process, residues of solvents after the extraction process). The different classes of compounds analysed in vegetable oils are discussed below.

Because of the complex matrix of vegetable oils, different analytical techniques are used for their identification and quantitative determination; however, the most widespread technique used is high-pressure liquid chromatography (HPLC) and high-resolution gas chromatography (HRGC). These techniques allow for both the qualitative and quantitative identification of fatty acids, triacylglycerols, sterols, tocopherols and hydrocarbons. The application of capillary columns significantly improves resolution, sensitivity, and accuracy of determinations; qualitative and quantitative assays, and also shortens the time of analysis.

Fatty acid composition, degree of unsaturation and "*trans*" isomers

One of the main indicators of the quality and freshness of vegetable oils is their fatty acid composition. Because long

chain organic acids are not very volatile compounds, for analytical purposes they are converted into their more volatile derivatives – fatty acid esters. The esterification reaction is very often used in the analysis of fats. In gas chromatography, fatty acids are generally analysed in the form of methyl esters [Chiou *et al*., 1995]. They are usually obtained following procedures described in the Polish Standard [PN-EN ISO 5509:2001] by direct esterification of fatty acids using methanol in the presence of HCl, H_2SO_4 or BF_3, or by esterification with diazomethane. The mechanism of the esterification reaction catalyzed by acids is the reversal of the hydrolysis reaction of the ester bond [Drozdowski, 2002]. Before the esterification reaction, a fat sample should first be saponified in the reaction of fatty acids with NaOH in methanol (30 min, 60°C), and then the esterification reaction should be conducted in methanol with, *e.g*., boron trifluoride. The resultant methyl esters are then extracted into the hexane phase and dried in nitrogen atmosphere. Thus prepared sample is injected into a gas chromatograph with a flame ionization detector (GC/FID).

With the use of this method, not only the fatty acid composition of an analysed sample can be determined, but also degree of its hydrogenation. Hydrogenation of vegetable oils is one of the earliest and most widespread methods of lipids modification, used to change the melting point and solidification characteristics [Naglic & Smidovnik, 1997; Daniels *et al.*, 2006]. The process of partial hydrogenation is conducted mainly to achieve new organoleptic properties and better oxidative stability of the lipids obtained. However, as a result of the hydrogenation process, certain unfavorable products emerge in the form of fatty acid *trans* isomers. Using appropriate capillary column fillings, both emerging geometric and positional isomers can be determined [Naglic & Smidovnik, 1997]. Both *trans* isomers and conjugated linoleic acids (CLA) can be determined using gas chromatography coupled with infrared spectroscopy and with a chemometric analysis of the results obtained [Mossoba *et al.*, 2009]. Infrared profiles are obtained with a scanning within the range of 1000–850 cm^{-1}, as in this range CH bond vibrations appear, coming from fatty acid *trans* isomers as well as vibrations from deformed CH bonds in conjugated linoleic acids [Christy *et al*., 2003] (Figure 1).

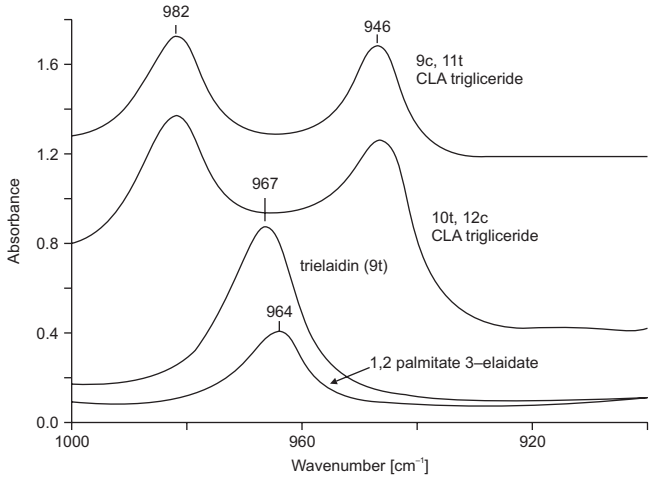

FIGURE 1. Infrared spectra of triglycerides of certain *trans* fatty acids in the region 1000 – 850 cm^{-1} showing their CH out-of-plane deformation absorptions. (Reproduced from Christy *et al*. [2003]).

Antioxidant content

Naturally appearing fats and oils, especially vegetable oils, contain natural substances, which protect fats from oxidation. These are non-volatile or only slightly volatile substances, however, they influence the changes in fats and the emergence of more volatile substances, through which they affect the sensory properties of the fats. Substances which slow down or stop the oxidation reaction are called antioxidants. This is a large group of chemical compounds which differ in their mechanisms of action. In this respect, they can be classified into two categories: primary and secondary antioxidants. Primary antioxidants stop the oxidation process by terminating the radical chain reaction by conversion of radicals into more stable compounds [Szukalska, 2003; Leclercq *et al.*, 2007]. A unique characteristic of antioxidants is that they are effective in very small concentrations (0.001–0.1%). Exceeding these optimal concentrations can diminish their activity or even enhance prooxidation. Among natural antioxidant substances appearing in vegetable oils, compounds from tocopherols [Nogala-Kałucka *et al.*, 2005], flavonoids as well as some vegetable sterols, among them brassicasterol appearing in rapeseeds [Rudzińska *et al.*, 2003], may be identified. The antioxidant activity can also be attributed to polyphenol substances appearing in rapeseed seeds as well as phenolic acids [Siger *et al.*, 2004, 2005], whose high content protects olive oil from oxidation.

Apart from primary antioxidants, secondary antioxidants are also distinguishable, which slow down lipid oxidation as a result of other process than the termination of the auto-oxidation chain reaction. The main mechanism of their actions depends on the ability to bind certain metal ions (chelating compounds, *e.g.*, EDTA), and oxygen, as well as on the absorption of UV rays, regeneration of primary antioxidants (*e.g.* ascorbic acid), creation of a protective border surface between oil and air (*e.g.* phospholipids), causing also a decomposition of peroxide to non-radical products or deactivation ("scavenging" or "quenching") of a singlet oxygen (*e.g.* β-carotene) [Szukalska, 2003].

Analytical techniques used to determine individual antioxidants naturally appearing in vegetable oils are presented below.

Synthetic antioxidants most often used in oils, because of their chemical stability, low cost and availability, include *t*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-p-hydroxytoluene (BHT) and *t*-butyl hydroquinone (TBHQ) [Leclercq *et al.*, 2007; Yang *et al.*, 2002]. Their activity is most often indicated by exposing oil with an addition of antioxidants to the process of accelerated oxidation. The induction period in such samples can be indicated with the use of differential scanning calorimetry (DSC) in an isothermal stream (heat flux), with a continuous oxygen stream [Giuffrida *et al.*, 2007]. However, the generation of free radicals can be observed with electron spin resonance spectroscopy (ESR) using N-*tert*-butyl-α-phenylnitrone dissolved in an oil sample as a spin trap [Velasco *et al.*, 2005]. Both methods are also

widely used in analysis of the activity and stability of antioxidants in high temperatures [Giuffrida *et al.*, 2007]. Oxidative stability of oils, after adding synthetic antioxidants, can also be determined with the use of thermogravimetric analysis (TGA). The insusceptibility of oil to oxidation was determined by measuring sample mass increase, a consequence of the absorption of molecular oxygen, temperature during the maximum sample mass as well as temperature at the initiation of the oxidation process. The technique enables achieving repeatable results, requires a small sample amount and is time-efficient [van Aardt *et al.*, 2004].

A more traditional technique based on gas chromatography was used by Yang *et al*. [2002] for direct, simultaneous determination of BHA, BHT and TBHQ in food samples containing fats. Because of the potential threat to health, the quantity of these substances in food should be monitored. The proposed method is simple, fast (one analysis lasts 18 min) and yields reliable results. Also, sample preparation is simple and therefore time- and work-efficient – the sample simply needs to be dissolved in diethyl ester and injected directly into the chromatograph. The separated analytes are detected with the aid of a flame ionization detector (FID) [Yang *et al.*, 2002]. For quantitative determinations of synthetic antioxidants with regard to additional 2,6-di-*t*-butyl-4--hydroxymethylphenol (Ionox 100) as well as ethoxyquin (EQ) in vegetable oils, a mass spectrometer was additionally used, and analytes were extracted from oil samples with ethanol. Just like before, the analysis lasted 18 min, and detection limits of individual antioxidants oscillated at about 1 μg/L [Guo *et al.*, 2006]. A few methods exist which use liquid chromatography to determine antioxidants, however, because of the difficulty of dissolving fats in conventional mobile phases, these techniques require laborious, multi-step and time-consuming sample preparation procedures for analysis. A possibility exists, however, of converting the oil samples into microemulsions , which can be separated and indicated with the help of micellar liquid chromatography (MLC). Phases used in the technique are non-toxic, not expensive, biodegradable and allow for simple solubilize of both hydrophilic and hydrophobic samples. Vegetable oils are dissolved in water/sodium dodecyl sulphate (SDS)/n-pentanol microemulsion and are dosed into the liquid chromatograph with a micellar mobile phase. This quick (15 minutes) and simple method allows for the direct injection of hydrophobic samples into a liquid chromatograph and allows for the determination of antioxidants with good repeatability and a detection limit below concentrations permissible both in Europe as well as in the USA [Noguera--Orti *et al.*, 1999].

Phenolic compounds content

The highest content of phenolic compounds is noted in olive oils. These compounds represent five different classes: simple phenols (hydroxytyrosol, tyrosol), secoiridoids (oleuropein, ligstroside, and their hydrolysis derivatives), lignans ((+)-pinoresinol, (+)-acetoxypinoresinol), flavonoids (luteolin, apigenin), and phenolic acids (*p*-cumaric, vanillic, *etc*.) [Carrasco-Pancorbo *et al.*, 2007]. The presence of those substances influence aroma and taste (bitter, tart) of fats, but also their oxidative stability, through the quenching of free radicals and the properties of chelative metals [Romero *et al.*, 2002]. Oxidative stability of olive oils is related, to a large extent, with the content of total phenolic components as well as with individual phenolic compounds. The composition of phenolic substances in oil differs from the composition found in olive fruits, based on the change which phenolic compounds undergo during the ripening of the fruit [Bonoli *et al.*, 2004], during the process of extraction, time and method of storage [Caruso *et al.*, 2000], and also with regard to the proceeding enzymatic reactions [Gomez-Alonso *et al.*, 2002]. The composition of a phenolic profile can also serve as a tool for differentiating and classifying types of olives. The composition of phenolic compounds in olive oil is determined with the use of high-pressure liquid chromatography with a UV-DAD detector or mass spectrometer [Carrasco-Pancorbo *et al.*, 2007; Małecka, 1995; Ocakoglu *et al.*, 2009; Fu *et al.*, 2009]. The content of total phenolics can be determined using the Folin-Ciocalteau reagent, whose proper amount is added to a water-methanol extract, and following a specified time period sample absorption is measured at 725 nm. The content of o-diphenol is similarity determined, combining a water-methanol phenolic extract with 5% sodium molybdate dihydrate solution in ethanol/water, and then measuring absorption at 370 nm. Another fast tool for routine analyses of phenolic content in olive oil is capillary zone electrophoresis (CZE). It offers the possibility of qualitative and semi-quantitative analysis. Likewise liquid chromatography, for identifying analysed compounds, a UV-DAD detector is used and similarly to HPLC, diminishing phenolic compounds content is found along with an increase in the level of fruit ripeness. However, in contrast to HPLC, the method allows for a routine analysis, conducted in a time that is 7.5 times shorter (10 min) [Bonoli *et al.*, 2004; Carrasco-Pancorbo *et al.*, 2007]. Another method was used by Caruso *et al.* [2000] to identify phenolic compounds and determine their influence on the oxidative stability of olive oil. The procedure is based on atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). Using a clean methanol extract of phenolic compounds from olive oil, their profile can be determined and the commercial quality of the analysed oil can be evaluated [Caruso *et al.*, 2000].

Tocopherols content

Vitamin E (tocopherol) is a natural component of vegetable oils and appears in them in quantities, which enable their protection from oxidation processes [Warner, 2005]. It is therefore a primary antioxidant. α-Tocopherol is used as an additive to food, simultaneously fulfilling the function of a vitamin E supplement as well as a natural antioxidant, whereas other isomers of vitamin E (β-, δ-, γ-tocopherols) solely fulfill the role of inhibitors to the oxidation process. Because of its antioxidant properties, the highest activity is attributed to δ-tocopherol, whose additional benefit is the fact that it exhibits pro-oxidative properties only at very high concentrations. This is a reason of frequent use of δ-tocopherol in food products [Leclercq *et al.*, 2007]. In considering the influence of tocopherols on the oxidative stability of oils, one should also take into consideration the interactions between tocopherols in the sample matrix. It has been stated that α-tocopherols, appearing in large quantities in sunflower oil, are the most

effective in quenching free radicals, and therefore are suitable for protecting oil from photo oxidation processes, whereas δ-, γ-tocopherols appearing in large quantities in soybean oil are better for protecting it from autooxidation [Warner, 2005]. In the case of olive oil, α-tocopherols display synergy with certain phenolic components present in such oil, thus actively protecting it from oxidation by quenching peroxide radicals [Deiana *et al.*, 2002]. Simultaneously, analysis of natural tocopherols appearing in oils can provide information regarding the purity or level and type of adulteration of oils, based on the fact that these oils differ significantly in their natural tocopherols profiles. They are determined with the use of high-pressure liquid chromatography (HPLC) with a fluorescent or UV detector [Warner, 2005; Romero *et al.*, 2007; Stevenson *et al.*, 2007; Gutierrez & Fernandez, 2002] or with the use of nanoliquid chromatography with UV-vis detection [Cerretani *et al.*, 2010]. This is a standard method recommended by the American Oils Chemists Society (AOCS). It is most often used to separate analytes in a normal phase mode, which guarantees complete separation of all phenolic compounds, and the analysed oil sample is dissolved in hexane with a small addition of 2-propanol. The benefit of using the reversed phase set-up is a faster balance of the system and better repeatability of retention time of individual analytes; but incomplete separation of β- and γ-tocopherols as well as the necessity of using during analysis temperatures higher than room temperature are its drawbacks. The oil sample is dissolved in such a case in a hexane mixture with a high quantity of methanol, ethanol or an acetonitrile-methano-propanol mixture [Gliszczyńska-Świgło & Sikorska, 2004].

Sterol content

Phytosterols (campesterol, stigmasterol, β-sitosterol) appear in many vegetable products. In the case of oil substrates, the higher phytosterol content is in corn seeds as well as rape and cotton grains. In recent years, an increase in phytosterol interest has been observed, because of its application in counteracting heart disease as well as hypercholesterolemia by decreasing the cholesterol level in blood. They are also biologically active in the prevention of cancer-related illnesses [Rudzińska *et al.*, 2003; Lopez-Lopez *et al.*, 2008]. The antipolymericic activity of these compounds during the frying process has also been confirmed. Unfortunately, these compounds, similar to cholesterol and other fat-components, are subject to the oxidation process. With regards to the similar chemical structure of oxidated phytosterlos, they are claimed to display some adverse activities, likewise cholesterol derivatives (mutagenes, carcinogens, cytotoxins, atherogenes) [Rudzińska *et al.*, 2003]. Increased phytosterol consumption requires strict control of these compounds and their oxidated derivatives in food products. Sterol content is determined using gas chromatography coupled with mass spectrometry (GC-MS) or a flame ionization detector (GC-FID). Ethyl extracts from the fraction of unsaponified lipids are analysed directly, whereas in the case of oxidized phytosterols, it is necessary to conduct their transesterification, extraction with chloroform, and then cleaning using solid phase extraction (SPE) [Rudzińska *et al.*, 2003]. In order to purify the sterol fraction, ethyl extracts from the unsaponified fraction can be separated with the help of thin-layer chromatography (TLC) [Lopez-Lopez *et al.*, 2008].

Analysis of the sterol fraction can also be conducted with the aim of discovering adulterated olive oils, oil from pine nuts, or for classifying oils based on the origin of the fruit and oil quality [Lopez-Lopez *et al.*, 2008]. On-line coupling of high pressure liquid chromatography and liquid chromatography-gas chromatography can also be used, which enables the removal of most of the undesirable sample components during the LC step, because of which, it can be better separated in the GC [Senorans *et al.*, 1996; Villen *et al.*, 1998]. Different transfer techniques have also been applied, as well as coupling techniques for both chromatograph interfaces, yet most LC-GC applications require a normal phase set-up (NP-LC). Analysis in a reversed phase set-up, which widens the field of application of this technique and provides the possibility of simultaneous analysis of compounds from different groups, is possible after applying an evaporizer located between the two chromatographs with a programmed temperature. Before analysis, the oil samples are dissolved in dichloromethane [Senorans *et al*., 1996]. This technique has been also applied for determining squalene. This compound appears in olive oil in significantly larger amounts than in other vegetable oils and therefore, its content can be used for determination of oils adulteration [Villen *et al.*, 1998].

Coupling both chromatographic techniques and applying them for direct vegetable oil analysis enables the elimination of the traditional sample preparation steps (multi-step solvent extraction and saponification), allows reducing the use of organic solvents lowering the detection limit with regards to a larger sample volume undergoing analysis. A LC-GC coupling enabled a simultaneous increase in selectivity and method output, which is required in the analysis of complex samples [Senorans *et al*., 1996; Villen *et al.*, 1998].

Glucosinolate content

Thioglycosides – secondary plant metabolites are present in rapeseed oil, broccoli, cauliflower or cabbage. Detrimental effects can only by exerted by products from decayed aliphatic glucosinolate with goitrogen activities, causing damage to the liver and kidneys. The content of these compounds in rapeseed seeds should not exceed 25 μmol/g of the dry protein mass [PN-90/R-66151]. However, useful anticarcinogenic activities of indole glucosinolates have been documented as well [Rotkiewicz *et al.*, 2000; Stoewsand, 1995; Troczyńska 2005].

Unstable thioglycosides undergo hydrolysis under the influence of thioglycosidases (myrosinase), which splits off glucose, simultaneously releasing aglycone, which undergoes further decomposition. As a result of the degradation of glucosinolates, thioamide, nitriles, episulfide, isothiocyanates, thiocyanates, vinyl oxazolidinethiones, oxazolidinethiones emerge [Drozdowski, 1988].

The amount of glucosinolates and their degradation products to a large extent depends on the type of plant, climatic and agrotechnical conditions, for example, sulphur content in soil. The most often occurring hydrolysis products are isothiocyanate-3-butenyl, 5-vinyl-oxazolidine-2-thiones, isothiocyanate-4-penthyl and isothiocyanate-2-phenylethyl. Both isothiocyanates (ITC) and vinyl oxazolidinethiones (WOT)

are solubilized in fats, thus affecting deterioration of the nutraceutical value of oil, its taste and aroma. They are also strong catalytic poisons during the process of fat hydrogenation (they influence the reaction kinetic and also its mechanism – selectivity and isomerization) [Drozdowski, 1988].

The content of individual glucosinolates in oil samples is determined on the basis of the reference method described in the Polish Standard [PN-93/R-66166]. The sample is prepared for analysis with desulfatation, and then undergoes separation with the use of high-pressure liquid chromatography with a UV-DAD detector at a wavelength of λ=229 nm. Another method used to assay glucosinolates content is near infrared spectrometry (NIR), based on the ability to absorb infrared rays through individual sample components. The method indicates a high cofactor correlation with the HPLC technique and can be widely used in glucosinolates content determination in samples [Koprna *et al.*, 2002].

Determination of pollutants after oil extraction and refinement

Many pollutants appear in oils during the oil extraction process, for example during extraction with organic solvents. Due to that trace amounts of solvent, *e.g.* hexane, can be found in oils. Using headspace analysis, as well as a static technique and solid-phase microextraction the trace of selected solvents can be detected. The second technique, thanks to analytes enrichment on the fiber, enables the detection of volatile solvents at very low levels [Michulec & Wardencki, 2004; Michulec & Wardencki, 2005]. Chloro-derived organic solvents usually emerge from incomplete tank and cistern cleaning during a multi-step cleaning process. Fat is better dissolved with the aforementioned compounds, therefore a very important step in tank preparation for oil is careful rinsing of the tank with water. Because of their volatility, these compounds can be determined both quantitatively and qualitatively, coupling solid-phase microextraction with gas chromatography and an electron capture detector (SPME/GC/ECD) [Michulec & Wardencki, 2004].

Another significant source of oil pollution is its packaging, based mainly on synthetic products [Cecchi *et al*., 2010]. During oil storage, a portion of the volatile compounds can be diffused from the surface of the packaging into the oil. This is why raw, unrefined oils are stored especially in glass bottles made from dark glass, simultaneously protecting them from ultraviolet radiation, thus restricting the oxidation process.

Checking the authenticity as well as level and type of adulteration

Application of high-resolution gas chromatography enables detection of adulterations in vegetable oils with significantly better sensitivity than traditional techniques. This problem is especially important in the case of more expensive and higher quality oils (olive oils, pumpkin seed oils), in the case of which even the slightest addition of inexpensive and ordinary oils significantly increases incomes. Therefore the authenticity and oil purity have to be controlled [Wenzl *et al.*, 2002].

One method used for discovering adulterations is determining the composition of fatty acids on the basis of chromatographic analysis of their methyl esters [Casas *et al.*, 2009]. However, such an approach is connected with the risk of false interpretation because of the similarity of fatty acids in certain vegetable oils. In addition, many factors influence the fatty acid profiles, such as climate, region of plant preparation and growth environment, which additionally renders the evaluation of oil authenticity more difficult [Wenzl *et al.*, 2002; Camin *et al.*, 2010].

The content of linoleic acid is a good indicator of purity in the case of adulterated sunflower oil with less expensive soybean or rapeseed oils. Similarly the content of *trans* isomers of oleic, linoleic and linolenic acids in oil above the maximum permissible level for olive oil can be an indicator of adulteration by hydrogenated oil with seeds, esterified olive or inappropriately treated oil during the extraction process [Aparicio & Aparicio-Ruýz, 2000]. The evidence of the rapeseed oil addition can be the presence of erucic acid, characteristic of rapeseed, in the acid profile of the analysed oil. Webster, Simpson, Shanks and Moffat [2000] demonstrated the authenticity of olive oil on the basis of hydrocarbons presence and their concentrations. The addition of rapeseed and sunflower oils can be detected by the indication of n-nonacosane and n-hentriacontane [Webster *et al.*, 2000].

The most difficult is determination of additives of hazelnut oil because of their high chemical similarity to olive oil. The best analytical tool for fingerprinting complex samples, such as olive oil, appears to be tandem mass spectrometry, thanks to which the chromatographic separation step is eliminated, which significantly shortens analysis and sample preparation time [Gomez-Ariza *et al.*, 2006] (Figure 2). Direct infusion electrospray ionization mass spectrometry (ESI-MS) as a fingerprinting analysis was applied by Catharino *et al.* [2005] for evaluating the authenticity of olive oil, as well as soybean, corn, rapeseed, sunflower and cotton oils. It also allows to determine the origin and geographical region of the oil plants. This fast and selective technique enables classification, quality control, freshness evaluation and discovery of adulterations in vegetable oil samples. In addition, the sample preparation step is minimized, just to the production of water-methanol extracts, allowing for the simultaneous detection of fatty acids and polar phenolic components, two of the most important compound groups in vegetable oils [Catharino *et al.*, 2005] (Figure 3).

Similarly, sterol content can be used in purity determination and adulteration of oils [Lerma-Garcia *et al.*, 2009]. Only 5% addition of rapeseed oil into sunflower oil allows for the discovery of brassicasterol in adulterated oil, a characteristic rapeseed component. It is also an indicator in situations when there is suspicion that rapeseed oil was adulterated by soybean oil [Aparicio & Aparicio-Ruýz, 2000]. The purity of pumpkin oil can be determined on the basis of phytosterol content (stigmasterol, campesterol, β-sitosterol), whose content, in contrary to other vegetable oils, is not high in pumpkin oil [Wenzl *et al.*, 2002]. Pumpkin seed oil is most often adulterated by the addition of sunflower or rapeseed oils, which are practically undetectable by the consumer because of their dark green colour and intense odour originating from pyrazine, which is typical of pumpkin oil [Wenzl *et al.*, 2002].

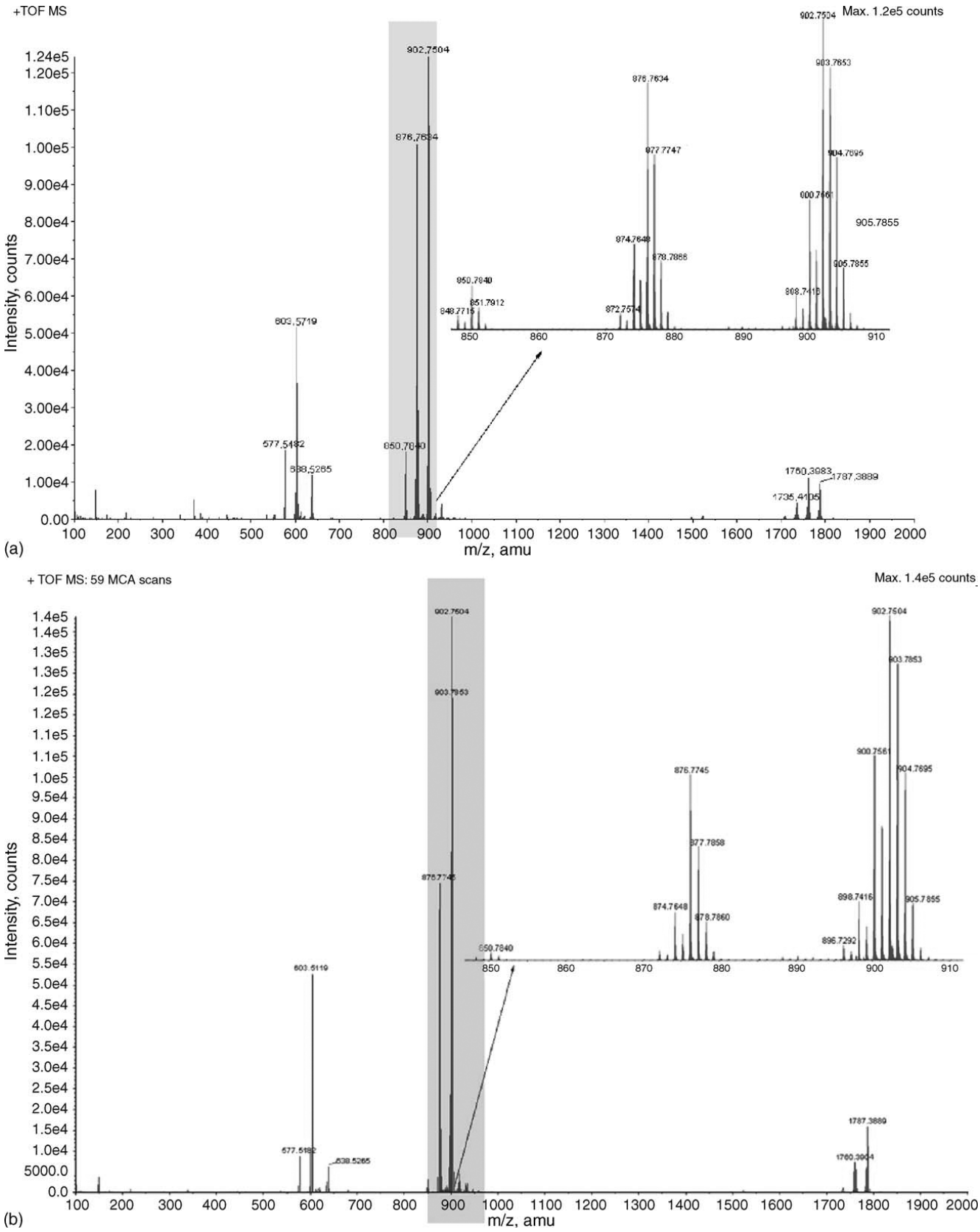

FIGURE 2. Full-scan ESI-MS: (a) olive oil and (b) hazelnut oil. The region between m/z 800 and 900 is magnified (Reproduced from Gomez-Ariza *et al*. [2006]).

Another approach in evaluating authenticity or adulteration of vegetable oils is proposed by Zhang, Ni, Churchill and Kokot [2006], and is based on the analysis of physiochemical properties, such as: acid value, peroxide value, saponification value, as well as colour, density, refractive index, humidity and volatility. Next, a transformation of the obtained data is con-

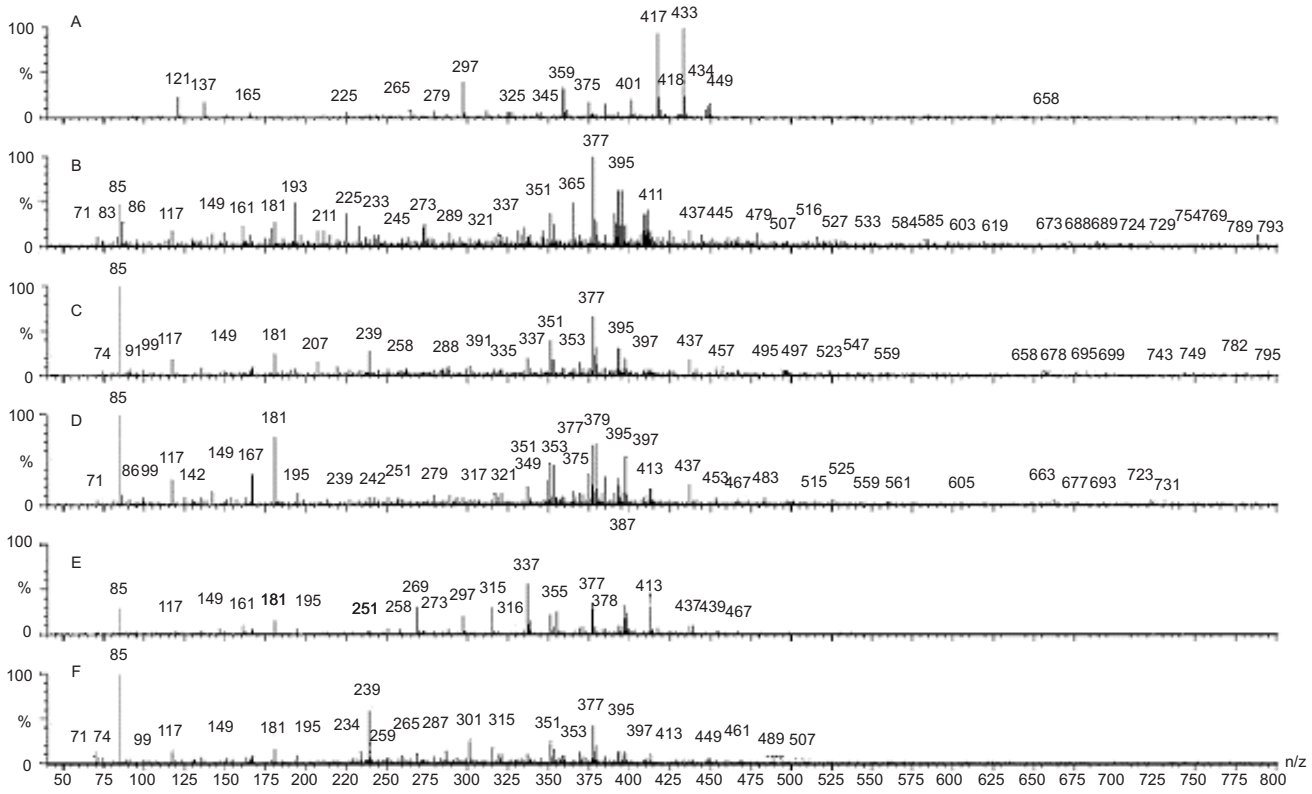

FIGURE 3. ESI-MS fingerprints in the positive ion mode of methanol/water extracts of the following: (A) olive, (B) soybean, (C) corn, (D) canola, (E) sunflower, and (F) cottonseed oil. (Reproduced from Catharino *et al*. [2005]).

ducted with the help of chemometric techniques, such as principal component analysis (PCA), partial least squares (PLS) or radial basis function – artificial neural network (RBF-ANN). These techniques enable not only discovering the adulteration of oils, but also additionally evaluating their freshness and quality [Zhang *et al.*, 2006]. Chemometric techniques have also been used to evaluate the authenticity of olive oil on the basis of lipid fraction analysis, with the aid of gas chromatography (peak surface area of individual analytes) with a mass detector. The proposed chemometric model enabled elaborating a three-step olive oil identification method which allows: to evaluate whether the sample was adulterated, to identify which oil it was adulterated with (sunflower, corn, peanut or coconut), and to determine the degree of adulteration (how much external oil was added) [Capote *et al.*, 2007].

Determining the stability of oxidated oils

Vegetable oils contain many unsaturated compounds (unsaturated fatty acids, sterols, squalene, *etc*.), which undergo oxidation under the influence of different factors, such as increased temperature, increased humidity, UV-radiation, microorganism activity, or natural enzymes. The oxidation process is hindered by substances naturally appearing in plant tissues, named antioxidants (tocopherols, phenolic compounds, carotenoids), however, during the fruit and seed collection stage, their storage, processing, and then storage of the final product (oil), activities of natural antioxidants are decreased.

Freshness and oxidative stability of fats are often determined on the basis of the induction period, meaning the period in which peroxide creation is untraceable or very small, until the point of its sudden increase in the volume of the analysed sample. Standard methods for indicating oxidation processes are relevant for only one class of compounds from a complicated mixture, generated during oxidation [Bester *et al.*, 2008]. Due to that such methods provide limited information regarding the oxidation process, yet they are normalized methods and often used for reference [Kardash-Strochkova *et al.*, 2001]. The peroxide value (PV), most often used for indicating the oxidation level, allows for the evaluation of the content of primary oxidation products in an analysed sample. Together with the determination of free fatty acids (FFA), the level of fat rancidity can be evaluated. These methods, based on titrimetric techniques, are strictly empirical, and their specificity depends on various factors, such as: strictly controlled reaction time, the intensity of the agitation, protection of the reaction components from light or atmospheric oxygen [Nouros *et al.*, 1999]. Another disadvantage of such methods is the time of analysis (only 6–8 samples can be determined within one hour), the necessity of maintaining strict time regimes during individual stages of analysis, as well as a large amount of organic solvents used (acetic acid and chloroform – not permitted in many countries).

In a further stage of analysis, first-rank products of the oxidation process (peroxides and hydro- peroxides) are transformed into volatile compounds (secondary oxidation products) determined with the help of the p-anisidine value (AV) [Lee *et al.*, 2007a; Coppin & Pike, 2001]. At this stage of the reaction, a simultaneous decrease in the peroxide value is observed [Smith *et al.*, 2007]. The emerging volatile products (short-chain hydrocarbons, aldehydes, ketones, esters, lac-

tones, alcohols and ethers) impart food characteristic aroma and rancid taste. Yet more important than deterioration of the sensory properties is a decrease in the nutritive value of such products [Smith *et al.*, 2007]. The content of essential unsaturated fatty acids (UFA) as well as these of carotenes and vitamin A are decreased. In the result of tocopherols oxidation, the biological activity of vitamin E also decreases. The products of further oxidation are, among others, toxic radicals which are responsible for a few pathological processes, such as: tumour changes, sclerosis, the degradation of biologically-active proteins, mutagenic activities on nucleophilic acids, negative cell changes related to the ageing process [Pellegrini *et al.*, 2001].

Currently in industry as well as in quality control laboratories, volatile secondary oxidation products are determined based on the Rancimat method [Płatek, 1995]. The procedure is automated and allows for the simultaneous evaluation of oxidative stability of a few samples. The analysis is based on the conductometric determination of products formed during the oxidation process of fats. Oil samples are oxidized at a higher temperature in air stream. Volatile products are then placed on a measurement dish containing demineralized water, equipped with electrodes for measuring conductivity. By the end of the induction period, there comes a moment when conductivity of water sharply increases with time, the result of the disassociation of volatile carboxylic acids [Hęś *et al.*, 2001].

Volatile oxidation products can also be determined using headspace phase analysis coupled with gas chromatography [Jeleń *et al.*, 2000; Gromadzka, *et al.*, 2008; Keszler & Heberger, 1998; Keszler *et al.*, 1998, 2000; Mildner-Szkudlarz *et al.*, 2003; Lee *et al.*, 2007b]. Volatile secondary products which emerge during the oxidation process are collected in the gas phase on the sample surface, from which they are collected and introduced into the chromatographic injector to be identified and quantitatively determined with the help of a mass spectrometer or a flame ionization detector. One advantage of such a method is the possibility of simultaneous determination of composition of the volatile phase as well as the content of individual compounds [Miraliakbari & Shahidi, 2008; Smith *et al.*, 2007]. Commonly appearing secondary products of oil oxidation process are aldehydes. Miraliakbari & Shahidi [2008], analysing tree nut oils which previously underwent accelerated oxidation through the application of temperature and light, determined that the compounds which were most often discovered were hexanal and nonanal [Miraliakbari & Shahidi, 2008]. Hexanal is a product of the oxidation of linoleic acid, while nonanal – of oleic acid. These compounds were also found in oxidized olive oil [Jimenez *et al.*, 2004; Vichi *et al.*, 2003; Cavalli *et al.*, 2004] and also when rapeseed oil is warmed [Jeleń *et al.*, 2007, 2000] and radiated with UV light [Gromadzka *et al.*, 2008]. Jimenez *et al.* [2004] proposed the application of one of these aldehydes, or their relative amount in a sample, as an indicator of the oxidation process of fats. Methods for analysing the volatile fraction of oxidation products are fast and suitable for the routine analysis of different samples. They additionally do not require the application of organic solvents or the work-consuming sample preparation stage prior to analysis [Richards *et al.*, 2005].

Another alternative for determining the stability of oxidized oils is the flow injection analysis technique (FIA), which assures a repeatable time for each stage of the analysis and a closed environment, allowing for the elimination of light and oxygen (factors influencing the oxidation process) [Thomaidis & Georgiou, 2000; Nouros *et al.*, 1999]. In this determination, the quantity of oxygen absorbed by the sample is monitored at a given temperature. The logarithm of the quantity of oxygen used during the oxidation process is reversibly proportionate to absolute temperature, because of which extrapolation of temperature-dependent data is possible in order to determine the stability of an analysed sample in different temperatures [Tian & Dasgupta, 1999]. For analysis of slow-moving reactions, such as the oxidation reaction, multiple incubation channels are used, where simultaneous incubation of 2 to 10 samples occurs. This technique was named parallel flow injection (PA-FI) and was used to determine the peroxide value (PV) and iodine value (IV) in olive oil. Detection takes place in an analytical-evaluative system of a spectrophotometer or a triiodide flow through a detector which measures the quantity of triiodide released from the reaction vessel [Saad *et al.*, 2006].

The proposed technique allows for the determination of the PV or IV values in 60 samples in one hour with 10 minute of incubation and is therefore ten-times faster than the classical peroxide or iodine value determination methods. In addition, the method is precise (at a level of 0.1–1.7% in comparison to the official AOAC method). It is also environment friendly since it decreases the use of organic solvents and eliminates the use of chlorine-derived ones [Thomaidis & Georgiou, 1999; Thomaidis *et al.*, 2000].

CONCLUSIONS

On the basis of the analysed literature, it can be concluded that there is still a need for determining different edible oils components which may influence on oil oxidative stability. Some of them may be good indicators of oil pollution or adulteration. It can also be remarked that beside standard methods new instrumental techniques become more popular in edible oils analysis.

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