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Purification and Identification of Endogenous and Exogenous Minor Constituents from Vegetable Oils

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Minor constituents are ubiquitous in plant origin sources, particularly plant derived oils. According to the origins of the minor constituents, factors that are responsible for their occurrence can be categorized into endogenous and exogenous factors. To date, extensive researches have been carried out to separate, purify, identify and analyze them to further asses their value as well as advise their thorough utilization or decontamination. These issues emphasizing on hydrocarbons, aldehydes, ketones, pesticides, herbicides, and trace metal compounds as well as their isolation methodologies via physical and chemical means will be covered in this review.

KEYWORDS Hydrocarbons, aldehydes and ketones, pesticides and herbicides, trace metal compounds

INTRODUCTION

The term "minor constituents (also known as other compounds)" in this study is addressed to the ones found in plant origin oils other than neutral lipids (viz. free fatty acids, *trans*-fatty acids, monoacylglycerides, diacylglycerides, and triacylglycerides) and bioactive compounds (such as tocopherols, sterols, squalene, flavonoid, and phenolic compounds). According to their origin, they can be classified into endogenous and exogenous minor constituents. The endogenous minor constituents principally act as fingerprint

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of the plants, while the exogenous ones commonly act as impurities, even unanimously regarded as the pollutants. The plant origin oils that will be discussed here include edible and non-edible as well as crude, refined and vegetable oil deodorizer distillate. In the past decades, numerous works have been done on vegetable oils such as corn, cotton, olive, palm, pumpkin seed, rapeseed, rice bran, soybean, and sunflower oils.

Sample fractionation selectively removes the undesired matrices that may interfere with analysis, providing a better picture of the composition of target substance. However, Lercker and Rodriguez-Estrada (1) stated that a serial fractionation often does not grant a complete description of the real composition. On the other hand, artifact formation, as the result of sample manipulation, can be diminished by taking into account several analytical precautions, as well as by introducing some corrections to limit the corresponding problems.

Via subjection to chemical-modification such as saponification and physical modification such as column chromatography, minor constituents can be fractionated directly into one group or several groups and ease their identification. Frega and Lercker (2) saponified lipids obtained from the exocarp and endocarp–mesocarp of olive oil drupes at different ripening stages. They isolated and fractionated unsaponifiable matters by using thin layer chromatography (TLC) preparative method. The purified fractions were analyzed as trimethylsilyl (TMS) derivatives, except for the hydrocarbons (HCs), in a non polar capillary GC column. Unlike saponification, column liquid chromatography (HPLC) offers less harmful feature for heat-labile and volatile compounds and better selectivity albeit it requires longer time and solvent consumption. Recently, HPLC offered numerous benefits such as providing various enhanced stationary phases, time-effective, improved selectivity and detection. It can be employed to do numerous routine assays, all of which are superior to the classical low pressure column chromatography.

The highlighted minor constituents are as follows: HCs, aldehydes, ketones, pesticides, herbicides, and trace metal substances—all of which are ubiquitous in plant origin oils. This review article summarizes the current state of isolation and purification techniques of minor constituents as well as their identification/characterization to promote their more proper and environmentally friendly utilization from miscellaneous sources of vegetable oils, thus meeting the "green chemistry" challenge.

ENDOGENOUS MINOR CONSTITUENTS

These constituents may play a role as a distinctive characteristic of their plant origin or as evidence of a chemical change due to storage and processing. In vegetable oil, these compounds can give contribution to detect adulteration

and can also give evidence of what kind of process to which the vegetable oil has been subjected.

Hydrocarbon (HC) Compounds

The amount of ubiquitous HCs in natural lipid systems is quite small, mostly only 0.2% of total lipids amount. One exception is virgin olive oil, where HCs content is approximately 0.5% and is predominantly composed of squalene (1). HCs present in vegetable oils can be generally divided into four groups: aliphatic HCs (alkanes, alkenes, and alkynes), steroidal HCs, terpene HCs (monoterpene, triterpene (squalene), and sesquiterpene HCs), and aromatic HCs (volatile, polycyclic and chlorinated ones). HC fractions are commonly isolated by saponification and further subjected to chromatographic fractionation and analysis.

Bastić et al. (3) succeeded in isolating hydrocarbons and other weakly polar unsaponifiables from various vegetable oils (crude rapeseed oil, crude olive oil, crude pumpkin seed oil, crude sunflower oil, and soybean oil). The oils were saponified and subjected to modified TLC fractionation. The fractions obtained from these oils were composed of *n*-paraffins, isoprenoidal polyolefins, squalene, diterpene, and triterpene HCs and compounds with oxygen. Table 1 shows the contents and composition of HCs and other weakly polar constituents in several common vegetable oils. The identification of these compounds was performed by using GC–MS. According to Bastić et al. (3), the difference in the HC fractions of these oils suggested they may be used for characterization.

Aliphatic HCs

Aliphatic HCs are composed of alkane, alkene, and alkyne groups, which exist in the form of either linear, branch, or cyclic chain. Most HCs in vegetable oils consist of aliphatic HCs (*n*-alkanes) with 12 to 35 carbon atoms (4). It is worth noting that *n*-alkanes are endogenous to a plant because of the possibility of *n*-alkanes formation as a result of decarboxylation of long-chain fatty acids (5, 6). Note that some *n*-alkanes may be a potential irritant toward humans. Based on the material safety data sheet (MSDS), *n*-alkanes containing less than 16 C atoms are potential irritants; but for those containing 16 or more C atoms, they are not hazardous, according to Directive 67/548/EEC as their structure is more stable, more nonpolar, and less reactive.

Isolation Methods of Aliphatic HCs

Isolation of aliphatic HCs can be carried out via classical silica gel column chromatography with *n*-hexane as the eluting solvent. McGill et al.

TABLE 1 Distribution of HCs and Other Weakly Polar Constituents in Several Common Vegetable Oils[a]

Oil Matrices	Unsap. content in w% of oil	HCs content in w% of unsap.	Composition of HCs and other weakly polar unsap.
1. Olive	1.54	65	*n*-paraffins with 14–27, 29–32 C atoms, isoprenoidal polyolefins with 18, 19, 21, 23 & 26 C atoms, squalene, hexahydrofarnesylacetone, allofarnesene, α-farnesene, 6,10-dimethyl-1-undecene[b]
2. Pumpkin seed	1.06	50	*n*-paraffins with 16–26, 28–33 C atoms, isoprenoidal polyolefins with 18, 19, & 23 C atoms, squalene, hexahydrofarnesylacetone, kaurene
3. Rapeseed	0.98	19	*n*-paraffins with 12–29, 32 C atoms, lactone, 4,8,12,16-tetramethylheptadecan-2-one, 2,6,10,14,18,22-hexamethyltrocosan-12-one, Δ3,5-sitostadiene-7-one, 24-methyl-Δ3,5,22-cholestatriene-7-one, 24-methyl-Δ3,5-cholestadiene-7-one
4. Soybean	1.38	28	*n*-paraffins with 14–36 C atoms, squalene, hexahydrofarnesylacetone
5. Sunflower seed	0.79	13	*n*-paraffins with 15–33 C atoms, kaurene, squalene, lactone, cyclic diterpeneketone, hexahydrofarnesylacetone, 7,11,15-trimethylhexadecan-2-one, 4,8,12,16-tetramethylhepta-decan-2-one
6. Sesame seed[c]	1.1–1.3	41.8–46.2	*n*-paraffins with 12–28,30,32 C atoms, squalene[b]

[a]Bastić et al. (3).
[b]Lanzón et al. (32).
[c]Mohamed and Awatif (109).

(7) determined the composition and concentration of *n*-alkanes in various edible oils (olive and extra virgin olive, sunflower, sesame, corn, walnut, peanut/groundnut, hazelnut, sweet almond, pistachio, mustard seed, safflower, grape seed, soya, and cod liver oils) via simple column chromatography, followed by a HPLC step to remove aromatics. Various concentrations of *n*–alkanes ranged from 15 to 33 C atoms in these common oils were detected and reported, such as those in olive and extra virgin olive oils (28–99 mg/kg), sunflower oil (105–166 mg/kg), sesame oil (22–82 mg/kg), corn oil (26–33 mg/kg), walnut oil (7–30 mg/kg), peanut oil (27–40 mg/kg), hazelnut oil (14 mg/kg), sweet almond oil (44 mg/kg), pistachio oil (21 mg/kg), mustard seed oil (74 mg/kg), safflower oil (61 mg/kg), grape seed oil (52 mg/kg), soybean oil (17 mg/kg), and cold liver oil (16 mg/kg).

Bastić et al. (3) identified the composition of *n*-alkanes isolated from soybean oil by using saponification and found that the carbon number of

n-alkanes were distributed from 14 to 36 (see Table 1). Gunawan et al. (8) reported that during the isolation of squalene from soybean oil deodorizer distillate (SODD), *ca.* 40–50% of nonpolar lipid fraction (NPLF) consisted of HCs. Their preliminary results suggested that the HCs contained in NPLF could be categorized into four main groups, which are aliphatic, steroidal, sesquiterpene, and triterpene (squalene) HCs (see Figure 1). These aliphatic HCs, which were isolated from SODD via modified soxhlet extraction followed by modified silica gel column chromatography, were later identified as a combination of *n*-alkanes and irresolvable complex mixture (unpublished work).

Analysis of Aliphatic HCs

A precise quantitation of *n*-alkanes is already well-established and can be simply done via GC/FID with internal or external calibrations of the HCs standard. The response of versatile FID is mostly proportional to the carbon number of the analyte. Unfortunately, it will vary considerably if nonlinear

FIGURE 1 TLC fractionation of HC groups obtained from non polar lipid fraction (NPLF) and polar lipid fraction (PLF) of SODD. Spot identification: (A) aliphatic HCs; (B) steroidal HCs; (C) unknowns; (D) squalene; and (E) fatty acid steryl esters. The chromatogram was developed with pure *n*-hexane as the mobile phase, sprayed with a ferric chloride specific reagent prepared according to Fried (108). (Chromatogram is courtesy of Gunawan et al. (8)).

HCs are present and FID operating condition is changed. Watanabe et al. (9) succeeded in developing more precise quantitation using post-column reaction GC/FID system. In this system, two micro-reactors were attached in series to a capillary GC/FID system: the first one oxidized the analytes effluent to carbon dioxide; the second one reduced the carbon dioxide to methane. Relative error of the analyte response was less than 1% and only one standard substance was required in this system.

Another quantitative determination of aliphatic HCs that worth mentioning is the one using heteronuclear 2D-NMR instead of 1D ^{1}H- and ^{13}C-NMR analyses, which are often interfered by the overlapped signals in the analyte spectra (10). Compared to the less-likely-to-do-quantitation of 1D-NMR, the 2D-NMR results are more feasible to apply in the quantitation. However, its precision is somewhat poor, which is ascribed to low signal-to-noise ratio and the existed variation of J coupling constants of the analytes.

Steroidal HCs

Steroidal hydrocarbons are formed in vegetable oils as the dehydration or hydrolysis products of · 5-phytosterols due to bleaching with acidic earth and deodorization at high temperature during the refining process. Steradienes and steratrienes are steroidal HCs that have two double bonds and three double bonds in their steroid ring system, respectively (see Figure 2), in which their parental sterols have the same pattern as well (the same position where the parental sterols loss their OH functional group, as depicted in Figure 3).

Generally, there is only single OH functional group attached to the sterol ring system which later will be hydrolyzed to form the steradienes (see Figure 3a). Unlike steradienes, steratrienes are derived from the hydroxyl derivative of sterol, not from sterol (see Figure 3b). After subject to hydrolysis, both steradienes and steratrienes undergo intramolecular isomerization to form more stable isomers, which possess a conjugated system in sterol rings (11). Therefore, the occurrence of 3,5-isomer is more preferable and more abundant in vegetable oils.

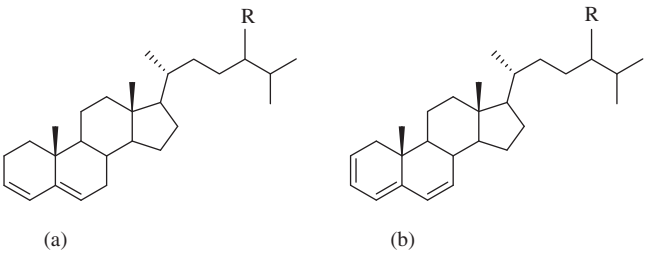


FIGURE 2 Molecular structure of steroidal HCs: (a) steradienes, (b) steratrienes.

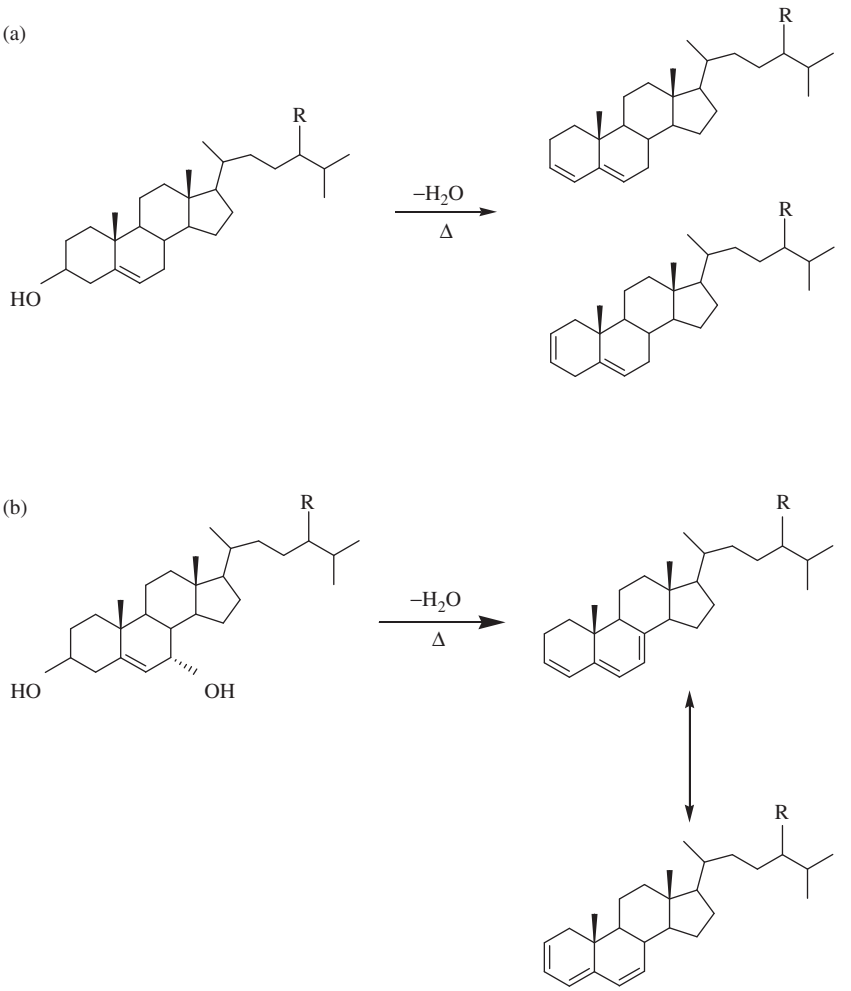


FIGURE 3 Postulated mechanism of sterol dehydration to form (a) steradienes and (b) steratrienes. R is designated as alkyl chain. (Figure 3a is courtesy of Kasim et al. (11)).

Isolation of Steroidal HCs

Cert and Moreda (12) developed and optimized a new silver-ion stationary phase for column chromatography allowing the isolation of sterenes from refined olive oils. The presence of sterenes in refined olive oils might be interfered with by isoprenoid olefins deriving from the isomerization of squalene. Using column chromatography in which silica gel was effectively loaded with 23% water and 10% silver nitrate, separation between 3,5-, 2,4-, and 2,5-sterene isomers was achieved.

Mennie et al. (13) isolated HCs fraction from several vegetable oils (maize, sunflower, rapeseed, palm, and soybean oil) by using a silica gel column, and then eliminated the aromatic substances by employing HPLC

with a calibrated Lichrosorb Si-60 column. Subsequent analysis by GC–MS suggested the presence of steroidal HCs at different stages in the refining process of vegetable oils, favorably during bleaching, deodorization, and hydrogenation processes; and succeeded in identifying -3,5-; -2,5-; -4,6-; -3,5,22-; and -4,6,22-sterenes using a combination of TLC, GC, and GC–MS tools (see Table 2).

TABLE 2 Steroidal HCs Concentration (μg/g oil) and Composition in Different Stages of Refining Processes of Vegetable Oils[a]

Oil matrices	Total content	Steroidal HCs composition
1. Maize:		
Bleached	3.7	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene
Dewaxed	4.3	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene
Deodorized	162.6	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene, 24-ethylcholesta-2,5-diene, 24-methylcholesta-2,5-diene, 24-ethylcholest-2-ene, 24-ethylcholesta-4,6-diene, 24-ethylcholesta-4,6,22-triene, 24-methylcholesta-4,6-diene
2. Olive[b]		
Virgin	0.10	24-ethylcholesta-3,5,22-triene
Refined	12.40	24-ethylcholesta-3,5,22-triene
3. Palm		
Bleached	5.2	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene
Deodorized	5.2	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene, 24-ethylcholesta-2,5-diene
4. Rapeseed		
Bleached	4.7	24-ethylcholesta-3,5-diene, 24-methylcholesta-3,5-diene, 24-methylcholesta-3,5,22-triene
Deodorized	12.5	24-ethylcholesta-3,5-diene, 24-methylcholesta-3,5-diene, 24-methylcholesta-3,5,22-triene, 24-ethylcholesta-2,5-diene, 24-methylcholesta-2,5-diene
5. Soybean		
Bleached	1.8	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene
Deodorized	5.7	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene, 24-ethylcholesta-2,5-diene, 24-methylcholesta-2,5-diene
Deodorizer Distillate	24,600 (2.46%)[c]	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene, 24-ethylcholesta-2,5-diene, 24-ethylcholesta-2,4,6-triene.
6. Sunflower		
Bleached	7.5	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene
Deodorized	29.3	24-ethylcholesta-3,5-diene, 24-ethylcholesta-3,5,22-triene, 24-methylcholesta-3,5-diene, 24-ethylcholesta-2,5-diene, 24-ethylcholesta-4,6-diene, 24-methylcholesta-4,6-diene

[a]Mennie et al. (13).
[b]Bonveh et al. (110).
[c]Kasim et al. (11).

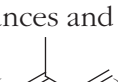
Grob et al. (14) employed an on-line HPLC–HPLC–GC–MS on two consecutive silica gel columns to identify 3,5-, 2,4-, 2,5-steradienes, 3,5-cyclo-6-enes, and 2,4,6-trienes in refined olive oil. The first column separated sterenes from triacylglycerides and other polar compounds, which is commonly done via saponification method. The second column separated and identified different sterene fractions with UV detector; steradienes at 235 nm and steratrienes at 309 nm. Several fractions isolated by the LC–LC system were transferred to the GC–MS equipped with a methylsilicone or carbowax-type column. The proposed methodology is more favorable than saponification because artifact formation in chromatographic techniques is greatly reduced.

Determination of Steroidal HCs Concentration

The steroidal HC contents in vegetable oils can be used as a means of identifying the oil origin, since their composition reflects the composition of their parental sterols, and therefore, for detecting adulteration (15). The legal limit of steroidal HCs content in virgin olive oil is between 0.01 and 4 mg/kg, which is regulated by the International Olive Oil Council; in particular, the upper limit of 3,5-stigmastadiene content in extra virgin, virgin, and lampante (kerosene) olive oils have been fixed at 0.1, 0.1, and 0.50 mg/kg, respectively (16), amending the previous regulation (17) wherein the stigmastadiene upper limit was set at 0.15, 0.15, and 0.50 mg/kg, respectively. The quantitative determination of stigmastadienes content in crude vegetable oils is prevalently carried out via GC on a fused-silica capillary column coated with 5% phenyl-methylsilicone. The method has been standardized by IUPAC (18, 19) and adopted also by the International Standard Office (20).

Cert et al. (21) and Verleyen et al. (22) reported the presence of 3,5-stigmastadiene in olive oil and compared GC and HPLC techniques in quantitation of stigmastadiene in terms of accuracy and precision. Using HPLC equipped with reversed-phase (RP) column and UV-Vis detector at wavelength 235 nm is a promising alternative to the standard GC procedure. Bortolomeazzi et al. (23, 24) detected the existence of steradienes and steratrienes in olive oil from the unique occurrence of ions at m/z 255 and 213, and at m/z 253 and 211, respectively, in the GC–MS chromatogram.

TERPENE HCs

Terpene HCs, an extensive class of HC compounds which are widely used in fine fragrances and resins, are derived biosynthetically from building units of isoprene () with molecular formula C_5H_8. Terpene HCs are prone to

autoxidation on exposure to air. Oxidized product of terpene HCs are widely known as terpenoids. Based on the amount of isoprene unit constructed, terpene HCs can be classified as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, and polyterpenes that consist of one, two, three, four, five, six, eight and more than eight units of isoprene, respectively.

Isolation of Terpene HCs

Terpene HCs (mainly mono- and sesquiterpenes) are utilized as natural fragrance compounds in a wide range of cosmetics, household and industrial products. Most terpenoid products derived from mono- and sesquiterpenes exert an allergenic activity and therefore have potential to be the cause of contact allergy in dermatitis patients (25, 26). This issue was investigated thoroughly by Karlberg et al. (27) and Matura et al. (28). Some natural allergenic terpenoids, viz. sesquiterpene lactones, were discovered and isolated by Asakawa et al. (29) in 14 species of *Frullania* (liverworts) via TLC, GC, and GC–MS.

Extreme care must be adhered in isolating terpene HCs as they are quite volatile and possess low boiling points. Miller and Kirchner (30) improved the chromatographic methods for terpenes by employing chromatostrip prior to running the column. Chromatostrip principally acts as disposable miniature of LC and can be utilized as a fast-and-simple set of trials by which the key factors influencing LC selectivity were evaluated. Ikeda et al. (31) collected and determined monoterpene HCs composition from 29 non-citrus essential oils. They isolated monoterpene HCs by silicic acid chromatostrip procedure and then analyzed it by gas-liquid chromatography (GLC).

Virgin olive oils contain a substantial amount of squalene and small quantities of volatile monoterpene and sesquiterpene HCs (32–35). Lanzón et al. (32) identified and quantitated those terpene HCs by means of GC–MS and GC/FID measurements, respectively. Gunawan et al. (8) mentioned that SODD contained some unknown HCs, one of them was tentatively identified as sesquiterpene HCs. Using headspace solid-phase micro extraction (SPME) coupled to GC–MS, Vichi et al. (35) succeeded in isolating 15 monoterpene and 30 sesquiterpene HCs simultaneously. Due to less sensitivity of the headspace SPME technique in isolating higher boiling compounds, hydrodistillation and saponification methods can be viable alternatives (33, 34). Bortolomeazzi et al. (33) fractionated sesquiterpene HCs from the unsaponified olive oil by employing silica gel column chromatography followed by silica gel $AgNO_3$ column chromatography.

Lycopene is a tetraterpene that possesses antioxidant activity but no provitamin A activity. It acts as a key intermediate in the biosynthesis of most carotenoids, is responsible for pigmentation in fruits and vegetables, and was attributed to a decreased occurrence of prostate and intestinal cancers

(36–38). Recently, there are numerous works have been done on the extraction of lycopene from tomato via nonpolar solvent (e.g., hexane, ethanol, benzene, and chloroform), and supercritical fluid carbon dioxide (ScF-CO_2) extractions (39, 40). Although ScF-CO_2 extraction gives lower extraction yield than the nonpolar solvent, it is more environmentally friendly and nontoxic.

Analysis of Terpene HCs

The structure of terpene HCs, viz. squalene, in the unsaponifiable fraction of olive oil was elucidated by Baeten et al. (41) via FT-Raman spectroscopy. Raman scattering of pure squalene, which showed intense bands at vicinity of 2910, 2860, 1665, and 1435 cm^{-1}, were shifted slightly in the unsaponifiable fraction of olive oil, indicating its independence from olive oil variety. Nevertheless, this phenomenon is somewhat tilted in other kinds of oil such as corn, soybean, sunflower, and hazelnut oils, which can be ascribed to observable shift of squalene absorption. Note that in different oils squalene, in fact, demonstrates variable shift of Raman scattering, indicating that FT-Raman spectroscopy can be a powerful tool for detecting oil adulteration.

Table 3 depicts the distribution of terpene HCs detected in some essential oils as well as its isolation and analytical procedures. Terpene HCs, such as limonene, α- and γ-terpinene, farnesene, α- and β-pinene, myrcene, *p*-cymene, and copaene are commonly found in essential oils. These terpene HCs are one of the predominant constituents of essential oils. Limene is the most abundant compound in citrus peel (73–82%), while squalene is the major compound in olive oil, either virgin or refined one. Bortolomeazzi et al. (33) were able to isolate and identify some major sesquiterpene HCs in olive oil, such as α-farnesene, α-copaene, eremophylene, and α-muurolene (see Table 3) by using GC–MS. Quantitative determination of these sesquiterpene HCs by GC/FID resulted in a total concentration of about 2 to 37 mg/L (ppm), in which α-farnesene constituted approximately 90% of the total sesquiterpene HCs.

Aldehydes and Ketones

Aldehydes and ketones are carbonyl compounds formed by either autoxidation or decomposition of lipids. They play both positive and negative roles in lipid-rich foods (42). These carbonyl compounds commonly exist in crude oil, especially in soybean oil, contribute to sensory notes (flavor and odor), and hence to oil quality. These compounds are removed at early stages (bleaching and deodorization) of the soybean oil refining process. However, it was reported that a trace amount of ketone compound was accumulated in the unsaponifiable fraction of soybean oil (3), indicating a more careful manner in removing them needs to be addressed.

TABLE 3 Composition of Terpene HCs Isolated from Some Essential Oils

Oil matrices	Terpene HC compound	Isolation – Analysis	Ref.
Citrus peel	*d*-Limonene, α- and γ-terpinene, (*E*)- and (*Z*)-β-farnesene, myrcene, α- and β-phellandrene, valencene, α- and β-pinenes, α-fenchene, camphene, sabinene, δ-3-carene, (*E*)-β-ocimene, *p*-cymene, α- and β-cubebene, α-ylangene, α-copaene, α-cedrene, β- and γ-elemene, β-caryophyllene, α-humullene, germacrene-D, sesquiphellandrene	Cold press – GC–MS	(111)
Lime peel	α-thujene, α- and β-pinene, camphene, sabinene, myrcene, α-phellandrene, α-and γ-terpinene, *p*-cymene, limonene, δ- and β-elemene, (*E*)- and (*Z*)-α-bergamotene, caryophyllene, α- and β-farnesene, α-humulene, β-copaene, germacrene B	Preparative HPLC – GC–MS	(112)
Virgin olive	α- and β-pinene, δ-3-carene, myrcene, α- and γ-terpinene, *dl*-limonene, (*E*)- and (*Z*)-β-ocimene, *p*-cymene, α-copaene, 4,8-dimethyl-1, 3,7-nonatriene, (*E*)- and (*Z*)-alloocimene, 6,10-dimethyl-1-undecene, eremophylene, α- and γ-muurolene, (*E,E*)-α-farnesene, δ-cadinene, *allo*-farnesene (Z2, E4, E6), *allo*-farnesene (E2, E4, E6), squalene,α-cedrene, (*E*)- and (*Z*)-β-farnesene	Saponification, column chromatogr. – GC–MS; hydrodistillation, SPME – GC–MS	(32–35)
Other essential oils	α- and β-pinene, sabinene, δ-3-carene, myrcene, α- and γ-terpinene, *dl*-limonene, *p*-mentha-1,5,8-triene, (*E*)- and (*Z*)-β-ocimene, *p*-cymene, α-copaene, 4,8-dimethyl-1, 3,7-nonatriene, (*E*)- and (*Z*)-alloocimene, 6,10-dimethyl-1-undecene, α- and β-cubebene, calarene, cyclosativene, sativene, α-cedrene, calacorene, δ-cadinene, (*E*)-α-bergamotene, β- and γ-gurjunene, β-caryophyllene, (*E*)- and (*Z*)-β-farnesene, γ-curcumene, β-acoradi, calamenene, β-sesquiphellandrene, α-selinene, α-zingiberene, δ-guaiene, valence	Headspace SPME – GC–MS	(113–121)

Isolation of Aldehydes and Ketones

Hill and Hammond (43) studied the flavor compounds of autoxidized soybean oil. The flavor compounds of soybean oil in the early stages of autoxidation were isolated by molecular distillation and then analyzed by GC and organoleptic analysis. The distillate contained an aqueous layer and an oily film. The oily film (hexanal, vinylamyl ketone, and (*Z,E*)-2,6-nonadienal) did not reproduce the autoxidized flavor when added to freshly deodorized oil. The aqueous layer (vinylethyl ketone combined with other carbonyl compounds) reproduced the autoxidized flavor when added to freshly deodorized oil. Recently, Kasim et al. (44) also detected aldehyde compound in saponified polar lipid fraction of SODD.

The existence of volatile aldehyde and ketone compounds in virgin olive oil could be utilized to show that oil authentication is closely associated with olive variety from which the oil is obtained by strictly physical means (45, 46). As previously mentioned in the subsection about isolation of terpene HCs, a dynamic headspace technique was applied to recover the volatiles. The key points that must be addressed to optimize this method are by selecting a cold trap injector coupled to GC and connecting a cooled fused silica trap to the effluent of GC (47). As for nonvolatile compounds, molecular distillation and liquid-liquid partition chromatography can be the options.

Another possible pathway in the formation of aldehydes is via oxidation of fatty acid ester by ozone, as hypothesized by Morrison and Nazaroff (48). They exposed linseed and tung oils to air-free ozone, collected the samples and analyzed by thermal desorption GC–MS. The aldehyde compounds identified in general are *n*-nonanal, hexanal, pentanal, and particularly 2,4-nonadienal and 2-heptenal emitted from the ozone-oxidation of tung oil (see Table 4). Kiritsakis (49) reviewed the flavor compounds of olive oil, whose formation was related to cell destruction of olive fruit where a number of enzymatic reactions (hydrolysis and oxidation) occurred. The reactions proceeded at a high rate, depending on pH and temperature. 13-Hydroperoxy-9,11-octadecadienoic acid is formed by the enzymatic oxidation of linoleate. Subsequently, hexenal is formed by the catalytic action of the enzyme aldehyde-lyase. In another process, *(E)*-3-hexenal and *(Z)*-2-hexenal are formed via enzyme aldehyde-lyase (50).

Characterization of Aldehydes and Ketones

An unpredicted role of ketones and aldehydes as fingerprints in detecting oil adulteration is apparently useful. Using TLC fractionation of unsaponifiable matters, Bastić et al. (3) characterized several compounds with oxygen, which are aliphatic and alicyclic ketones, conjugated steroid ketones, esters and lactones. They reported that the composition of them could attribute

TABLE 4 Isolation, Analysis and Composition of Volatile Aldehyde and Ketone Compounds in Some Vegetable Oils

Oil matrices	Aldehyde and ketone compounds	Isolation – Analysis	Ref.
Citrus peel	*l*-carvone, decanal, menthone, octanal, 6-methylhept-5-en-2-one, α- and β-thujone, citronellal, (*Z*)-2-decenal, neral, dodecanal, (*Z*)-2-undecenal, perillaldehyde, tridecanal, isopiperitone, (*Z*)-2-dodecenal, tetradecanal, β-ionone, β-sinensal, octadecanal	Cold pressing – GC–MS	(111)
Linseed	Nonanal, 3,6-nonadienal, 3-hexenal, hexanal, decanal, 2-octenal, octadienal	Thermal desorption GC–MS	(48)
Soybean	Pentanal, hexanal, vinylamyl ketone, (*Z,E*)-2,6-nonadienal, heptanal, 4-pentenal, formaldehyde, 2,4-dinitrophenylhydrazone, acetaldehyde, 2-propenal, 2-propanone, 2,3-butadione, butanal, 2-butanone, 2-butenal, 2-petanone, 2-hexanone, 4-methyl-2-pentanone, 2-hexenal, 2-heptenal	Oxidation, column chromatography – IR spectroscopy; distillation – GC/TCD; GC–MS headspace	(43, 53, 56, 122)
Sunflower	Pentanal, hexanal, (*Z*)-2-hexenal, 2-heptanone, heptanal, (*Z*)-2-heptenal, (*Z*)-2-octenal, (*Z*)-2-nonenal, 5-butyl-2(5*H*)-furanone, 2-decanone, 5-pentyl-2(3*H*)-furanone, (*E,Z*)-2,4-decadienal, (*Z,Z*)-2,4-decadienal, 5-pentyl-2(3*H*)-furanone, 2-nonanone, 4-hydroxy-(*Z*)-2-nonenal, 2-octanone, 4,5-epoxy-2-decenal, 3-octen-2-one	SPME – GC–MS	(58)
Tung	2,4-nonadienal, 2-heptenal, nonanal, hexanal, pentanal, octanal, decanal, 3-hexenal, 2-octenal, dimethylamine	Thermal desorption GC–MS	(48, 52)
Virgin olive	2-butanone, 3-methylbutanal, 3-pentanone, 4-methylpentan-2-one, pentanal, hexanal, 1-penten-3one, 2-methylbuten-2-al, (*E*)-2-pentenal, (*Z*)-2-pentenal, (*E*)-3-hexenal, (*Z*)-3-hexenal, heptan-2-one, (*E*)-2-hexenal, (*Z*)-2-hexenal, octan-2-one, 6-methyl-5-hepten-2-one, nonan-2-one, (*E,E*)-2,4-hexadienal, 2-octenal, heptanal, octanal, nonanal, decanal, dodecanal	Headspace extraction; co-distillation with H_2O, solvent extraction, dry column chromatogr. – GC–MS; headspace GC/FID	(45, 46, 49, 123–127)

to characterization of the oils. For instance, hexahydrofarnesylacetone and kaurene in sunflower and pumpkin seed oils, as well as hexahydrofarnesylacetone and 2,6,10,14,18,22-hexamethyltricosan-12-one in rapeseed oil. Table 4 summarizes the isolation, analysis and composition of aldehyde and ketone compounds in some vegetable oils. It can be seen that aldehyde and ketone groups exist together, and the predominant group is the aldehyde. Benzaldehyde (51) was reported as one of the major volatile compounds in almond oil. This type of compound and its derivatives were claimed to be the common volatile of essential oils extracted from plants. A possible explanation for the formation of benzaldehyde could be amygdaline hydrolyses under the conditions employed in aqueous enzymatic extraction.

Debruyne (52) hypothesized that the formation of aldehyde and ketone compounds, contributing to flavor reversion, in soybean oil might be incorporated with oxidation of linolenic acid, a phosphate reaction wherein trimethylamine oxide reacts with linoleic acid and hydroperoxides and releases formaldehyde and dimethylamine, and addition of other oils. Mookherjee and Chang (53) characterized 21 mono-carbonyl compounds in reverted-but-not-rancid soybean oil by conversion of carbonyl compounds into their 2,4-dinitrophenyl-hydrazones, passing them through an alumina column followed by a celite column and subjecting them to liquid-liquid partition chromatography to collect each fraction and submit to IR-spectroscopy for analysis. A total of 20 carbonyl compounds were identified with a peroxide number of 2.7 meq/kg and an organoleptic flavor score of 6.0. Six of the carbonyl compounds were saturated methyl ketones, 10 were saturated aldehydes and 5 were 2-enals (see Table 4).

Thermal desorption GC–MS can be employed to analyze the volatile aldehydes and ketones. This tool is quite sensitive as it can detect trace concentration of volatile compounds in μg/kg level. Morrison and Nazaroff (48) detected several volatile aldehyde and ketone compounds in linseed and tung oils after the oils exposure to ozone. According to Montedoro et al. (50), the aldehyde content in green and black olive oils are 50% and 75%, respectively. Hexanal, *(Z)*-2-hexenal, 1-hexanol and 3-methylbutan-1-ol are the major volatile compounds in olive oil.

EXOGENOUS MINOR CONSTITUENTS

These compounds may originate from contaminated soils, water, and air in the plants uptake and due to their chemically stable characteristics, are somewhat persistently retained in plants and their parts. Overall, the compounds that commonly reside in plants are aromatic HCs, pesticides and herbicides, and trace metal compounds. Although they are categorized as environmental pollutants, the fact that these pollutants are usually present in vegetable oil in minor quantities is the solid reason to include these pollutants in this review.

Aromatic HCs

Two major groups of aromatic HCs that are usually present in vegetable oils as minor constituents are volatile and polycyclic aromatic HCs. Due to its stable aromatic ring structure; they are categorized as one of the most widespread organic pollutants, which remain in the environment for a long time. In crude vegetable oil, obtained either by cold pressing or solvent extraction, small quantities of pollutants (VAHs, PAHs, chlorinated HCs, pesticides, etc.) can be detected.

Volatile Aromatic HCs (VAHs)

Trace amount of benzene (1–10 μg/kg) and a total amount of benzene, toluene, and C_2-benzenes of 15–250 μg/kg has been detected by using capillary GC, which can be considered as potential interferences in determination of VAHs (54). The presence of VAHs or so-called low-molecular-mass-aromatic hydrocarbons in the volatile fraction of edible oils can cause public concerns. In Germany, the upper limits suggested for benzene, toluene, ethylbenzene, and xylene are 50 μg/kg, 180 μg/kg, 50 μg/kg and 280 μg/kg, respectively (54). Detection of VAHs in the oil matrices can be performed by using GC–MS measurement.

Isolation of VAHs

Due to its high volatility, VAHs are usually isolated by using a dynamic headspace technique. A nitrogen stream was passed through an oil sample and the volatiles were then trapped on activated charcoal. The trapped compounds were desorbed with carbon disulfide and fractionated by a silica gel column, and finally analyzed by GC–MS.

Via a trap-purge-desorption system, Morchio et al. (55) isolated and transferred the volatiles in edible oils, particularly virgin olive oils, into GC. The quantitation was done by means of a selected-ion monitoring GC–MS of the ion appearing at m/z 78, 91, 92, and 106. Pinnel and Vandegans (56) analyzed VAHs contained in soya oil. They mentioned that the analysis of VAHs had been used to estimate the degree of oxidation of vegetable oil. They carried out a dynamic headspace technique without heating the sample during purge time of the analysis. This technique was useful to recover VAHs and other volatiles contained in the oils. Many researchers have extensively studied the VAHs detection in various kind of seed oils using this technique, such as Pićurić-Jovanović and Milovanović (51) work in almond and plum kernel oils, Biedermann et al. (54) in virgin olive oil, Pinnel and Vandegans (56) in soybean oil, Vichi et al. (57) in north-eastern Italy virgin olive oil, and Guillén et al. (58) in sunflower seed oil. The latter two works tried to

modify the isolation of volatiles by headspace SPME technique followed by GC–MS to enhance the recovery of the extracted volatiles.

Analysis of VAHs

Ethylfuran and 3-(4-methyl-3-pentenyl) furan, which are heterocyclic aromatic compounds, were detected in virgin olive oils by using a dynamic headspace technique followed by thermal desorption GC/FID quantitation with isobutyl acetate spiked into the samples. These furan compounds were predicted to be responsible for sweet (ripe fruit) reception and their corresponding concentrations in the olive oil were 0.060 and 0.057 mg/L (ppm). Some volatile VAHs that were also detected in virgin olive oils are methylbenzene, ethylbenzene, ethenylbenzene, and 1,2,4-trimethylbenzene. Methylbenzene and ethenylbenzene were detected in the ripe fruit; ethylbenzene was responsible for the bitter taste; on the other hand, 1,2,4-trimethylbenzene was undesirable due to its high flammability and strong odor (46).

Investigation was carried out by Biedermann et al. (54) into the sources of contamination of virgin olive oil, which showed high concentration of benzene, toluene, and C_2-benzene. They concluded that up to 10 μg/kg benzene and 250 μg/kg total sums of VAHs must be considered as possible background concentration because of their high concentration in olives picked from tree. Gasoline vapor might be responsible for the contamination by oil exposure to contaminated air, as well as contamination during oil storage, processing and transportation. Vichi et al. (57) mentioned that overall concentration of VAHs (677 μg/kg) was higher than that of light PAHs (80 μg/kg), suggesting that it might be ascribed to the relatively high VAHs concentration in atmospheric air. They also claimed to detect C_3- and C_4-benzenes, which had never been reported before. The major compound in the C_3-benzenes was trimethylbenzene, while substances belonged to C_4-benzenes were 1,3-dimethyl-5-ethylbenzene, 1,2-dimethyl-4-ethylbenzene, and 1,2,3,5-tetramethylbenzene.

Polycyclic Aromatic HCs (PAHs)

Polycyclic or polynuclear aromatic hydrocarbons (PAHs) are HC compounds comprising two or more fused carbocyclic aromatic rings, which may or may not have substituent groups attached to one or more rings. PAHs are generally formed by incomplete combustion and high temperature pyrolysis reactions, such as the burning of coal, oil and other forms of organic matters (59). Due to their lipophilic properties and wide distribution in environment, PAHs can easily contaminate edible oils.

Larsson et al. (60) summarized the possible routes of its contamination, namely uptake by oil plants from contaminated soils and polluted

atmosphere, direct drying of oilseeds with combustion gases, uptake from the petroleum-based solvents introduced in oils extraction from their seeds. Some of these compounds have extraordinary toxic properties and are highly stable contaminants. A sensitive determination of PAHs in edible oils is imperative because studies have shown that PAHs coming from oils and fats origin is one of the most contributing food groups to PAHs intake (constitute 14–34% of total food groups in Europe) (61).

Based on EPA recommendation due to their toxicological relevance and their typical distribution profile, PAHs containing up to 3–4 fused aromatic rings are called as "light" PAHs, meanwhile those containing 5 or more fused aromatic rings are called as "heavy" PAHs. U.S. EPA (62) has regulated 16 PAH-based pollutants, viz. acenaphthene, acenaphthylene, anthracene, benzo(*a*)anthracene, benzo(*a*)pyrene, benzo(*b*)fluoranthene, benzo(*g,h,i*)perylene, benzo(*k*)fluoranthene, crysene, dibenzo(*a,h*)anthracene, fluoranthene, fluorine, indeno(1,2,3-*cd*)pyrene, naphthalene, phenanthrene, and pyrene. Among them, three compounds have been classified as suspected human carcinogens: benzo(*a*)anthracene, benzo(*a*)pyrene and dibenzo[*a,h*]anthracene (62). The molecular structure of 16 EPA-priority PAHs are shown in Figure 4.

Isolation of PAHs

Various methods for isolation and purification of PAHs have been reported. One of the common methods is the liquid-liquid partition technique (63) with dimethylformamide/water (90:10) and cyclohexane for the isolation of PAHs. After extraction, the sample was cleaned by silica gel chromatography and gel permeation chromatography. Finally, identification of PAHs was performed by GC–MS. Fisher et al. (64) proposed employing size-exclusion chromatography as an alternative prior to GC–MS analysis. Bazylak and Maslowska (65) employed HPLC with UV-Vis and fluorescence detectors as an alternative of the analysis. Details of PAHs concentration in vegetable oils were reported by Speer et al. (66). They also mentioned that the German Society for Fat Science regulated a 25 μg/kg limit for total PAHs (sum of phenanthrene, anthracene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, anthanthrene, benzo[*g,h,i*]perylene, dibenzo[*a,h*]anthracene, coronene]) and a 5 μg/kg limit for the total heavy fraction.

According to Guillén et al. (58), light PAHs can be recovered by SPME followed by headspace GC–MS. After oil sample was introduced into heating process for a certain time to reach equilibrium, a SPME fiber commonly coated with Tenax or divinylbenzene/carboxen/ polydimethylsiloxane was inserted into the headspace of the sample and maintained for 60 min. The fiber was later desorbed, separated, identified, and quantitated by using a gas chromatograph.

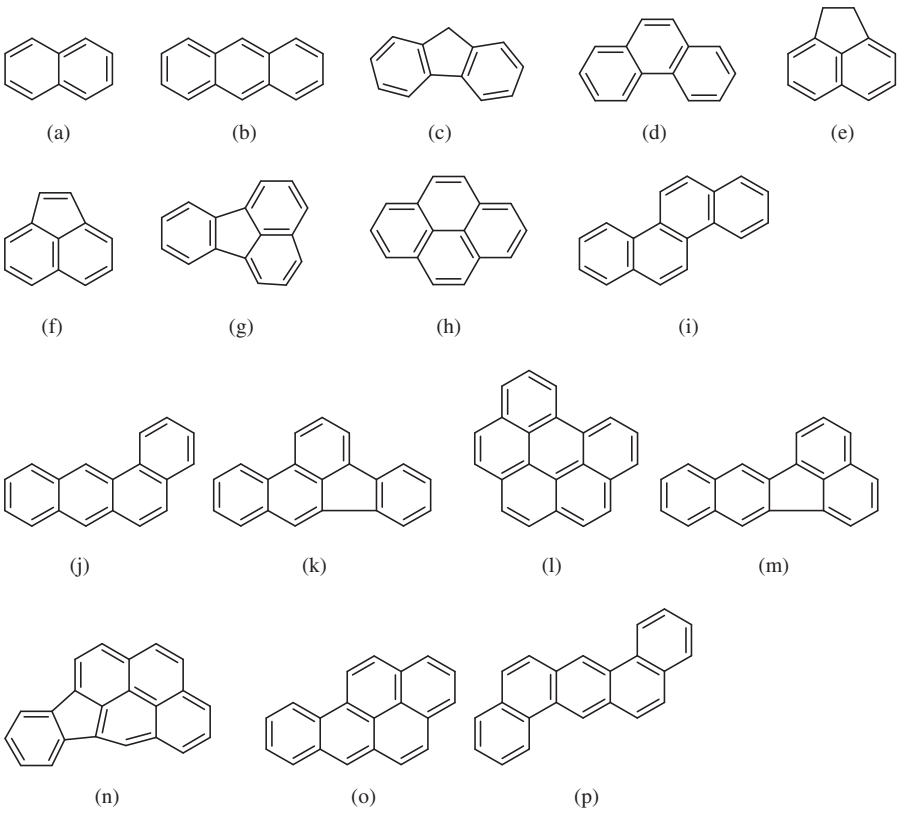


FIGURE 4 Molecular structures of several PAHs: (a) naphthalene, (b) anthracene, (c) fluorene, (d) phenanthrene, (e) acenaphthene, (f) acenaphthylene, (g) fluoranthene, (h) pyrene, (i) chrysene, (j) benzo(*a*)anthracene, (k) benzo(*b*)fluoranthene, (l) benzo(*g,h,i*)perylene, (m) benzo(*k*) fluoranthene, (n) indeno(1,2,3-*cd*)pyrene, (o) benzo[*a*]pyrene, (p) dibenzo(*a,h*)anthracene.

The refining process (based on deodorization step) of the oils can greatly reduce the level of lower molecular weight PAHs such as fluoranthene, while no corresponding effect is observed for the higher molecular weight PAHs. The concentration of the latter might be reduced by filtration through activated carbon and followed by bleaching treatment (60). This cleanup procedure has been implemented in the commercial oil-refining process (67).

Determination of PAHs Concentration in Vegetable Oils

PAHs are commonly present in vegetable oils (safflower, sunflower, maize germ, sesame, linseed, and wheat germ oils) at low concentrations. For example, the total PAHs content measured in crude and deodorized soybean oils are 33–56 and 5–17 μg/kg, respectively, while in crude and deodorized

rapeseed oils are 43–61 and 5–12 μg/kg, respectively (60); and one exception in olive oils that show significantly higher contamination by light PAHs ranging from 53 to 105.6 μg/kg (66).

This exclusive phenomenon can be explained by variation in oil processing. Although other vegetable oils are often subjected to steam treatment, which can decrease the level of light PAHs, the International Olive Oil Agreement permits only physical processes (e.g., cleaning, decantation, centrifugation, and filtration) for olive oil extraction (66). Due to direct copra drying via combustion gases, similar phenomenon was observed in crude coconut oils whose total PAHs content ranged from 2600 to 3700 μg/kg (60). The distribution of light PAHs in virgin olive oil was predominantly represented by substances containing 2 and 3 aromatic rings with average concentration of 36 and 29 μg/kg, respectively; while 4-rings PAHs were present in lower mean concentrations, 15 μg/kg. Naphthalene and phenanthrene were the major PAHs in virgin olive oil (57).

Guillén et al. (58) proposed a possibility of light PAHs formation by oxidation of edible oils at room temperature. The mechanism could be explained in two ways: either by generation of unsaturated alkyl free radicals to produce aromatic products in the presence of oxygen; or by reaction between secondary oxidation products that are unsaturated. Distribution of light PAHs in sunflower oil was dominated by 1-methylnaphthalene, naphthalene, 2-methylnaphthalene, fluorene, 1,6-dimethylnaphthalene, and *o*-terphenyl; all of which were the most accelerated PAHs formed among others after certain prolonged storage time.

Pesticides and Herbicides

Pesticides are harmful or toxic substance or mixture of substances synthesized to kill pests. The most common one used in soybean farming is organochlorine pesticides (OCPs). They could reach soil surface, where they eventually degraded, gradually spread over, or are trans-located to other environments, such as plant-derived products, and even human themselves. OCPs are more persistent, but have less acute toxicity than the organophosphate (OP) ones. Due to their properties, accurate and effective routine method to monitor them is needed.

Isolation of pesticides and herbicides

A thorough study of biological, chemical, and physical properties of pesticides and herbicides must be the first consideration in devising their isolation and analytical methods. Gunther and Blinn (68) emphasized that a successful analytical isolation technique employed on one plant or its part may give unsatisfactory performance when used on another. Therefore, isolation and analytical assays of pesticide residues from various plants

should be individually investigated. The isolation techniques evaluated well by Gunther and Blinn (68) are physical isolation such as steam distillation, distillation, partition distribution, chromatography, and crystallization; and chemical isolation such as oxidation, reduction, hydrolysis, dehydrohalogenation, and combustion.

A number of extraction methods of OCPs from vegetable oils has been published, such as sample soaking with 1% NH_4Cl followed by cold soxhlet extraction with hexane-acetone (69), solid-phase extraction with various stationary phases (70, 71), size-exclusion chromatography on zeolite (64), gel permeation chromatography (72), LC-GC coupling (73), and direct coupling of supercritical fluid extraction with tandem supercritical fluid chromatography and GC (74). Meanwhile, their quantitative and qualitative determination can be performed by GC/electron capture detection (ECD) and GC–MS. The efficacy of different extraction methods might vary with oil matrices, but most literatures reported comparable performance for those methods. Recently, solid phase extraction (SPE) has become the most reliable and fastest method to screen for OCPs prior to analysis. The application of SPE technique with various stationary phase (e.g. RP-C18, C8, C2 and polar alumina, Florisil, and silica) in the isolation of pesticides from various fatty matrices was well-reviewed by Picó et al. (75)

A simpler qualitative detection of pesticides can be carried out by using chemically bonded 1D-TLC, such as with cyano, amino, and diol normal phase (NP) layers and C18 and water wettable C18 RP layers (76). Meanwhile for detection of herbicides and fungicides, 2D-TLC on chemically bonded cyanopropyl silica gel stationary phase, using an organic mobile phase in the 1st development and an aqueous RP mobile phase in the 2nd development, will be preferable (77). Sherma (76) reviewed the application of TLC for qualitative and quantitative determination of pesticides, OP pesticides, insecticides, herbicides, and fungicides.

Distribution of Pesticides and Herbicides in Vegetable Oils

Chaudry et al. (78, 79) investigated the distribution of OCPs (hexachlorobenzene isomers, heptachlor, heptachlor epoxide, aldrin, dieldrin, DDT, DDD, and DDE) in soybeans, its oil, and its by-products during processing. Soybean samples were analyzed for OCPs content of oil from different fractions of soybean oil (crude, refining, bleaching, and deodorization). Soybean cotyledons were found to contain lesser amount of pesticide residues than whole beans, hulls, hypocotyls and fines. Deodorization of oil (at 250 °C and 1–5 mmHg) was noted to be the most effective in removing pesticides. Deodorizer distillate had high residue concentration, albeit its levels in the oil decreased somewhat after each processing step; meanwhile pesticide residues were not detected (up to detection limits of instrument) in refined soybean oil (78).

Halbert and Archer (80) investigated dioxin and furan content in tocopherols isolated from deodorizer distillate of soybean, cottonseed, and safflower oils. The total toxic equivalence of those in soybean, cottonseed, and safflower oil were 1.6–5.5 pg/g, 8.1 pg/g, and 0.93 pg/g, respectively. Filek and Lindner (71) purified hexachlorobenzene and dieldrin in pumpkin seed and its oil by using classical silica gel column chromatography with a mixture of petroleum benzine and *tert*-butylmethyl ether (98:2, v/v) as eluent and later quantified them via GC/ECD measurement. They reported that the contents of hexachlorobenzene and dieldrin in pumpkin seed were 1–310 ng/g and 2–335 ng/g, respectively; while in its oil were 5–90 ng/g and 4–8 ng/g, respectively.

Natural pesticides and herbicides are more desirable alternatives as they give much less adverse effect to environment and human health than the chemically made ones; while at the same time still give comparable performance. One of the most famous natural insecticides is azadirachtin, which is omnipresent in neem plant and to date, has been widely used around the world (81, 82).

Trace Metal Compounds

The worrisome concern that there is small quantities of metal compounds still occur in vegetable oils need to be carefully handled and addressed as it may have adverse effect on the stability of edible oils, and thus imply the deterioration of oil quality. Some metals are essential at low level, but may become hazardous at higher concentrations (83).

To date, numerous works have been done in either isolation followed by determination or direct determination of trace metals level in oils. Table 5 summarizes works reported on the levels of trace metals in several common vegetable oils. As shown in Table 5, the magnitude of trace element contents in the oils follows the following order: Fe > Zn ~ Cu > Cd > Pb ~ Cr ~ Mn. This order is in agreement with the relative abundance order of elements in Earth's crust (84).

Isolation of Trace Metal Compounds

The procedure to isolate trace metals varies with its matrices. Buldini et al. (85) removed organic matrices that often interfere with analysis of trace metals by using saponification followed by oxidative UV photolysis. They claimed that this method was simple and required less reagents compared to other sample pretreatment procedures. Its analysis was accomplished via ion chromatography with dual detectors: conductivity and UV-Vis detectors. The most prevalent sample preconcentration procedure in vegetable oils is the one performed prior to atomic absorption spectrometric (AAS) analysis.

TABLE 5 Level of Trace Metals (μg/kg oil) in Several Vegetable Oils

Ref.	Analytical method[a)]	Oil[a)]	Cr	Mn	Fe	Cu	Zn	Cd	Pb
Fedelli et al. (101)	Dilution MBIK – FAAS	O			320	60			
		Su			570	50			
		P			350	50			
Black (102)	Char ashing – FAAS	So			3296.7	64.3			
	Direct aspiration – FAAS	So			3346.7	50.3			
	Carbon rod – EAAS	So			3336.7	47.3			
Elson et al. (103)	Digestion-EAAS (Cu); FAAS (Zn)	R				40	2700		
Hon et al. (106)	Diluted propionic acid – EAAS	Co			1500				
		P			1430				
Elson et al. (91)	Digestion-EAAS (Cu); FAAS (Zn)	R				230	64900		
Ooms and Van Pee (128)	Char ashing – FAAS	Co			154	15	5		
Sun (100)	Diluted MBIK – Graphite FAAS	So			44–124	11–36			
		Co			72	26			
		Su			62	25			
Carbonell et al. (93)	Char ashing – FAAS	V-o			27000	90			
	Flow injection – FAAS	V-o			28000	1130			
Garrido et al. (87)	Char ashing – FAAS	Co			640	70	200		
		O			1010	90	170		
		R			620	60	190		
		So			590	80	150		
		Su			550	60	160		

Buldini et al. (85)	Saponification - oxidative UV – ion chromatography	O			392	12.7	<25	<100	<50
		P			1257	48.6	125	<100	<50
		So			2675	25.5	<25	<100	<50
		Su			575	18.3	<25	<100	<50
Karadjova et al. (89)	1,4-dioxane – EAAS	O	n.d.- 30	11–47	600–1540	50–220			n.d- 22
Pera et al. (99)	HCl extraction – stripping chronopotentiometry	Ci		407.2–930.6		102.5–190.5		<0.6	57.2–118.6
Marfil et al. (88)	Diluted MBIK – EAAS	V-a	10.3–55.3	15–70.8	800–4000	158.4–695.7			28.5–450
Demirbas (105)	Oxi-acidic & thermal extraction – FAAS	T-s			2204	15980	71900	666	422

[a)]Denotation: FAAS = flame atomic absorption spectrometry; EAAS = electrothermal AAS; Ci = citrus; Co = corn; O = olive; P = peanut; R = rapeseed; So = soybean; Su = sunflower; T-s = tomato seed; V-o = virgin olive; V-a = virgin argan

Prior to AAS analysis, sample pretreatment must be conducted to remove the interference of lipid matrices during the analysis. There are three important sample pretreatment steps: ashing or charring, extraction of the ash, and dilution. Many variations of the techniques have been developed such as ashing followed by high-temperature dry ashing and extraction of the ash with dilute acid (86, 87); oil dilution with MIBK (88), 1,4-dioxane (89), or $BuNH_2$-water-THF (90) followed by direct aspiration; oil extraction with nitric acid (91), or nitric acid-EDTA (92) followed by direct analysis; or direct analysis of the oil samples (93). In principle, these procedures can remove the lipid matrices effectively.

Based on using a solid powder Pb-piperazinedithiocarbamate complex for extraction and a potassium cyanide solution for back extraction, Bati and Cesur (94) introduced an alternative method for preconcentration and separation of copper in edible oils prior to flame AAS determination. Piperazinedithiocarbamate reagent was chosen instead of other dithiocarbamates for the SPE of copper in an organic matrix because its chelates are polymeric, stable, and insoluble in water and numerous organic solvents by means of two-CS_2 groups.

Lunde (95) determined the distribution of trace metal compounds in some vegetable oils via autoradiography and γ-spectroscopy. The oils were saponified and subjected to wet destruction, a treatment with warm sulfuric acid, nitric acid, and hydrogen peroxide. After mixing with hexane and HCl at pH 2, the activated trace metals were extracted into the water phase and registered for analysis.

Analysis of Trace Metal Compounds

The occurrence of metals in oils is due to two factors: endogenous and exogenous. Endogenous factor is defined as when the trace metals are originated from soil, fertilizers (the first two sources are inherited from parental plant that takes the metals for growth need), and chelating association with phospholipids (PLs); while the exogenous factor may originate from refining processes, transportation, and storage of oils. McLaughlin et al. (83) reviewed food safety issue related to the distribution of trace metal compounds in crops and addressed some important factors affecting the distributions such as soil, climate, plant genotype, and agronomic management.

The indicated level of certain trace metal compounds, such as Fe and Cu, in oils can be alternatively determined using acidity and peroxide values (87, 96). Elements Fe and Cu are one of the factors responsible for the initiation and acceleration of lipid oxidation, which will lead to rancidity of oil. As for metal incorporated with PLs, its distribution in the oils is influenced by PLs content because PLs are widely known as chelating agents (95). Therefore the removal of PLs will greatly eliminate trace metals, especially Fe and Cu, and hence enhance the oxidative stability of oil (97). The

presence of small quantities of water in oil may enhance the concentration of trace metal compounds by providing small area with a higher water concentration where the trace metals can be concentrated (95).

Several analytical techniques such as γ-spectroscopic (95, 97), arc spectrographic (98), derivative stripping chronopotentiometry (99), ion chromatographic (85), and AAS methods have been extensively reported in determining trace metals in edible oils (87). AAS has been the most selective and sensitive technique, and therefore has been the most prevalent method in conducting analysis of trace metal compounds in oil matrices. To date, two types of AAS atomizer have been employed: flame atomizer (87, 91, 93, 94, 100–105), and electrothermal atomizer (88, 89, 91, 102, 103, 106) (see Table 5).

American Oil Chemists' Society (107) has standardized this AAS-based analytical technique for determining various trace metal compounds in vegetable oils, such as AOCS Official Method Ca 15-75 for determination of chromium, copper, iron and nickel in vegetable oils by AAS; AOCS Official Method Ca 18-79 for analysis of chromium, copper, iron, nickel and manganese in triglyceride oils by AAS using a graphite furnace; AOCS Official Method Ca 18d-01 for determination of cadmium by direct graphite furnace AAS; and AOCS Official Method Ca 18c-91 for determination of lead by direct graphite furnace AAS.

The distribution of trace metals in various vegetable oils is shown in Table 5. Low concentration of trace metal compounds were detected in refined soybean, olive, rapeseed, sunflower, peanut, and corn oils in μg/kg level up to 2000; however, the concentration of these metals in unrefined soybean and olive oils is particularly the highest among others, which is up to 28000 μg/kg. Another noteworthy thing is that metals Fe and Cu were predominantly present in all types of oil.

CONCLUSIONS AND FUTURE ENDEAVORS

From the preceding coverage, we can conclude that for trace pollutants, there is significant improvement on the detection limit of the analytical instrumentation chronically and hence amending the compliance regulation concerning the maximum tolerance level of the pollutants in plant origin oil. Therefore, it will prompt better techniques in the refining process, transportation, and storage of edible oils.

As for other less harmful constituents, an apparent enhancement on the separation techniques and analytical instrumentation by either modifying some parts of the instrument or coupling several instruments has substantially contributed to obtaining highly purified constituents and depicting their characteristics.

The future endeavor in this research scope will be expected to focus on improving the sample preparation, methods of extraction, detection limits of analytical instrumentation and profiling a complete picture and (bio) safety assessment of some minor constituents that are still currently unknown.

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