APPLICATION NOTE

Atomic Absorption

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Trace Elemental Characterization of Edible Oils with Graphite Furnace Atomic Absorption Spectrophotometer

Introduction

The determination of the inorganic profile of oils is important because of the metabolic role of some elements in the human organism. On the one hand, there is knowledge of the food's nutritional value, which refers to major and minor elements. On the other hand, there is the concern to verify that the food does not contain some minerals in quantities toxic for the health of the consumers, regardless whether this presence of minerals is naturally occurring or is due to contamination during the production processes. Oil characterization is the basis for further nutritional and food technological investigations such as adulteration detection[1]. The most common adulteration is an addition of a cheaper vegetable oil to expensive oil. Authenticity is a very important quality criterion for edible oils and fats, because there is a big difference in prices of different types of oil and fat products. Adulteration detection is possible by determining the ratio of the contents of some chemical constituents and assuming these ratios as constant for particular oil. In regard to adulteration detection, approaches based on atomic spectroscopy can be attractive[2]. The quality of edible oils with regard to freshness, storability and toxicity can be evaluated by the determination of metals. Trace levels of metals like Fe, Cu, Ca, Mg, Co, Ni and Mn are known to increase the rate of oil oxidation. Metals like As, Cd, Cr, Se etc. are known for their toxicities. The development of rapid and accurate analytical methods for trace elements determination in edible oil has been a challenge in quality control and food analysis. However, sample pretreatment procedures are required in order to eliminate the organic matrix. These include wet, dry or microwave digestion, dilution with organic solvent and extraction methods[3]. The content of metals and their species (chemical forms) in edible seed oils depends on several factors. The metals can be incorporated into the oil from the soil or be introduced during the production process. Hydrogenation of edible seed oils and fats has been

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performed using nickel catalysts. The presence of copper and iron can be caused by the processing equipment as well. Different digestion methods were applied for oil digestion prior to spectrometric measurements. Many of the used wet or dry digestion methods are not recommended for use in high fat material because of the associated safety hazards. GFAAS is a suitable and widely used technique for the trace level determination of metals due to its selectivity, simplicity, high sensitivity, and its capability for determination in various matrices.

This paper reports the development of a simple method for the analysis of edible oil samples by using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). Sample preparation has been done by using a microwave digestion system. Metals like Fe, Cu, Mn, Ni, Cr, As, Cd, Pb, Se and Zn were analyzed using the developed method.

Experimental

The measurements were performed using the PerkinElmer® AAnalyst™ 800 Atomic Absorption Spectrophotometer (PerkinElmer, Inc., Shelton, CT, USA) (Figure 1) equipped with WinLab32™ for AA Version 6.5 software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. PerkinElmer high efficiency double beam optical system and solid-state detector provide outstanding signal-to-noise ratios. The AAnalyst 800 features longitudinal Zeeman-effect background correction for furnace and the solid-state detector which is highly efficient at low wavelengths. The AAnalyst 800 uses a transversely heated graphite atomizer (THGA) which provides uniform temperature distribution across the entire length of the graphite tube. This eliminates the memory effect inherent with the high matrix sample analysis. The THGA features an integrated L'vov platform which is useful in overcoming potential chemical interference effects common to the GFAAS technique. EDL lamps were used whenever available.

Figure 1. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

A Multiwave™ 3000 Microwave system (PerkinElmer/Anton-Paar) was used for the microwave-assisted digestion. This is an industrial-type oven which is equipped with various accessories to optimize the sample digestion.

The samples were digested in the Rotor 8XF100 in eight 100 mL high pressure vessels made of PTFE-TFM protected with its individual ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compare to conventional PTFE. This vessel has a "working" pressure of 60 bar (870 psi) and temperatures of up to 260 ˚C. A Pressure/Temperature (P/T) Sensor Accessory was also used for this work. The P/T sensor simultaneously measures temperature and pressure for one vessel. All vessels' temperatures were monitored with the IR Temperature Sensor Accessory. This measures the temperature of the bottom surface of each vessel liner remotely during the digestion process, thus providing the over-temperature protection to each vessel.

Figure 2. PerkinElmer/Anton Paar Multiwave 3000 Microwave Digestion System.

Standards, Chemicals and Certified Reference Material

PerkinElmer single element calibration standards for Atomic Spectroscopy were used as the stock standards for preparing working standards. All the working standards were prepared daily in Millipore® water (18.2 MΩ cm) acidified in 0.2% Suprapur® nitric acid. Suprapur® nitric acid used for preparing the diluent for standards was from Merck® (Darmstadt, Germany). Chemical modifiers were prepared from stock solutions, by diluting with acidified Millipore® water and were added automatically to each standard, blank and sample by the autosampler AS 800, an integral part of the AAnalyst 800. Standards were prepared in polypropylene vials (Sarstedt®) and were prepared on volume-by-volume dilution. Micropipettes (Eppendorf®, Germany) with disposable tips were used for pippetting solutions. Certified Reference Standard for trace metals in soybean oil from High Purity Standards (Lot # 0827322) was used for quality control. Multi-element ICP standard for trace metal ions in nitric acid from Spex Certiprep®. (New Jersey, USA), prepared at midpoint of the calibration curve was used as quality control check standard.

Sample Preparation

Three common edible oils: coconut oil, sunflower oil and soybean oil were bought from a local supermarket and were used without any pre-treatments. ~0.25 g of each sample, accurately weighed in duplicate was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done in accordance with the program given in Table 3.

The digested samples were diluted with 0.2% HNO_3 and made up to 25 mL in polypropylene vials. Plastic bottles were cleaned by soaking in 10% (v/v) HNO_3 for at least 24 hours and rinsed abundantly in de-ionized water before use.

The instrumental conditions for furnace experiments are given in Table 1, and the graphite furnace temperature programs are listed in Appendix I. A heated injection at 90 ˚C was used for all the experiments. Pyrolytically coated graphite tubes with integrated platforms were used. The autosampler cups were soaked in 20% nitric acid overnight to minimize sample contamination, and thoroughly rinsed with 0.5% HNO_3 acid before use. Five point calibration curves (four standards and one blank) were constructed for all the metal ions and the calibration curve correlation coefficient was ensured to be better than 0.999 before the start of the sample analysis.

Dilution of the order of 1000-10000 were required depending up on the metal ion analyzed.

Results and Discussion

The goal of this method development was to develop a simple method for the quantitative analysis of various toxic metals and other trace metals in edible oils. The validity of the developed method has been ensured by incorporating various Quality Control (QC) checks and analysis of Certified Reference Material (CRM).

The QC samples gave excellent recovery of between 94-111%. Excellent spike recovery for these QC samples ranging between 91-109% was also achieved. The recovery for the reference soybean oil was between 91-107%.

Method detection limits (MDLs) were calculated based on the standard deviation of seven replicates of the samples (student t-value of 3.14 for a confidence interval of 98%).

The excellent detection limits obtained shows the capability of AAnalyst 800 in analyzing difficult matrices at lower concentrations. The calibration curves obtained had correlation coefficient as good as 0.999 for all the metal ions under study.

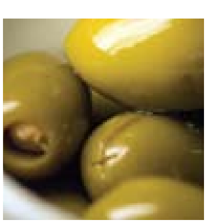
Conclusions

A simple method for the sequential quantitative determination of trace metal impurities in edible oil samples was developed. The patented THGA tube used in the AAnalyst 800 provides a uniform temperature distribution along its entire length. This eliminates cooler temperatures at the tube ends and removes most interference. There is no re-condensation, carry-over and memory effect is eliminated. With the THGA tube design, accuracy and sample throughput are improved by reducing the need for the time-consuming standard additions technique. With the longitudinal Zeeman-effect background correction, the amount of light throughput is doubled by eliminating the need for a polarizer in the optical system. All other commercial Zeeman designs incorporate inefficient polarizers that reduce light throughput and diminish performance. With this unique design, the AAnalyst 800 provides the lowest detection limits available.

In conventional furnace systems, the heating rate during atomization depends on the input-line voltage. As voltage may vary from day to day, season to season or among laboratory locations, so may the heating rate. The high-performance AAnalyst 800 uses enhanced power control circuitry to maintain a uniform heating rate, irrespective of the location of the instrument, one can be sure that it provides outstanding, and consistent performance.

The AAnalyst 800 atomic absorption spectrophotometer also produces highly accurate, fast and reproducible results with difficult matrices such as edible oil. The developed method has been validated by using reference material and the method has been successfully applied for the analysis of different edible oil samples.

The Multiwave 3000 microwave digestion system has proven to be an excellent tool for digesting difficult matrices such as edible oils.



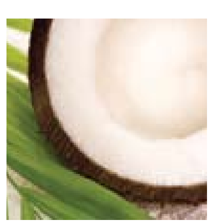
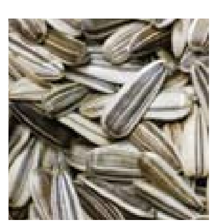
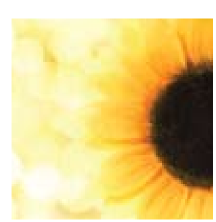





Table 1. **Experimental Conditions of AAnalyst 800.**

Element	Cd	Pb	As	Se	Ni	Cu	Fe	Mn	Cr	Zn
Wavelength (nm)	228.8	283.3	193.7	196	232	324.8	248.3	279.5	357.9	213.9
Slit (nm)	0.7	0.7	0.7	2.0	0.2	0.7	0.2	0.2	0.7	0.7
Mode	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG	AA-BG
Calibration	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.	Linear w/ Calc. int.
Lamp	EDL	EDL	EDL	EDL	HCL	HCL	HCL	HCL	HCL	HCL
Current (mA)	230	440	380	280	25	15	30	20	25	15
Standards (µg/L)	0.5, 1.0 1.5, 2.0	20, 30 40, 50	20, 30 40, 50	25, 50 75, 100	20, 30 40, 50	5, 10 15, 25	5, 10 15, 20	2.5, 5 7.5, 10	4, 6 8, 10	0.4, 0.8 1, 2
Correlation coefficient	0.999831	0.999799	0.999656	0.999789	0.999735	0.999533	0.999035	0.999581	0.999707	0.999972
Spike (µg/L)	1.0	10	10	10	5	2.5	5	1.5	4	0.5
Read time (sec)	5	5	5	5	5	5	5	5	5	5
Measurement	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area
Injection Temp (˚C)	90	90	90	90	90	90	90	90	90	90
Sample Volume µL	20	20	20	20	20	20	20	20	20	20
Matrix modifier	0.05 mg $NH_4H_2PO_4$ & 0.003 mg $MgNO_3$	0.05 mg $NH_4H_2PO_4$ & 0.003 mg $MgNO_3$	0.005 mg Pd and 0.003 mg $MgNO_3$	0.005 mg Pd and 0.003 mg $MgNO_3$	Nil	0.005 mg Pd and 0.003 mg $MgNO_3$	0.015 mg $MgNO_3$	0.005 mg Pd and 0.003 mg $MgNO_3$	0.015 mg $MgNO_3$	0.005 mg $MgNO_3$
Modifier volume µL	5	5	5	5	0	5	5	5	5	5

Table 2. **Program used for Edible Oil Digestion with MDS.**

Sequence	Power	Ramp Time (min.)	Hold Time (min.)	Fan
1	600	5	2	1
2	900	5	2	1
3	1400	15	20	1
4	0	0	15	3
Weight Taken	~250 mg	HNO_3	5 mL	
H_2O_2	3 mL	Rate	0.3 bar/sec	
HCl	1 mL	Pressure	55 Bar	

Table 3. **Results of Edible Oil Analysis.**

Metal	Coconut Oil I µg/g	Coconut Oil II µg/g	Sunflower Oil I µg/g	Sunflower Oil II µg/g	Soybean Oil I µg/g	Soybean Oil II µg/g
Pb	<DL	<DL	<DL	<DL	<DL	<DL
Cd	<DL	<DL	<DL	<DL	<DL	<DL
Cr	0.40	0.43	1.1	1.4	0.39	0.42
As	<DL	<DL	<DL	<DL	<DL	<DL
Se	0.08	0.12	0.10	0.07	0.05	0.03
Zn	0.78	0.79	0.80	1.5	3.9	2.26
Cu	0.12	0.11	0.14	0.13	0.09	0.10
Mn	0.57	0.60	0.21	0.19	0.15	0.18
Ni	0.24	0.23	4.16	5.27	0.37	0.25
Fe	7.05	6.75	3.93	4.3	4.08	4.6

Table 4. **Results of CRM Analysis (Lot # 0827322).**

Metal	Certified Value (µg/g)	% Recovery
Pb	100 ±1.0	100.3
Cd	Not present	–
Cr	Not present	–
As	Not present	–
Se	Not present	–
Zn	100 ±1.0	94.0
Cu	100 ±1.0	96.1
Mn	Not present	–
Fe	100 ±1.0	91.1
Ni	100 ±1.0	107.3

Table 5. **Results of QC and Spike Recovery.**

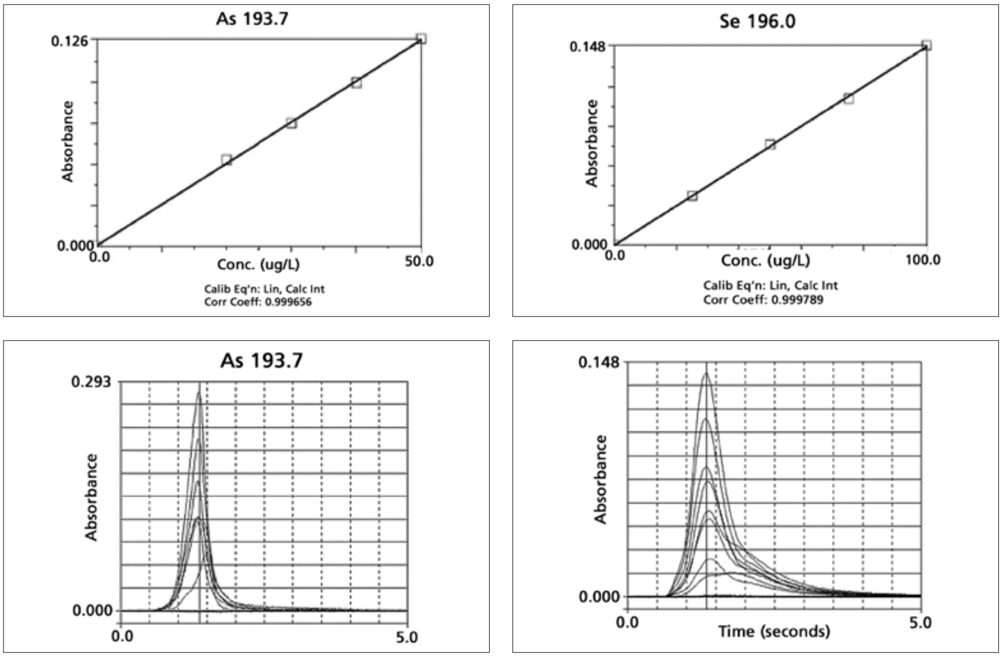
Metal	QC 1 (%)	QC 2 (%)	Spike Recovery (%)
Pb	104.6	102.9	99.9
Cd	102.0	105.6	100.1
Cr	101.8	99.0	101.6
As	100.6	103.1	109.4
Se	104.3	102.5	97.7
Zn	97.3	97.3	111
Cu	96.0	98.7	93.5
Mn	100.7	104.6	91.2
Fe	96.1	94.6	102.4
Ni	109.1	101.8	101.8

Table 6. **Method Detection Limits (MDLs).**

Metal	MDL (µg/kg)
Pb	19.8
Cd	0.8
As	48.4
Se	167.9

Appendix I. **Graphite Furnace Temperature Program.**						
Element	**Step**	**Temp ˚C**	**Ramp Time (Sec)**	**Hold Time (Sec)**	**Internal Gas Flow (mL/min)**	**Gas Type**
Se	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1300	10	20	250	Argon
	4	1900	0	5	0	Argon
	5	2450	1	3	250	Argon
Cd	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	500	10	20	250	Argon
	4	1500	0	5	0	Argon
	5	2450	1	3	250	Argon
As	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1200	10	20	250	Argon
	4	2000	0	5	0	Argon
	5	2450	1	3	250	Argon
Cu	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1200	10	20	250	Argon
	4	2000	0	5	0	Argon
	5	2450	1	3	250	Argon
Ni	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1100	10	20	250	Argon
	4	2300	0	5	0	Argon
	5	2450	1	3	250	Argon
Fe	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1400	10	20	250	Argon
	4	2100	0	5	0	Argon
	5	2450	1	3	250	Argon
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	Argon
	5	2450	1	3	250	Argon
Zn	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	700	10	20	250	Argon
	4	1800	0	5	0	Argon
	5	2450	1	3	250	Argon
Cr	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1500	10	20	250	Argon
	4	2300	0	5	0	Argon
	5	2450	1	3	250	Argon
Mn	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1300	10	20	250	Argon
	4	1900	0	5	0	Argon
	5	2450	1	3	250	Argon

Appendix II. **Examples of Typical Calibration Curves and Atomization Profiles.**



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