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Determination of Metal Contents in Edible Vegetable Oils Produced in Iran Using Microwave-assisted Acid Digestion

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Abstract

The concentration of heavy metals in vegetable oils is an important criterion for the assessment of oil qualities with regard to freshness, keeping properties, storage and their influence on human nutrition and health. Trace heavy metals in vegetable oils are known to have an effect on the rate of oil oxidation. In this work, the contents of lead (Pb), cadmium (Cd), nickel (Ni), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) in four varieties of edible vegetable oils (olive oil, canola oil, sunflower oil and soybean oil) collected from Iran were determined using atomic absorption spectrometry (AAS). The samples were digested in microwave digestion system The concentration of nickel, manganese, zinc, copper, iron, calcium and magnesium were observed in the range of 0.91–2.17, 0.14–1.76, 3.58–9.54, 0.18–0.68, 7.78-28.93, 21.42–78.52, 5.34–36.49 μg/g, respectively. Lead and cadmium were found to be 4.56–15.82 and 1.87–8.58 μg/kg. We found that the content of the heavy metals in all of the tested oils was lower than the maximum values recommended for FAO/WHO.

***Keywords**: Heavy metals, Vegetable oils, Microwave-assisted digestion.*

Introduction

There is currently considerable interest in the determination of trace elements in edible oils. The determination of elements in edible oils is important because of both the metabolic role of metals and possibilities for adulteration detection and oil characterization. The oils and fats are major components of the human diet, along with carbohydrates and proteins. They are energy dense, useful in achieving a

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necessary level of caloric intake. Fats and oils are necessary for the delivery and absorption of the fat-soluble vitamins (A, D, E and K). They are of vegetable or animal (including milk) or marine origin. Vegetable oils are widely used in the cooking and the processing, cosmetics, pharmaceutical and chemical industries [1]. One of the most important quality criteria of vegetable oils is its metal content. The presence of metals in vegetable oils is due to both endogenous factors, connected with the plant metabolism and exogenous factors due to contamination during the agronomic techniques of production and the collection of olives and seeds during the oil extraction and treatment processes, as well as systems and materials of packaging and storage [2, 3]. Specific to refining is the introduction of nickel, which is used as a hardening agent [4]. The presence of metals in the final refined oil is undesirable because the metals can facilitate oxidative degradation of the oil and decrease shelf life. Trace levels of metal ions are known to have adverse effects on the flavor, color and odor. Copper and iron in particular greatly reduce the oxidative stability of oil. Transition metals catalyze the decomposition of hydro-peroxides, aldehydes, ketones and acids. These compounds may develop pathological effects on the digestive system [5]. They also increase carcinogenic effect by reacting with other food components such as proteins and pigments. The presence of calcium and magnesium in crude oils reduces the efficiency of degumming and refining operations. Nickel, an artifact of hydrogenation, must be removed from the oil for health, stability and safety considerations. The U.S. Food and Drug Administration has recently expressed interest in defining lead level exposure from edible oils, because even trace levels in oils that have high consumption can result in significant exposure. The exposure to very low levels of lead and cadmium has been shown to have cumulative effects since there is no homeostatic mechanism which can operate to regulate the levels of these toxic substances. Lead intoxication has been reported to be associated with neurological problems, renal tubular dysfunction and anemia [6]. Cadmium is a highly toxic element that accumulates in biologic systems and has a long half-life. This toxic element is easily transferred from soil to plants, which are increasingly contaminated by cadmium from phosphate-based fertilizers. Cadmium can also be present in edible oils, as a result of contamination from the environment, the refining process, the storage tank or the packing material (e.g., as a colorant or stabilizer in plastics) [7]. Therefore, accurate determination of trace metal contents is very important in evaluating deteriorating effects.

This study focuses on determination of lead (Pb), cadmium (Cd), nickel (Ni), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) levels

in edible vegetable oils produced in Iran by atomic absorption spectrometry (AAS).

Experimental

Samples preparation

In this study, 40 samples of edible vegetable oils produced in Iran, corresponding to four different species were used for analysis. The oil samples taken from some food supply markets in Iran include olive oil, canola oil, sunflower oil and soybean oil. The collected oil samples were packed in polyethylene bags and stored below -20 °C until analysis.

Reagents, standards and digestion samples

All reagents and standard stock solutions used were from Merck. The nitric and sulfuric acid used for sample preparations were ultrahigh purity grade. All laboratory ware including pipette tips and autosampler cups were cleaned thoroughly with detergent and tap water, rinsed with distilled water, soaked in dilute nitric acid then rinsed thoroughly with deionized distilled water. To avoid contamination of the specimens, all steps in the sample preparation procedure were carried out in a laboratory equipped for trace element analysis.

All calibration curves were based on five standards. The element standard solutions used for calibration were freshly prepared by diluting stock standard solutions for each element (1000 mg/L) in a nitric acid solution (0.1% v/v) immediately before use. The calibration ranges were selected according to the expected concentrations of the elements of interest and depending on the technique applied.

1 gr samples were digested with 6 ml of concentrated HNO_3 (65%) and 2 ml of concentrated $H2O_2$ (30%) in microwave digestion system for 32 min and finally diluted to 10 ml with 2% HNO_3. All sample solutions were clear. A blank digest was carried out in the same way. Digestion conditions for microwave system were applied as 3 min for 500 W, 5 min for 800 W, 8 min for 1000 W, 10 min for 1300 W, vent: 8 min, respectively [8]. Blank solutions were also prepared by using the digestion procedure given above to check the possible analytic contaminations in the reagents used for the sample preparation.

Determination of metals

Lead, cadmium, nickel, manganese and copper were determined by the graphite furnace atomic absorption spectrometry (GF-AAS) (SpectrAA 220, GTA 110, Varian, Australia), equipped with pyrolytically coated graphite tubes and deuterium background correction. Flame atomic absorption spectrometry (F-AAS) (SpectrAA 220, Varian, Australia), equipped with deuterium background correction is the method of choice for determining of zinc, iron, calcium and magnesium. The $NH_4H_2PO_4/Mg(NO_3)_2$ was used as chemical modifier for determination

of lead and cadmium in vegetable oils. The other metals were measured without modifier. All instrument-operating parameters were summarized in Tables 1 and 2.

Table 1. Instrument settings for determining of zinc, iron, magnesium and calcium by FAAS.

Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Fuel gas	Oxidant gas
Zn	213.9	1.0	5.0	Acetylene	Air
Fe	248.3	0.2	5.0	Acetylene	Air
Mg	285.2	0.5	4.0	Acetylene	Air
Ca	422.7	0.5	10.0	Acetylene	Nitrous oxide

Table 2. Selection of lines, pretreatment and atomization temperatures for GFAAS.

Element	Wavelength (nm)	Pretreatment temperature (°C)	Atomization temperature (°C)
Pb	283.0	500	2200
Cd	228.8	500	1800
Ni	232.0	900	2400
Mn	279.5	900	2300
Cu	324.8	900	2500

Method validation

The characteristic parameters of the analytical methods were determined for each metal by means of analysis of blanks, standard solutions, and standard reference material (SRM). These parameters included the recovery of the microwave-assisted digestion, limit of detection, precision (minimal and intermediate) and accuracy.

– The recovery of the microwave-assisted digestion method was checked by the spiked levels of metals in oils samples (a test material with a known addition of analyte).

– Method Detection limit (MDL), defined as three times the standard deviation of the blank signal, and calculated by $C_m=3\delta_b/m$, where C_m is detection limit, δ_b is standard deviation of the blank signal, and m is slope of the regression line.

– Method quantification limit (MQL), calculated by $C_m=10\delta_b/m$.

– Minimal precision was assessed by analysis of SRM multiple times (n=10) over a single run.

– Intermediate precision was evaluated from the analysis of SRM in 1-week periods for 5 weeks. The results were expressed as percent relative standard deviation.

– The accuracy of the measurement was evaluated based on recovery studies and

analysis of two biodiesel reference materials NIST SRM 2772 (soybean biodiesel) and NIST SRM 1570A (trace elements in spinach leaves). Accuracy was above %95 for all of the metals.

The performance of the method was evaluated, then the analytical parameters were checked (Table 3). The concentrations of heavy metals in the all samples were above their respective detection limits. The data revealed an excellent coefficient of correlation range; linearity (R^2) of all metals was above 0.99.

All results were expressed as mean values ± SD, standard deviation refers to the mean concentration of trace elements for each group.

Table 3. Analytical characteristics for trace metals determination in oil samples.

Element	Recovery (%)	Method detection limit (µg/L)	Method quantification limit (µg/L)	Minimal[a] precision %R.S.D.	Intermediate[b] precision %R.S.D.
Pb	100.7	0.56	1.84	4.3	4.9
Cd	98.9	0.74	2.44	5.1	5.5
Ni	96.2	0.45	1.49	3.8	4.6
Mn	104.5	0.49	1.61	2.5	3.5
Zn	98.4	0.03*	0.10*	2.1	3.0
Cu	109.8	0.62	2.04	1.8	2.7
Fe	99.0	0.04*	0.13*	2.2	3.5
Ca	96.7	0.18*	0.59*	3.3	4.6
Mg	97.1	0.12*	0.40*	2.7	3.1

[a] n= 10

[b] The analyses were carried out in 1-week periods for 5 weeks.

*mg/L

Results and Discussion

Results were obtained for all samples, were classified in the following tables for better comparison of each of them. The average amounts of trace elements in four groups of edible vegetable oils supplied from different markets are given in Tables 4 and 5. The concentration of Ni, Mn, Zn, Cu, Fe, Ca and Mg were observed in the range of 0.91-2.17, 0.14-1.76, 3.58-9.54, 0.18-0.68, 7.78-28.93, 21.42-78.52, 5.34-36.49 µg/g, respectively. Pb and Cd were found to be 4.56–15.82 and 1.87–8.58 µg/kg.

In this study, the highest metal concentrations were found in olive oil for Zn (9.54 µg/g), Ca (78.52 µg/g) and Ni (2.17 µg/g), canola oil for Cu (0.68 µg/g), Mg (36.49 µg/g) and Cd (8.58 µg/kg), sunflower oil for Pb (15.82 µg/kg) and soybean oil for Mn (1.76 µg/g) and Fe (28.93 µg/g). The olive oil contains lower amounts of Pb (4.56 µg/kg), Mn (0.14 µg/g) and Mg (5.34 µg/g) compared to the other vegetable oils. The

lowest contents of Fe (7.78 µg/g) and Ca (21.42 µg/g) were found for canola oil, Ni (0.91 µg/g) for sunflower oil and Cd (1.87 µg/kg), Cu (0.18 µg/g) and Zn (3.58 µg/g) for soybean oil.

Table 4. Results for the determination of trace element levels in edible vegetable oils by GFAAS.

Vegetable oil	Metal concentrations (mean±SD)				
	Pb (µg/kg)	Cd (µg/kg)	Ni (µg/g)	Mn (µg/g)	Cu (µg/g)
olive oil	4.56±0.98	4.66±0.34	2.17±0.61	0.14±0.03	0.42±0.03
canola oil	10.57±1.95	8.58±0.82	1.53±0.31	0.45±0.03	0.68±0.03
sunflower oil	15.82±2.06	3.95±0.11	0.91±0.22	0.91±0.03	0.25±0.02
Soybean oil	6.78±1.75	1.87±0.48	1.26±0.21	1.76±0.12	0.18±0.01

The number of oil samples for each group: 10

Table 5. Results for the determination of trace element levels in edible vegetable oils by FAAS.

Vegetable oil	Metal concentrations (mean±SD)			
	Zn (µg/g)	Fe (µg/g)	Ca (µg/g)	Mg (µg/g)
olive oil	9.54±0.13	20.65±3.32	78.52±4.65	5.34±1.06
canola oil	4.87±0.16	7.78±2.17	21.42±2.64	36.49±4.98
sunflower oil	7.24±0.19	17.66±2.25	32.54±1.88	29.10±1.69
Soybean oil	3.58±0.10	28.93±2.32	55.65±5.79	11.36±1.39

The number of oil samples for each group: 10

Food chemical hazards remain a worldwide concern with implications for both health and trade. The FAO/WHO has set a limit for heavy metals intakes based on body weight. For an average adult (60 kg body weight), the provisional tolerable daily intake (PTDI) for copper, zinc, iron, Ni and lead are 3 mg, 60 mg, 48 mg, 100–300 µg/g and 214 µg/g, respectively [9, 10]. The increase in international trade in food is one factor that drives international regulation of trace elements as contaminants in food. Monitoring of trace elements in oils is essential in order to prevent excessive build-up of these metals in the human food chain. The companies to treat products before selling them to markets should take the appropriate measures during the production process. Many countries have regulations to the effect that foods offered for sale may not be contaminated to an extent

that can cause disease or poisoning. In the industrial countries, most hazardous chemical in edible oils appears to be under control. In most developing countries, lack of monitoring systems does not allow a direct assessment of the health impact of chemical contamination.

Trace element contamination of food grade fats and oils is regulated by Codex Alimentarius and national standards. Maximum permissible concentration of lead in vegetable oils is 0.1μg/g [11]. The obtain results show that the mean concentration of lead in vegetable oils produced in Iran is according to European food standards. Lead can trigger both acute and chronic symptoms of poisoning. Acute intoxications only occur through the consumption of relatively large single doses of soluble lead salts. Chronic intoxications can arise through the regular consumption of foodstuffs only slightly contaminated with lead. The danger of chronic intoxications is the greater problem. Lead is a typical cumulative poison. It is known that some living organisms possess the ability to take in and accumulate lead in their structures. Cadmium is today regarded as the most serious contaminant of the modern age. It is a major problem for foodstuffs because of its toxicity. The presence of cadmium in vegetable oils depends on many factors. It might originate from the soil, fertilizers or presence in the industry or highways near the plantations. Cadmium, like lead is a cumulative poison. The danger lies primarily in the regular consumption of foodstuffs with low contamination. This element is concentrated particularly in the kidneys, the liver, the blood-forming organs and the lungs. It most frequently results in kidney damage (necrotic protein precipitation) and metabolic anomalies caused by enzyme inhibitions. We found that the concentration of this hazardous heavy metal in all of the tested oils was very low. Some trace elements in food, which so far generally have been disregarded as contaminants in food, such as nickel, may provoke allergies in particularly sensitive consumers. People suffering from a contact allergy to nickel may have been sensitized to an extent that otherwise insignificant amounts of nickel in food or drink may provoke an attack of the allergy [12]. According to international requirements, the approved contents of these metals in oils are: 0.2 μg/g (Ni) and 0.05 μg/g (Cd) [13].

Some trace elements such as copper, zinc, iron, manganese, magnesium and calcium are essential in very small concentrations for the survival of all life forms. These elements can also be quite toxic in higher concentrations. In addition, small amounts of heavy metals in edible oils are well known to have serious deteriorative effects on the stability of these oils. The difference in the concentrations of these elements can be used for adulteration determination. Mean values obtained in this work for Cu and Fe are lower than the

maximum values recommended for FAO/ WHO [14] (0.1 μg/g for Cu and 1-1.5 μg/g for Fe).

Table 6 compares the contents of heavy metals in vegetable oil samples in Iran with the other countries as cited in literatures. Based on the element analysis, trace elements levels in vegetable oil samples of Iran compared favorably with those reported in the other countries. This study may help in generating data needed for surveillance programs aimed at ensuring the safety of the food supply and minimizing human exposure. It is hoped that monitoring of these metals regularly will increase the consumer confidence in edible vegetable oil products from Iran.

Table 6. Reported contents of heavy metals (μg/g) in vegetable oil samples.

Country	Oil samples	Pb	Cd	Ni	Cu	Fe	Zn	Reference
Spain	Olive oil	----	----	----	0.03-0.33	0.49-2.20	0.06-0.43	15
	Sunflower oil	----	----	----	0.03-0.19	0.27-1.31	0.08-0.33	
	Soybean oil	----	----	----	0.04-0.12	0.38-0.76	0.08-0.27	
China	Olive oil	0.009-0.016	2.39-2.78[a]	0.046-0.061	0.24-0.27	32.8-35.4	1.24-1.58	8
	Sunflower oil	0.006-0.015	3.34-3.75[a]	0.028-0.041	0.052-0.068	27.3-29.8	1.04-1.33	
	Soybean oil	0.012-0.018	4.13-4.52[a]	0.043-0.064	0.048-0.056	21.6-23.1	0.671-0.804	
Italy	Olive oil	0.03	0.15[a]	----	----	----	----	1
	Sunflower oil	0.005-0.016	0.51-4.15[a]	----	0.055-0.153	----	0.175-0.310	
	Soybean oil	0.011-0.043	2.53-5.03[a]	----	0.44-0.854	----	0.03-0.042	
Turky	Olive oil	0.04 -0.10	43 -58[a]	----	0.06-0.11	1.53 -1.99	1.25-1.58	16
	Sunflower oil	0.05 -0.11	35 -52[a]	----	0.04-0.10	1.30-1.71	1.08-1.39	
	Soybean oil	0.04 - 0.10	38 -57[a]	----	0.05-0.13	1.33 -1.68	1.07-1.36	
Pakistan	Sunflower oil	0.79-4.29	1.70-6.18	----	----	----	----	17

[a] ng/g

Conclusion

An important deciding factor in the assessment of quality on edible oils is the heavy metals composition. It is known that heavy metals affect the oils rate of oxidation, nutritional value, preservation properties, and storability. As such, the determination of heavy metal in vegetable oils is very important. Our

researches on vegetable oil samples produced in Iran showed that there is no accumulation of heavy metals in oils.

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