way (reaction 4), this large difference would not be expected from results obtained in this study. Clearly more work is needed in this area to clarify these very interesting results.

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Determination of Trace Metal Content in Corn Oil by Atomic Absorption Spectroscopy

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ABSTRACT

Atomic absorption spectrophotometry is used for the determination of Cu, Fe, K, Na, Ni and Zn in industrial refined corn oil. The results of five different analytical techniques are compared. Fair agreement is obtained between the char-ashing method and the dilution procedure. Char ashing is a sensitive and useful technique, if handled correctly. Dilution, coupled to carbon-rod atomization, is a reliable method. To eliminate errors, due to sample loss, a proper injection technique is required. Extraction methods suffer from incomplete recoveries, owing to deficient break up of the organometallic compounds in oil.

INTRODUCTION

Small amounts of metals in edible oils are well known to have serious deterioration effects on the stability of these oils (1). The altered oil characteristics are expressed in changes of color, odor and flavor. Copper and iron in particular will greatly reduce the oxidative stability of oil (2). Therefore, exact determination of trace metal contents is very important in evaluating deteriorating effects.

Due to its high sensitivity and specificity, atomic absorption spectrometry has been used extensively to study trace wear metals in used engine oils (3).

Despite the established procedures and instrumental refinements, the determination of metals in vegetable oils is still a problem. This is especially due to extremely low metal concentrations in refined edible oils.

Trace metal contents of oils can be performed using aqueous or organic matrices (4). For aqueous matrices, techniques such as char ashing, wet ashing, acid extractions and chelation are commonly used. Direct analysis of metals in oils requires dilution of the sample by an appropriate organic solvent. Depending on sample volume, sensitivity, detection range and the specific metal concentration, either a conventional burner or a carbon-rod atomizer can be employed (5).

Several workers (6-8) compared ashing, extraction and solvent dilution and discussed the advantages and disadvantages of each. Dry ashing (9) is simple and direct, although several elements are partially lost during heating. Wet ashing (10) causes no loss of inorganics but the risk of contamination and explosion (if perchloric acid is used) (11) is higher. Various extraction methods, including acid extraction by hydrochloric acid (12) or nitric acid (13) and acid/EDTA extraction (12) have been used.

Diluting the sample with methyl-isobutyl-ketone (MIBK) (14) reduces the metal concentration, but has some practical advantages: it is fast and simple, with little chance of contamination. Because of the very low metal contents, enrichment procedures using ashing or extraction methods may be more sensitive than diluting in addition with direct analysis.

The methods used in this study, which reports the levels of several trace elements in a typical commercial salad-grade corn oil, are described below. The metals under investigation are: copper (Cu), iron (Fe), potassium (K), sodium (Na), nickel (Ni) and zinc (Zn).

EXPERIMENTAL

Reagents

All reagents used in solutions were analytical-reagent grade (E. Merck). Glassware was cleaned with 6M nitric acid to remove metal contamination. Aqueous standard solutions were prepared by dissolving inorganic salts (E. Merck reference material for atomic absorption spectrometry) in doubly distilled water.

Organic standard solutions were made by dissolving the appropriate organometallic salts in a combination of xylene, hexanoic acid and MIBK. Except for tris (1-phenyl-1,3-butanediono) iron, all organometallic salts were cyclohexane butyrates.

The industrial refined corn oil was stored under nitrogen in a brown glass container and kept in a refrigerator.

METHODS

Char Ashing

Thirty g of oil were weighed in a 100-mL quartz crucible covered by a Pyrex watch glass, supported on Pyrex hooks, and charred for ca. 24 hr on a hot plate at 300 C. In this manner, the sample is protected from accidental contamination. When the sample stopped smoking, ashing continued in a muffle furnace at 400 C for 2 hr and was completed at 500 C for 20 hr.

After cooling, ash was dissolved in 5 mL of 2M nitric acid, warmed on a steam plate for 30 min and carefully transferred into a 10-mL volumetric flask. The procedure was repeated and the volumetric flask was made to volume with 2M nitric acid. The blank sample consisted of 2M nitric acid.

Extraction

Extractants. Preliminary tests about the extraction of radioactive Zn in oil with several acid extractants with or without chelating agents led to three distinct extractants. (a) 5.1 mol/L HCl-0.02%, w/v EDTA: dissolve 0.02 g EDTA in 50 mL of doubly distilled water and add 50 mL HCl (sp gr 1.16). (b) 5.1 mol/L HCl: dilute 50 mL of HCl (sp gr 1.16) to 100 mL with doubly distilled water. (c) 1.5 mol/L HNO_3: dilute 10 mL of HNO_3 (sp gr 1.40) to 100 mL with doubly distilled water.

These radioactive measurements, where Zn was chosen as a model for bivalent metal ions, revealed that the distribution coefficient $K = [Zn]_{aq}/[Zn]_{or} = 300$, where $[Zn]_{aq}$ is the zinc concentration in the aqueous-extraction layer and $[Zn]_{or}$ is the zinc concentration in the oil layer.

After n extractions, the number of millimoles remaining in the organic solution (X_n) is given by the expression (15):

$$X_n = \left[\frac{V_{or}}{V_{aq} \cdot K + V_{or}}\right]^n \cdot a$$

where V_{or} = ml of organic solution, V_{aq} = ml of aqueous solvent, and a = mmol of metal ions.

Taking V_{or} = 100 mL and V_{aq} = 8 mL, the remaining metal concentration in the organic solution is 4% for x_1, 0.16% for x_2 and 0.006% for x_3.

Procedure. A 100-g sample was weighed in a 250-mL flask, 8 mL extractant was added and the resulting mixture was warmed on a steam plate for one hr. The flask was then stoppered and the contents shaken continuously for 20 hr. After separation of the phases (4 hr), the lower aqueous layer was pipetted out into a 25-mL volumetric flask. The procedure was separated twice and the volumetric flask was made to volume with extractant.

Blanks were made up by the corresponding extractants.

Dilution

A sample of 5 g oil was diluted to exactly 25 mL with MIBK and directly analyzed.

Because metal-free base oil is hard to obtain, matrix-matching is difficult in oil analysis using this technique. The influence of sample matrix was determined by comparative measurements using the standard addition method where automatic matching is achieved. Base line measurements were performed on pure MIBK.

INSTRUMENTATION

Apparatus

The atomic absorption spectrophotometer (Model AA-6, Varian Techtron) was equipped with recorder readout facilities, scale expansion capabilities from 0.3x to 50.0x, a carbon-rod atomizer (Model 63, Varian Techtron) the appropriate hollow cathode lamps and a hydrogen continuum light source to correct for nonatomic absorbance.

Instrumental parameters and temperature programs, used for both aqueous and organic matrices, are summarized in Table I.

TABLE I

Instrumental Parameters and Furnace Operating Conditions

	Cu	Fe	K	Na	Ni	Zn
Lamp current (mA)	3	5	5	5	5	5
Wavelength (nm)	324.7	248.3	404.4	330.3	232.0	213.9
Slit width (nm)	0.2	0.2	0.5	0.5	0.2	0.2
Dry time (sec)	30	30	30	30	30	30
temperature (C)	100	100	100	100	100	100
Ash time (sec)	15	15	15	15	15	15
temperature (c)	600	600	600	600	600	600
Atomization time (sec)	3	3	3	3	3	3
temperature (c)	2200	2400	2000	2000	2400	1200

TABLE II

Metal Content in Corn Oil

Method	Cu (ng/g)	RSD (%)	Fe (ng/g)	RSD (%)	K (ng/g)	RSD (%)	Na (ng/g)	RSD (%)	Ni (ng/g)	RSD (%)	Zn (ng/g)	RSD (%)
Char ashing	15.2	32.9	154	6.5	972	2.4	1220^a	7.4	8.4	15.9	4.6	10.9
Extraction HCl/EDTA	6.7	20.9	112	3.6	404	2.5	308^a	6.8	6.1	16.4	2.0	10.0
HCl	4.0	30.0	114	10.5	322	2.5	205^a	9.3	6.0	17.4	1.4	14.3
HNO_3	8.2	18.3	100	8.0	806	1.1	285^a	7.0	6.5	16.2	1.7	11.8
Dilution calibration curve	8.1	24.7	208	13.0	940	1.2	920	3.2	7.6	14.0	4.0	9.1
Standard addition method	7.8	11.5	216	7.9	925	0.9	962	7.3	7.4	8.6	3.9	9.0

^{a}Analyzed by flame.

Sodium in an aqueous matrix was determined with a 4-in. laminar-flow single slot burner and an air-acetylene flame because no linear relationship between absorbance and metal concentration was obtained when using the carbon rod. Under these circumstances, the instrument was operated with a lamp current of 5 mA at a wavelength of 589.0 nm and a slit width of 0.2 nm.

Injection Procedure

A carbon-tube atomizer as furnace was preferred because maximum sensitivity and freedom from spectral interferences was required. To protect the furnace from oxidation, the tube was bathed in dry oxygen-free nitrogen.

Aqueous samples were injected with a 5 μL Eppendorf pipette. This injection technique was not used for organic aliquots because the oily solution tends to slip out of the carbon rod due to the low surface tension. Therefore 5 μL samples were injected with a 25 μL Hamilton syringe, cleaned with 5% nitric acid and dried prior to use. To prevent sample loss, the organic solution was injected during the dry stage of the carbon rod program at a rate of 0.5 μL/s.

CALCULATIONS

The average absorbance value for five independent measurements was calculated for each concentration. These mean values with their net weight made up the linear absorbance/concentration curve. A least-squares procedure was used for evaluating the slope and, when using the standard addition method, the intercept on the concentration axis.

Relative standard deviations (RSD) were calculated using the commonly employed equations of the standard deviation for a finite number of measurements taking into account the propagation of errors during mathematical operations.

Calculations were performed on a HP 9815A desk-computer.

RESULTS AND DISCUSSION

The concentration of the metals in industrial refined corn oil, determined by five analytical methods are shown in Table II.

As far as carbon rod atomization data are concerned, char ashing and dilution give, in broad outlines, corresponding results, while the extraction methods clearly suffer from incomplete recoveries. The most successful extractants are HNO_3 and HCl/EDTA. The former give nearly complete extraction of Cu and K, while the latter give better results for Fe, Na and Zn. The role of EDTA as a chelating agent in such highly acid media is unknown (16), but metal recovery with HCl without added EDTA is much lower.

The lower efficiency of these extraction methods can be explained by supposing a tight bond between the metal ions and the oil constituents or the combination of cations as organometallic compounds that are difficult to break up. By using more vigorous procedures such as refluxing in the presence of one of the proposed extractants, quantitative recovery is possible (12,16).

Char ashing is a tedious operation and the entire process takes about three days to prepare a sample. Moreover, oil samples tend to puff during carbonization, so careful control of temperature programming is indispensable. Partial volatilization of Zn and contamination with Na has been reported (7). On the other hand, enrichment is possible and the procedure, including the preparation of standards, is fairly simple.

Either a conventional burner or the more expensive carbon-rod atomizer could readily be used for aqueous media. However, the carbon rod offers the advantages of speed, higher sensitivity and very small sample requirement.

Proper temperature programming for every element is necessary to avoid incomplete atomization. Sodium contamination is possible. Since aqueous standards of sodium do not give a linear calibration plot, the determination of sodium in aqueous media by carbon-rod is impossible.

Measurements or organic solutions by carbon-rod and aqueous solutions by flame pose no problem. Under these circumstances, we suppose that the concentration of sodium in oil is too high for successful use of the carbon-rod atomizer.

Dilution is a rapid and simple method and reduces the manipulation of the oil, minimizing its possible contamination. However, this method is less sensitive and necessitates a metal-free base oil or the use of the standard addition method for matrix matching. Organometallic salts for organic standard solutions are expensive and not always readily available. When using the flame mode for analysis, unburned oil tends to accumulate and clog the burnerhead, so the use of a carbon-rod atomizer is necessary. Proper injection of the sample is essential for reproducible measurements. Injection during the drying stage of the furnace gives the best results.

It is noted that the standard addition method, where matrix-matching is achieved, leads to the same results as the classical calibration curve method. This is true for all elements. This is in agreement with the measurements of Olejko (17) who stated that, regardless of oil type, no matrix-matching is necessary.

Generally, background stability is better for organic matrices. The apparatus works at the upper detectable limit for the determination of Fe and Zn by the char-ashing method. Less sensitive spectral lines for Fe (371.9 nm) and Zn (307.7 nm), in contrast to those for Na (330.3 nm) and K (404.4 nm), give unstable measuring results. By using the dilution method for the analysis of Cu and Ni, one reaches the lowest detection level.

Concentrating is excluded by using this analyzing technique. The 1% absorption sensitivity for Zn is considerably superior to that for Cu and Ni. A volatilization error of Zn is possible by using improper temperature programming of the carbon rod. The higher level for sodium by char ashing is attributed to contamination during char ashing or atomizing (7).

Relative standard deviations are more correlated to an element than to a method. This implies that metal content determinations by atomic absorptions spectrometry strongly depend on the relative sensitivity for each element with respect to absorption measurements.

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Study of Triglyceride-Protein Interaction Using a Microemulsion-Filtration Method[1]

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ABSTRACT

Interactions between simple triglycerides and a variety of native and chemically modified proteins were studied. A new procedure was developed to evaluate hydrophobic binding of proteins at oil/water interfaces. The method involves emulsifying a triglyceride ultrasonically in water and equilibrating the microemulsion with a protein solution. The protein adsorbs to the surfaces of the globules as a stabilizing interfacial layer. Relative stability of different emulsions is evaluated under standard conditions of partition with a polycarbonate membrane filter. Either gas liquid chromatography or a radioactive technique is used to determine the amount of triglyceride stabilized by protein through hydrophobic binding. The procedure was used to compare interactions of six different triglycerides with bovine serum albumin (BSA) and interactions between trioctanoin and 26 different native and chemically modified proteins. In general, an increase in molecular weight of saturated triglycerides or an increase in double bonds of unsaturated triglycerides reduced hydrophobic binding. The order of binding of trioctanoin for the series of proteins (on a weight basis) compared to BSA taken as 100%, ranged from 160% for casein to 1% for lysozyme. Addition of nonpolar residues, including methyl, cyclohexyl, cyclopentyl and benzyl groups, to casein increased binding by 20-30%. Effects of protein mixtures, and changes in pH and ionic strength also were studied. The results demonstrate that interactions between a wide variety of triglycerides and proteins can be investigated by the microemulsion-filtration method.

INTRODUCTION

Interactions between lipids and proteins represent a major field of biological and technological interest (1). Different types of intermolecular forces may be involved: (a) covalent binding, (b) electrostatic binding, (c) polarization interaction, (d) dispersion interaction, and (e) hydrophobic binding (2,3). Hydrophobic lipid-protein interactions in aqueous systems are of special importance for the stability, conformation and function of biological macromolecules. However, hydrophobic interactions are difficult to study

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