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1. Introduction

It is widely known that trace metals have negative effects on the oxidative stability of olive oil. Natural composition of olive fruit, natural contamination from soil, fertilizers, industrial applications or highways near the plantations are the main sources of metals in olive oils. The olive oil may also be contaminated with the metals during the production process and contact with storage materials. The level of trace metals in olive oil is one of the quality parameters and also effective on oil oxidation and human health. Oxidation leading to the development of unfavorable odours and taste is one of the major reasons of deterioration of olive oils. The factors that most affect the rate of oxidation are the degree of unsaturation, the amount of oxygen, temperature, light and the presence of metals (mainly transition metals such as Fe and Cu) (Meira et al., 2011; Sikwese & Duodu, 2007). The trace metals enhance the rate of oxidation of edible oils by increasing the generation of free radicals from fatty acids or hydroperoxides. Benedet & Shibamoto observed that trace amounts of Fe, Cr, Pb and Cd contribute oxidative effects to lipid peroxidation (Benedet & Shibamoto, 2008).

The determination of metals has been a difficult analytical problem because of the hard organic content of the oil matrix. The analytical techniques used for metal determinations in oils are both emission and absorption spectrophotometry. ICP-OES (Allen et al., 1998; Angioni et al., 2006; Anthedimis et al., 2005; Costa et al., 2001; De Souza et al., 2005; Murillo et al., 1999; Zeiner et al., 2005), FAAS (Bati & Cesur, 2002; Carbonell et al., 1991; Köse Baran & Bağdat Yaşar, 2010; Nunes et al., 2011), ETA-AAS (Karadjova et al., 1998; Kowalewska et al., 1999; Zeiner et al., 2005), GFAAS (Allen et al., 1998; Ansari et al., 2009; Calapaj et al., 1988; Chen et al., 1999; Cindric et al., 2007; De Leonardis et al., 2000; Hendrikse et al., 1988, 1991; Lacoste et al., 1999; Martin-Polvillo et al., 1994; Matos Reyes & Campos, 2006; Mendil et al., 2009; Nash et al., 1983; Van Dalen, 1996), and ICP-MS (Benincasa et al., 2007; Bettinelli et al., 1995; Llorent-Martinez et al., 2011a, 2011b; Pereira et al., 2010; Wondimu et al., 2000) are the most commonly used techniques for the determination of metal contents in oils (Duyck et al., 2007).

In this chapter, recent determination techniques and sample pretreatment methods have been described and compared with each other. Additionally, a novel metal extraction procedure has been introduced in detail. In recent years, scientists have been interested in defining the bioavailable amount of metals more than total metal concentration. Taking into account this, fractionation and speciation analysis of metals in oil samples have also been discussed in the chapter.
2. Sample pre-treatment

The accurate determination of trace metals in olive oil is an analytical challenge due to their low concentration and the difficulties that arise because of the high organic content. Due to the high organic content, sample pretreatment is a critical step and frequently necessary in olive oil analysis. Sample pretreatment step provides the decomposition of organic matrix or the extraction of metals without matrix decomposition. On the other hand, oil sample can be diluted in a suitable solvent or emulsified with an appropriate emulsifier in a rapid pretreatment for direct determinations. The atomic spectrometers are the most commonly used devices but have some problems such as the reduced stability of the analytes in the solution, requirement of organometallic standards, the use of dangerous organic solvents or sample digestion with an acid or acid mixture (Nunes et al., 2011).

2.1 Acid digestion

Digestion procedures are regularly carried out with either open vessels using acid, acid mixture or basic reagents on hot plates or open- and closed-vessel microwave ovens. The decomposition in open system is hard, time consuming and prone to systematic error sources, i.e. contamination or analyte losses. In case of using microwave radiation, the high cost of instrumentation and dilution of the sample can be considered as disadvantages in the microwave assisted digestion system. Although the amount of sample in vessels is limited due to the generation of gaseous reaction products that can increase of pressure, the use of closed high-pressure vessels is appropriate for efficient sample digestion. On the other hand, in the use of open-focused microwave ovens, the advantages are decreasing the risk to the operator, possible introduction of reagents during procedure, opportunity to digest larger amounts of sample and low cooling time (Sant'Ana et al., 2007).

Microwave-assisted digestion has been performed to dissolve the oil sample for elemental analysis in a large number of papers (Angioni et al., 2006; Ansari et al., 2009; Levine et al., 1999; Llorent-Martinez et al., 2011a, 2011b; Mendil et al., 2009; Sant’Ana et al., 2007), while focused microwave assisted digestion for the same purpose has been employed in a few papers (Sant’Ana et al., 2007). As shown in Table 1, some investigation have been done on microwave digestion for olive oil using various procedures.

2.2 Dry ashing

In general, ashing methods may provide lower analyte recovery and exhibit poorer accuracy compared to acid digestion methods. Although dry ashing procedures are effective, they are time consuming and can often result in loss of analyte species that could occur during the preparation of the sample. Oil is decomposed by high-temperature dry ashing, subsequently the ash is dissolved in an acidic aqueous medium and the metal content of the aqueous phase can be measured by various detection techniques such as AAS, adsorptive stripping voltammetry (AdSV) and derivative potentiometric stripping analysis (dPSA) (Abbasi et al., 2009; Carbonell et al., 1991; Lo Coco et al., 2003). There are limited researches for metal determinations in oils after dry ashing of olive oil (Lo Coco et al., 2003).
Metal Determinations in Olive Oil

| Microwave digestion procedure | Reagent | Metals determined | Reference |
|-------------------------------|---------|-------------------|-----------|
| 130 °C 10 min. 140 psi, 150 °C 10 min. 200 psi, 10 min. cooling, 160 °C 20 min. 200 psi | HNO₃ | Mg, Ca, Cr, Fe, Mn, Cu, Ni, Zn, Cd, Pb | Bağdat Yaşar & Gücer, 2004 |
| 2 min. for 250 W, 2 min. for 0 W, 6 min. for 250 W, 5 min. for 400 W, 8 min. for 550 W, vent.: 8 min. | HNO₃–H₂O₂ | Fe, Mn, Zn, Cu, Pb, Co, Cd, Na, K, Ca, Mg | Mendil et al., 2009 |
| 250 W 2 min., 0 W 1 min., 250 W 2 min., 600 W 1 min., 400 W 5 min., vent.: 3 min. | HNO₃–H₂O₂ | Ca, Fe, K, Mg, Na, Zn, Al, Co, Cu, Mn, Ni, Cr, Pb | Cindric et al., 2007 |
| 25 °C-90 °C 5 min. 700 W, 90 °C-90 °C 10 min. 600 W, 170 °C-170 °C 7 min. 600 W | HNO₃ | Ag, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Ti, Tl, V | Llorente-Martinez et al., 2011a, 2011b |
| 300 W (83%) for 15 min., 600 W (75%) for 10 min., 1200 W (65%) for 15 min., 300 W (83%) for 5 min. | HNO₃–H₂O₂ | Cd, Cu, Pb, Zn | Angioni et al., 2006 |
| 750 W 90 °C 6 min., 750 W 90 °C 4 min., 1000 W 180 °C 8 min., 1000 W 180 °C 15 min. (35 bar), vent.: 20 min | HNO₃–H₂O₂ | Cu, Fe, Ni, Zn | Nunes et al., 2011 |
| 250 W, 4 min., 0 W 4 min., 250 W 5 min., 400 W 7 min., 700 W 6 min., 350 W 5 min. | HNO₃ | Be, Mg, Ca, Sc, Cr, Mn, Fe, Co, Ni, As, Se, Sr, Y, Cd, Sb, Sm, Eu, Gd | Benincasa et al., 2007 |

i: initial; f: final

Table 1. The summary of microwave digestion procedures for various metals in olive oil.

2.3 Extraction

Sample preparation involves acid extraction (Anwar et al., 2004; De Leonardis et al., 2000; Dugo et al., 2004; Jacob & Klevay, 1975), solid phase extraction (SPE) (Batı & Cesur, 2002) or extraction with special agents (Köse Baran & Bağdat Yaşar, 2010).

After the extraction of metals from oil with nitric acid, hydrochloric acid or acid mixture, the extracts are analyzed. Despite the fact that extraction method has the same advantage both in the separation and preconcentration of metals in oil samples, the recoveries are not satisfactory for many metals in most cases. Batı and Cesur described another method for the preconcentration and separation of copper in edible oils, based on using a solid Pb-piperazine-dithiocarbamate complex for extraction and a potassium cyanide solution for back extraction (Batt & Cesur, 2002).

Anwar et al. reported a simple acid-extraction method for the determination of trace metals in oils and fats. The method has been performed with the use of ultrasonic intensification and successfully applied for accurate determination of iron, copper, nickel and zinc in oils (Anwar et al., 2004). Many extraction procedures are available in literature, the summary of these is given in Table 2.
| Extraction method                                      | Metals determined | Detection technique | Notes                                      | Reference                  |
|--------------------------------------------------------|-------------------|---------------------|--------------------------------------------|----------------------------|
| Extraction with 10% HNO₃                                       | Fe, Cu            | GF-AAS              | Rec. % 94±23 (Cu); 97±12 (Fe)              | De Leonardis et al., 2000  |
| Extraction with CCl₄ + 2 N HNO₃ (ultrasonic intensification) | Fe, Cu, Ni, Zn    | FAAS                | Rec. % 92-98 (Fe); 91-100 (Cu); 92-97 (Ni); 93-101 (Zn) | Anwar et al., 2004         |
| Extraction with 35% H₂O₂ and 36% HCl (30 min., 90 °C)       | Cd, Cu, Pb, Zn    | dPSA                | Rec. % 96.5±2.1 (Cd); 97.0±2.7 (Cu); 95.0±1.8 (Pb); 93.5±1.7 (Zn) | Dugo et al., 2004          |
| Extraction with conc. HCl and 6% H₂O₂                        | Cu, Ni            | UV-Vis spec.        | Rec. % 90-118 (Cu); 96-100 (Ni)            | Hussain Reddy et al., 2006 |
| Extraction with 10% HNO₃ (50 Hz, 60 s)                      | Cu, Fe, Mn, Co, Cr, Pb, Ni, Cd, Zn | ICP-AES             | RSD %: < 10 (Cu); 5 (Fe); 15 (Mn); 8 (Co); 10 (Cr); 20 (Pb); 5 (Cd); 16 (Ni); 11 (Zn) | Pehlivan et al., 2008      |
| Extraction with CCl₄ and 2 N HNO₃ after pretreatment with conc. HNO₃ (ultrasonic bath, 30 °C) | Fe, Cu, Zn, Ni | FAAS                | Rec. % 96.5-97.5 (Fe); 96.5-97.1 (Cu); 95.8-97.5 (Ni); 96.0-97.8 (Zn) | Anwar et al., 2003         |
| Pb-piperazinedithiocarbamate SPE and KCN back-extraction    | Cu                | FAAS                | Rec. % 91-97                                | Batı & Cesur, 2002         |
| Zn-piperazinedithiocarbamate SPE                             | Cd                | FAAS                | Rec. % 93.1-100                              | Yağan Aşçı et al., 2008    |
| Ultrasonic-Assisted extraction with conc. HNO₃ and H₂O₂ (35 kHz) | Cu, Fe, Ni       | FAAS and ETAAS      | Rec. % 95.9-98.3 (Cu); 95.7-98.2 (Fe); 95.2-97.5 (Ni) | Ansari et al., 2008        |
| Ultrasonic-Assisted extraction with conc. HCl and 30% H₂O₂   | Cu, Pb            | SCP                 | Rec. % 82-107 (Cu); 84-105 (Pb)             | Cypriano et al., 2008      |
| Extraction with N,N′-bis(salicylidene)-2,2’-dimethyl-1,3-propanediaminato (LDM) | Fe, Cu            | FAAS                | Rec. % 100.2±5.6 (Fe); 99.4±2.8 (Cu)        | Köse Baran & Bağdat Yaşar, 2010 |

1Acc.: Accuracy; 2Rec.: Recovery; 3LOD: Limit of detection; 4RSD: Relative standard deviation
al., 2003; Tantaru et al., 2002; Ziyadanoğulları et al., 2008). The chemists have attended to the Schiff bases and their metal complexes because of their widespread applications in biological systems and industrial uses (Issa et al., 2005; İspir, 2009; Kurtaran et al., 2005; Li et al., 2007; Mohamed, 2006; Neelakantan et al., 2008; Prashanthi et al., 2008; Sharaby, 2007).

Although most techniques for metal determinations in edible oils require sample digestion, dilution or emulsification, the improved method can be employed for the same purpose without digestion. The procedure is based on efficient extraction of metals from oil to aqueous solution, and the determination of metals in aqueous phase by FAAS. The proposed approach has been applied for Fe, Cu, Ni and Zn successively. This method includes two main steps. Metal complexes with Schiff bases shown in Fig. 1 were investigated spectrophotometrically as a first step. In this step, the investigation of the complexation reaction as a driving force for the extraction is necessary to decide the appropriate pH and the equilibrium time in terms of complexation efficiency.

![Chemical structure of Schiff base used in the extractions](image)

As a second step, the experimental conditions affecting the extraction efficiency of metals should be researched. In the procedure of metal extraction with a Schiff base, the optimization of parameters -the ratio of Schiff base solution volume to oil mass, the stirring time and the temperature- for the metal extractions has been achieved by carrying out central composite design (CCD) as an optimization method.

As shown in Table 3, the CCD consisting of a combination of 2\(^3\) full factorial design and a star design was used, in which three independent factors were converted to dimensionless ones (x\(_1\), x\(_2\), x\(_3\)) with the coded values at 5 levels: -1.682, -1, 0, +1, +1.682.

| Factors | Levels |
|---------|--------|
| x\(_1\) (1\(^{st}\) factor) | VLDM / mol ratio (mL g\(^{-1}\)) | -1.682 | -1 | 0 | +1 | +1.682 |
| | 0.159 | 0.5 | 1 | 1.5 | 1.841 |
| x\(_2\) (2\(^{nd}\) factor) | Stirring time (minute) | 9.56 | 30 | 60 | 90 | 110.46 |
| x\(_3\) (3\(^{rd}\) factor) | Temperature (°C) | 13.18 | 20 | 30 | 40 | 46.82 |

Table 3. Variables, levels and the values of levels used in CCD (Köse Baran & Bağdat Yaşar, 2010)
Fifteen experiments should be done in a CCD. Additionally, to estimate the experimental error, replications of factor combinations are necessary at the center point (the level, 0). Experiment at the center point has been repeated five times. The total number of experiments in the CCD with three factors then amounts to 20 (Morgan, 1991; Otto, 1999). Accordingly, 20 experiments given in Table 4 were carried out in the extent of the CCD optimization procedure.

| Experiment no. | \( V_{LDM} / m_{oil} \) (mL g\(^{-1}\)) \( x_1 \) | Stirring time (min.) \( x_2 \) | Temperature (°C) \( x_3 \) |
|----------------|--------------------------------|----------------|----------------|
| 1              | -1                             | -1             | -1             |
| 2              | +1                             | -1             | -1             |
| 3              | -1                             | +1             | -1             |
| 4              | +1                             | +1             | -1             |
| 5              | -1                             | -1             | +1             |
| 6              | +1                             | -1             | +1             |
| 7              | -1                             | +1             | +1             |
| 8              | +1                             | +1             | +1             |
| 9              | 0                              | 0              | 0              |
| 10             | -1,682                         | 0              | 0              |
| 11             | +1,682                         | 0              | 0              |
| 12             | 0                              | -1,682         | 0              |
| 13             | 0                              | +1,682         | 0              |
| 14             | 0                              | 0              | -1,682         |
| 15             | 0                              | 0              | +1,682         |
| 16             | 0                              | 0              | 0              |
| 17             | 0                              | 0              | 0              |
| 18             | 0                              | 0              | 0              |
| 19             | 0                              | 0              | 0              |
| 20             | 0                              | 0              | 0              |

Table 4. The coded values of levels for the experiments in the extent of CCD

Organo-metallic standards in oil (Conostan code number; 354770 for iron, 687850 for copper) were used in CCD and metal concentrations of oil standards were fixed to be a certain concentration. The metal concentrations of the extracts gained from each experiment were determined by FAAS. The obtained results were used in order to establish recovery values for the extraction of metals from oil. The response values \( y \) were calculated from experimentally obtained recovery percentages. The empirical equations were developed by means of response values (Morgan, 1991; Otto, 1999). The following \( y \) equations were constructed based on the \( b \) values which were calculated by applying to the appropriate matrices.

\[
y = b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{123} x_1 x_2 x_3 \quad (1)
\]
New corresponding equations were obtained by equalization of the derivatives of $y$ equation in terms of $x_1$, $x_2$, $x_3$ to zero and solved using software to provide optimum extraction conditions. Optimum conditions are variable depending on the structure of Schiff base and significant metal. The found optimum conditions are given in Table 5 when LDM (Q and P = CH$_3$; X, Y and Z = H) was used as a Schiff base. The recovery values for the extraction of Cu and Fe from oil under the optimum experimental conditions were found to be 99.4(±2.8) and 100.2(±5.6)%, respectively ($n$=10). To test the applicability of the improved procedure, it was applied on spiked olive, sunflower, corn and canola oils. The recovery percentages were varied between 97.2-102.1 for Cu and 94.5-98.6 for Fe (Köse Baran & Bağdat Yaşar, 2010).

| Metal | $V_{LDM} / m_{oil}$ ratio (mL g$^{-1}$) | Stirring time (min.) | Temperature ($^\circ$C) |
|-------|---------------------------------|-------------------|-----------------|
| Cu    | 0.76                            | 73                | 31              |
| Fe    | 1.19                            | 67                | 28              |

Table 5. Optimum extraction conditions for determination of Cu and Fe in edible oils (Köse Baran & Bağdat Yaşar, 2010)

The improved determination strategy after the extraction with Schiff bases has main advantages like independency from hard oil matrix, elimination of explosion risk during decomposition, no requirement for expensive instruments, high accuracy, sensitivity, rapidity and cheapness.

3. Direct determination

The direct determination of metals in oils can be carried out by sample solubilization in an organic solvent, an emulsification procedure in aqueous solutions in the presence of emulsifiers such as Triton X-100 or a solid sampling strategy.

3.1 Dilution with organic solvent

The procedure of the dilution with organic solvents is an easy way to sample pretreatment before detection, but has some requirements: special devices for sample introduction e.g. for FAAS (Bettinelli et al., 1995), the addition of oxygen as an auxiliary gas in ICP-OES or ICP-MS (Costa et al., 2001). The volatile organic solvents have been directly introduced into ICPs for many years, but this can cause plasma instability, less sensitivity, less precision and high cost. Al, Cr, Cd, Cu, Fe, Mn, Ni and Pb contents of olive oil were investigated using diethyl ether, methyl isobutyl ketone (MIBK), xylene, heptane, 1,4-dioxane as solvent and N,N-hexamethylenedithiocarbamic acid, hexamethyleneammonium (HMDC-HMA) salt as a modifier by ETAAS (Karadjova et al., 1998). A transverse heated filter atomizer (THFA) was employed for the direct determination of Cd and Pb in olive oil after sample dilution with n-heptane (Canario & Katskov, 2005). Moreover, Martin-Polvillo et al. (1994) and List et al. (1971) determined trace elements in edible oils based on the direct aspiration of the samples, diluted in MIBK. In another research, the mixture of 2% lecithin-cyclohexane was used to introduce the oil samples to a polarized Zeeman GFAAS (Chen et al., 1999). Van Dalen was
also used lecithin and the organopalladium modifier solutions for the injection of the edible oils (Van Dalen, 1996).

### 3.2 Emulsification

Taking into account parameters such as economy, safety, environment, time, and low risk of contamination, emulsification appears beneficial over microwave assisted acid digestion. On the other hand, optimization of the particle size effect, slurry concentration and homogeneity are necessary in order to obtain good precision and recoveries with slurry techniques. In spite of optimization, complete destruction of the sample matrix in plasma and then liberation of analyte from the sample matrix are not always succeeded, causes unsatisfactory results. An alternative technique for introduction of oil sample directly into ICP is the on-line emulsification (Anthemidis et al., 2005). Direct introduction of oil samples in the form of emulsion into ICP facilitates the spray chamber and plasma torch owing to no need of extra oxygen or sophisticated desolvation device. In such a case, the use of stable emulsions with proper surfactant concentration is very important (Anthemidis et al., 2005).

Emulsification as sample preparation has been performed for the determination of trace metals in vegetable oils by ICP-OES (De Souza et al., 2005; Murillo et al., 1999), ICP-MS (Castillo et al., 1999; Jimenez et al., 2003), FAAS (List et al., 1971) and GF-AAS (Lendinez et al., 2001). Additionally, the use of microemulsion as sample preparation for vegetable oil analysis by High-Resolution Continuum Source FAAS (HR-CS FAAS) has been described by Nunes et al. (2011). The determination of Zn, Cd and Pb in vegetable oils by electrothermal vaporization in combination with ICP-MS (ETV-ICP-MS) was described in literature (Huang et al., 2001).

### 3.3 Direct solid sampling

Direct introduction of oil samples into the graphite furnace by solid sampling strategy is rarely used, providing an alternative methodology. Due to technical improvements in spectrophotometer and software capabilities of modern instrumentation, this method has not been entirely accepted (Sardans et al., 2010). Direct solid sampling has some advantages such as no sample dilution, satisfactory LOD levels, calibration probability with aqueous analytical solutions, simple analysis and no sample digestion or extraction. Other advantages of this method are reduced time and cost, required little amount of sample and the achievement of high sensitivity. Additionally, it reduces the risk of contamination due to the nonexistence of sample preparation and use of chemical reagents. Some disputes against the method are the difficulty of introducing small sample masses, faulty measurement of the results due to the heterogeneity of some natural samples and the limiting linear working range of AAS (Sardans et al., 2010). Despite these restrictions, direct solid sampling is a reasonable alternative for the determination of the total content of metals in oils, since it needs almost no sample preparation. A method for the direct determination of Ni and Cu in vegetable oils by GFAAS using the solid sampling strategy has been reported without sample dilution by Matos Reyes et al. (2006).

### 3.4 Flow injection

Various detection techniques like ETAAS, FAAS, ICP-OES, ICP-MS, voltammetry have been utilized for metal determination in oils. However, all of them have the need for sample
pretreatment procedures in common like: wet digestion, dry ashing, extraction and dilution with organic solvent in order to eliminate hard organic matrix. In the processing large numbers of samples, flow injection analysis (FIA) systems can be preferred for sample pretreatment. The FIA system for oil analysis is frequently based on the on-line preparation of oil-in-water emulsions by using ultrasonic bath with serious drawbacks in efficient preparation of stable emulsions. By this way, more concentrated emulsions (high oil concentration) can be introduced into the plasma and thereby the LODs were improved. A limited number of researches related to metal determination in oils by FIA systems have been presented. Jimenez et al. succeeded multi-element determination in virgin olive oil by flow injection ICP-MS using with HNO₃ and Triton X-100 as emulsifying agents (Jimenez et al., 2003). A magnetic-stirring micro-chamber has been developed for on-line emulsification and has been successfully employed by Anthemidis et al. to detect Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pb, TI and Zn in olive oil using flow injection ICP-OES (Anthemidis et al., 2005). The low concentration of analyte in the sample analysed and difficulty of obtaining stable emulsions with rich oil content were reported as the main problems. On-line emulsion preparation procedure was suggested as simpler, more effective, less time consuming, less labor intensive, less matrix interferences and less contamination risk over the other direct sample introducing procedures. The direct determination of Cu and Fe in edible oils based on the flow injection standard addition method by FAAS was performed without sample dilution in a previous study (Carbonell et al., 1991).

As mentioned above, various pretreatment procedure and detection techniques have been employed for the total determination of metals in olive oil. The researchers have dealt with metallic contents of olive oils during last few decades. As can be seen in Table 6, the concentration range of total amount is given for many metals.

| Metal | Concentration (µg g⁻¹) (ng g⁻¹) | References |
|-------|---------------------------------|------------|
| Minimum | Maximum | (Anthemidis et al., 2005; Benincasa et al., 2007; Buldini et al., 1997; Calapaj et al., 1988; Cindric et al., 2007); De Leonardis et al., 2000; Llorent-Martinez et al., 2011a, 2011b; Martin-Polvillo et al., 1994; Mendil et al., 2009; Nunes et al., 2011; Pehlivan et al., 2008; Zeiner et al., 2005) |
| Fe | 12.5* | 139.0 |
| Cu | 1.7* | 4.51 |
| Ni | 10.6* | 2.26 |
| Zn | 0.6* | 4.61 |
| Metal | Mean  | SD    |
|-------|-------|-------|
| Mn    | 0.7   | 0.15  |
| Pb    | 0.42  | 0.032 |
| Co    | 0.23  | 5.45  |
| Cd    | 0.6   | 0.15  |
| Cr    | 0.012 | 2.00  |
| V     | 0.005 | 0.46  |
| Ge    | 0.03  | 0.04  |
| Zr    | 0.01  | 0.04  |
| Ba    | 4.9   | 0.7   |
| Al    | 0.030 | 1.11  |
| Be    | 0.118 | 0.178 |
| Sc    | 49.94 | 747.9 |
| As    | 1.248 | 26.65 |
| Se    | 1.47  | 6.78  |
| Sr    | 1.52  | 48.9  |
| Y     | 0.082 | 0.331 |
| Sb    | 0.194 | 0.411 |
| Sm    | 0.004 | 0.226 |
| Eu    | 0.004 | 0.021 |
| Gd    | 0.003 | 0.094 |
| Sn    | 0.126 | 0.159 |
| Mg    | 0.056 | 4.61  |
| Ca    | 0.63  | 76.0  |
| K     | 0.05  | 2.14  |
| Na    | 8.7   | 38.03 |

Table 6. The metal levels for olive oils.
4. Speciation and fractionation

Fractionation was defined as “the process of classification of analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties”, and speciation of an element was also defined as “distribution of an element amongst defined chemical species in a system” by Templeton et al. (2000). The physicochemical form of an element, i.e. the actual species found in exposure medium and in the different body fractions, is frequently determinant in the evaluation of its bioavailability and toxicity (Flaten, 2002). An element can be found in various species: anionic or cationic inorganic forms, inorganic compounds, complex compounds with protein, peptide etc. Some organometallic compounds are much more toxic than the ions of the corresponding inorganic compounds. Hg, Pb and Sn obey this rule, for example, methyl-Hg and inorganic Hg are both toxic, but methyl-Hg show more toxicity than other (Templeton et al., 2000). In contrast to this, in the case of As and Se, most organo-arsenicals are less toxic than inorganic As species, organic forms of Se are ordinarily less toxic than Se(IV) (Kot & Namiesnik, 2000).

The determination of the total amount of an element in samples cannot give adequate information for understanding its bioavailability or toxicity, that’s why the fractionation and speciation of metals in oils are increasingly gaining importance. The fractionation and speciation analysis are more informative than total element determinations for all type of samples.

In general, many works dealing with the total amount of elements in oil samples are reported, but fractionation and/or speciation analysis in vegetable oils are less common in literature. To the best of our knowledge, magnesium fractionation analysis in olive and olive oil was cited firstly in 2004. The improvement of an analytical scheme for fractionation of magnesium in olive products and also the determination of Mg amounts absorbed in stomach and intestine was achieved by Bağdat Yaşar & Güçer (2004). It was reported that 3.37-8.47% of Mg was absorbed in the stomach (ionic and polar groups) and the remaining percentage of Mg was absorbed in the intestine (molecular and complexed structures) in olive oil. As can be seen, the Mg fraction in olive oil is almost absorbable in the intestine. This study can be accepted as a preliminary step for fractionation studies and the fractionation and/or speciation approach for other elements will be described in the future.

5. Detection techniques

Various researchers deal with determination of metals in oils at trace, ultra-trace levels using spectrometric and electrometric techniques. Mentioned detection techniques may be combined with some chromatographic systems. Oils have been analyzed for different metals using atomic absorption spectrometer (FAAS and GFAAS), inductively coupled plasma optical emission spectrometer (ICP-OES), inductively coupled plasma mass spectrometer (ICP-MS). ICP techniques have become more popular since the early 1990s. Although the use of AAS (flame, graphite furnace, hydride generation and cold vapour) has declined during the same period, it is still the most widely used technique (Rose et al., 2001).
Each technique has some special requirements, advantages and disadvantages according to its basic principle. GF-AAS is a sensitive, proper for direct introduction of oil samples in the form of emulsion and does not require a large amount of sample. FAAS and ICP-MS have a requirement of sample pretreatment, but ICP-MS is more sensitive and expensive when compared with FAAS. There are scarce researches dealing with oil samples related to voltammetric and potentiometric techniques such as Ad-SSWV, dPSA (Abbasi et al., 2009; Cypriano et al., 2008; Dugo et al., 2004; Galeano Diaz et al., 2006; Lo Coco et al., 2003).

6. Conclusion

Trace quantities of some metals are naturally present in olive oil. It could be possible to determine the levels of different trace metals with the help of precise and accurate analytical methods. In many cases, a sample pretreatment process is necessary to eliminate the oil matrix prior to the introduction of the sample into the instrument. A direct determination is also possible by sample solubilization in an organic solvent, an emulsification procedure or a solid sampling strategy when ETAAS, GF-AAS or ICP are used for the analysis of edible oils. Microwave-assisted wet digestion sample pretreatment is also employed combined with sensitive detection techniques. An alternative technique can be achieved efficiently and precisely by FAAS after the extraction of metals with a Schiff base ligand.

7. Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AAS          | Atomic Absorption Spectrometry |
| FAAS         | Flame Atomic Absorption Spectrometry |
| GF-AAS       | Graphite Furnace Atomic Absorption Spectrometry |
| ETAAS        | Electrothermal Atomic Absorption Spectrometry |
| ICP          | Inductively Coupled Plasma |
| ICP-OES      | Inductively Coupled Plasma Optical Emission Spectrometry |
| ICP-MS       | Inductively Coupled Plasma Mass Spectrometry |
| Ad-SSWV      | Adsorptive Stripping Square Wave Voltammetry |
| dPSA         | Derivative Potentiometric Stripping Analysis |
| SCP          | Stripping Chronopotentiometry |

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