Health risk assessment and micro determination of trace elements content in Egyptian olive oil using ICP-OES

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Abstract. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was applied to investigate multi elements (Zn, Cu, Pb and Cd) of Egyptian olive oil collected from different regions in Egypt. This process involves digestion using 10% nitric acid. The data showed that the concentration ranges were 0.6486-2.4166 μg/g, 0.0092-0.6919 μg/g, 0.0185-0.1026 μg/g and 0.0009-0.0186 μg/g for Zn, Cu, Pb and Cd, respectively. The results indicated that the highly toxic metals content did not exceed that recommended by FAO/WHO. The proposed method exhibited an excellent accuracy, adequate selectivity and high sensitivity. For the first time, this method described the direct determination of the previously mentioned elements and investigated the effect of both temperature and time for different samples in Egyptian olive oil.

1. Introduction
Olive oil is considered a major part of daily food regimes. Olive oils are commonly used in cosmetics, cooking processes, chemical industries and pharmaceutical [1]. Virgin olive oil can be extracted principally by pressure, other mechanical processes and without treatments [2]. Olive oil enhances the human health; it contains sufficient amounts of essential fatty acids and anti-oxidative compounds. The mechanism of olive oil in human body involves penetration rate of fatty acids in to artery wall and therefore, decreasing the risk of cardiovascular diseases and cancers [3, 4]. Spain, Italy and Greece are the major producer for olive oil [5]. The trace metal can be determined for evaluation of its quality [6]. Characterization and adulteration detection of olive oil is affected through trace element analysis [7]. Furthermore, the presence of metals in olive oils can be affected by many factors such as soil, plant genotype, environment, fertilizers and/or metal containing pesticides or by contamination from the metal processing equipment [7, 8]. Also, existing of these elements is essential for the traceability of oil. Therefore, the traceability of the oil is unaffected by transportation, storage, milling and retailing. Consequently, evaluation of trace-element represents a great interest which can be attributed to the potential toxicity of the elements [9].

The human body intakes heavy metals such as copper (3mg), zinc (60mg) and lead (214μg/g) [10]. Although copper metal is necessary for good health, high intake causes adverse health problem such as liver and kidney damage [11]. Simultaneously, the deficiency of copper leads to leucopenia, hypochromic anemia and osteoporosis especially in children [12]. Concerning zinc, it is well known that most metabolic pathways in humans required its presence and skin changes, loss of appetite, growth retardation as well as immunological abnormalities take place in case of its absence [13, 14].
Also, the highly toxic cadmium metal is present in soil and spread in the environment which may be attributed to human activities. High concentration of cadmium may cause rise to renal, skeletal, pulmonary, hepatic, cancer and reproductive effects [14 -17]. In addition, lead metal behaves similar to cadmium metal i.e. it has no useful role in human metabolism and consequently it produces high toxicity [18]. Also, it creates health disorders such as tiredness, sleeplessness, weight and hear loss [19].

It is worthy to mention that classical method ability for heavy metal determination in olive oil can be summarized as follows: wet digestion, dry ashing, acid extraction, closed vessel and focused open-vessel microwave dissolution and dilution [20, 21]. Recently, modern analytical techniques such as ICP-OES and electro analytical method [7, 13, 22, 23, 24] are applied.

The aim of this work is focused on the analysis of trace elements (Zn, Cu, Pb and Cd) in Egyptian olive oils using (ICP-OES).

2. Experimental

2.1. Instrumentation

Thermo Scientific iCAP 6200 inductively coupled optical emission spectrometer (ICP-OES) was used for simultaneous multi-element detection of Zn, Cu, Pb and Cd. The iCAP 6200 ICP-OES (Waltham, Massachusetts, USA) now incorporates a wider wavelength range of 175-847nm. The operating conditions of ICP-OES were reported in table 1.

Table 1. The operating conditions for ICP –OES analysis.

| Instrument | ICP6200 |
|------------|---------|
| RF power (W)| 1150W   |
| Gas flow rate (ml/min) | 0.5L/min |
| Auxiliary gas | 0.5L/min |
| Coolant gas | 12 L/min |
| Nebulizer Argon flow | 0.6 L/min |
| Center tube | 1.5 mm   |
| Torch | Axial |
| Elements monitored | Cu (324.754), Zn (213.856), Cd (214.438), Pb (220.353) |

2.2. Reagents

All reagents were of analytical reagent grade unless otherwise stated. Deionized water (Milli-Q Millipore 18.2 MX-cm resistivity) was used for all dilutions. Nitric acid (16 M) and HCl (11.65 M) were purchased from Merck, Germany. All containers, including test tubes with stoppers were cleaned with hydrochloric acid 5% followed by deionized water.

2.3. Olive oil samples

In this study, eleven Egyptian olive oils samples were purchased from Egypt supermarkets. Siwa, North Sinai, El Giza, Marsa Matruh and Alwahat are the most widely accepted and frequently consumed regions in Egypt. The collected oil samples were packed in polyethylene bags and below 4 °C until analysis.

2.4. Procedure

Aliquots (2.0-3.0 g) of olive oils were weighed directly into the test tube and 1ml nitric acid 10% was added. The oil-acid mixture was shaken at 50 Hz for 60 s with a test tube mixer until the layers were completely mixed. The capped test tube was placed in a shaking water bath at 50 °C for 2 h. After centrifugation at 2800 rpm for 10 min, the lower acid aqueous layer was withdrawn with a pipette and was filled to 25 ml by adding deionized water and then loaded directly into the auto sampler of the (ICP-OES) [25].
3. Results and discussion

3.1. Trace element determination

ICP-OES technique is considered a useful method for micro determination of metals in Egyptian olive oil samples, as shown in Table 2. The concentration of metal found in food could not be modified by traditional techniques and in some cases, it was found that washing may lower their content [26].

In this study eleven brands were collected from five Egyptian regions [Siwa (Altahhan, Al captain, Olive oil), North Sinai (Alqasimuh), Al Giza (Makkah, khoshala), Marsa Matruh (Ashbal, Alkhayrat) and Alwahat (Virgin olive oil, Waha, alwada)] were used for analysis.

The concentration range of copper in the present work is 0.0092-0.6919 μg/g. The concentration of Cu was partially higher than the recommended legal limits. These results are in agreement with that of other authors, whom quantified this metal in different olive oil samples at levels higher than the legislated limits [7, 27]. According to FAO/WHO organization and international requirements, the approved contents of these metals in oils are: 0.1 μg/g (Cu, Pb), 0.05 μg/g (Cd) and 0.04–0.70 μg/g (Zn) [10, 18, 21, 28, 29]. The copper level becomes safe up to 10 mg for a 60 kg adult [30].

Lead and cadmium, very toxic elements, may be responsible for environmental contamination. Specially, the content of lead would be warranted by the presence of highways, metallurgic industries near plantations or streets [17]. The highest content of cadmium and lead in our samples was 0.0186 and 0.1026 μg/g for Cd and Pb, respectively. As shown in table 2 all Cd and Pb concentrations were lower than the legal limits.

Zinc is essential in human nutrition, enhance physical growth and reduce child illness [31]. Zn content in tested samples is ranged from 0.6486-2.4166 μg/g. The recommended daily intake values of Zn are 15 mg for adult males and 12 mg for adult females [14].

| Sample | Sample weight | Cd(μg/g) | Cu(μg/g) | Pb(μg/g) | Zn(μg/g) |
|--------|---------------|----------|----------|----------|----------|
| 1      | 2.72          | 0.0092   | 0.6919   | 0.0738   | 1.8362   |
| 2      | 2.68          | n.d.     | 0.0092   | 0.0279   | 1.8216   |
| 3      | 2.69          | 0.0186   | 0.3169   | 0.0372   | 1.1245   |
| 4      | 2.70          | 0.0046   | 0.2870   | 0.0185   | 2.4166   |
| 5      | 2.71          | n.d.     | 0.0184   | 0.0277   | 2.0479   |
| 6      | 2.50          | 0.0020   | 0.4300   | 0.0900   | 2.3900   |
| 7      | 2.61          | n.d.     | 0.2107   | 0.0670   | 2.3754   |
| 8      | 2.71          | 0.0046   | 0.0331   | 0.0947   | 0.6486   |
| 9      | 2.69          | n.d.     | 0.0279   | 0.0465   | 1.5242   |
| 10     | 2.68          | 0.0009   | 0.1119   | 0.1026   | 1.8377   |
| 11     | 2.68          | n.d.     | 0.1306   | 0.1019   | 2.0336   |

n.d.=not determined

3.2. Effect of temperature

Digestion of Egyptian olive oil samples using HNO₃ under safe condition, is necessary and the temperature digestion was examined (40-80 °C). This test was applied for sample no. 8 as an example. It is obvious to note that by increasing the temperature digestion, the digestion efficiency is also improved within the range 40-60 °C. At 70 and 80 °C, the temperature digestion is presented as “0g” [32, 33], except in the case of zinc metal where an improvement in digestion efficiency was observed as shown in table 3. This behavior can be ascribed to the difference in density between zinc and other metals studied. In addition, it was observed that digestion efficiency for all metals was performed at 50 °C as an optimum temperature.
Table 3. The temperature effect on digestion efficiency for sample no. 8

| Sample weight | Cd(μg/g) | Cu(μg/g) | Pb(μg/g) | Zn(μg/g) |
|---------------|----------|----------|----------|----------|
| T₀₀          | 2.71     | 0.0046   | 0.0191   | 0.0845   | 0.5060   |
| T₀₅₀         | 2.75     | 0.0046   | 0.0331   | 0.0947   | 0.6486   |
| T₆₀₀         | 2.76     | 0.0054   | 0.2355   | 0.1232   | 0.8739   |
| T₇₀₀         | 2.73     | n.d.     | n.d.     | n.d.     | 0.8816   |
| T₈₀₀         | 2.70     | n.d.     | n.d.     | n.d.     | 0.9111   |

nd.=not determined

3.3. Effect of time

It is of great interest to note that, digestion efficiency increases as the time increases (1-4h), except in case of lead and zinc where the digestion decreases when the time reached 4h. This behavior is similar to that published recently according to Manjusha et al., (2019), applying ultrasound-assisted extraction of Pb, Cd, Cr, Mn, Fe, Cu, Zn from edible oils with tetramethylammonium hydroxide and EDTA followed by determination using graphite furnace atomic absorption spectrometer[34]. It is obvious to observe that the digestion efficiency of lead and zinc decreased above 4h as shown in table 4.

Table 4. Effect of time on digestion efficiency for sample no. 8

| Sample weight | Cd(μg/g) | Cu(μg/g) | Pb(μg/g) | Zn(μg/g) |
|---------------|----------|----------|----------|----------|
| 1h            | 2.71     | 0.0034   | 0.0326   | 0.0838   | 0.5346   |
| 2h            | 2.73     | 0.0046   | 0.0331   | 0.0947   | 0.6486   |
| 3h            | 2.69     | 0.0064   | 0.0856   | 0.1445   | 0.7677   |
| 4h            | 2.75     | 0.0101   | 0.1068   | 0.0727   | 0.5226   |

3.4. Comparison study

It is of great interest to note that the Italian olive oil is considered one of the best olive oils in the world, concerning its quality [35]. This behavior can be attributed to the low concentration of toxic heavy metals (Cd and Pb) according to national and international requirements. Whereas, high concentration of lead and cadmium was observed in Greece olive oil; the limits exceeded national and international requirements [36]. In addition, the Egyptian olive oil followed FAO/WHO for the toxic heavy metals (Cd and Pb) and therefore, it is favorable to use the Egyptian olive oil with sufficient safety, as shown in table 5.

Table 5. Comparison between heavy metals (μg/g) content in Egyptian olive oil and those published in European countries besides Turkey

| Country | Method  | Pb     | Zn     | Cd     | Cu     | Ref.  |
|---------|---------|--------|--------|--------|--------|-------|
| Spain   | ICP-MS  | -      | 0.06-0.43 | -      | 0.03-0.33 | [21] |
| China   | ICP-AES | 0.009-0.016 | 1.24-1.58 | 0.0023-0.0027 | 0.24-0.27 | [18] |
| Turkey  | ICP-AES | 0.04-0.10 | 1.25-1.58 | 0.043-0.058 | 0.06-0.11 | [36] |
| Italy   | ICP-MS  | 0.03   | -      | 0.00015 | -      | [1]   |
| Greece  | ICP-MS  | 1.09-7.13 | 0.32-2.54 | 0.01-0.21 | 0.01-0.23 | [35] |
| Egypt   | ICP-OES | 0.018-0.102 | 0.64-2.41 | 0.0009-0.0186 | 0.009-0.691 | This work |

4. Conclusion

This work describes determination of four heavy metals (Cu, Zn, Cd and Pb) in eleven varieties of Egyptian olive oils and determined using ICP-OES technique. The result indicated that a clear difference existed in some metals concentration among different varieties of Egyptian olive oil.
samples. The copper levels were found to be higher than the recommended legal limits in some olive oil sample. In addition, the estimated intakes of Cu, Zn, Cd and Pb from daily consumption of 25 g or weekly consumption of 175 g of the investigated olive oils should pose no risk to human health.

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