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Journal of Chromatography A, 789 (1997) 549–555

JOURNAL OF CHROMATOGRAPHY A

Determination of some inorganic species in edible vegetable oils and fats by ion chromatography

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Abstract

A procedure has been developed for the ion chromatographic determination of total chlorine, phosphorus and sulphur and of iron, copper, nickel, zinc, cobalt, lead and cadmium in edible vegetable oils and fats. The organic matrix which strongly interferes in analytical procedures, is completely removed by saponification followed by oxidative UV photolysis. This method is simpler and requires less reagents compared with other sample pretreatment procedures. To oil and fats, ethanol and potassium hydroxide were added, and they were saponified for half an hour. Hydrogen peroxide was added and the sample was subjected to UV photolysis at 85±5°C. In less than 1 h organic constituents were completely degraded, while inorganic constituents, except nitrates, iodides and manganese, remained unaffected by UV radiation. Chlorine, phosphorus and sulphur, present in different organic compounds, can be quantified as total amounts only, without speciation. The clear sample solutions were directly injected on to an ion chromatograph equipped both with a conductivity detector, for the determination of total chloride, phosphate and sulphate ions using a carbonate–hydrogencarbonate eluent, and a post-column reactor and a variable-wavelength UV–Vis detection system, for the determination of lead and cadmium using an oxalate eluent and iron, copper, nickel, zinc and cobalt using a pyridine-2,6-dicarboxylic acid eluent. The method has been tested on spiked and unspiked samples of vegetable oils and fats and was found to be satisfactory for the determination of the previously cited elements. Ion chromatographic responses were compared to voltammetric ones and were found to be in good agreement (±3%). © 1997 Elsevier Science B.V.

Keywords: Oils; Fats; Sample handling; Metal cations; Inorganic anions

1. Introduction

Edible vegetable oils and fats play a vital role in human nutrition and increasing attention is being paid not only to their organic composition, but also to presence of inorganic species [1,2]. It is important to have accurate data on anions like chloride, sulphate or phosphate [3,4] that are present in different organic compounds of the crude matter and/or are introduced during industrial manipulations. Heavy and transition metals [5,6] should not be present in such quantities that endanger the health or act as catalysts on the oxidative evolution of oils and fats.

Due to the different origin, characteristics and composition of oils and fats, a large number of samples must be accurately analysed for several parameters and the time-consuming steps necessary for their pretreatment [7–11] underline the need for reproducible, fully automatizable procedures capable of desegregating the fat matrix with minimum re-

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0021-9673/97/$17.00
PII S0021-9673(97)00822-4

Table 1
Recovery of various inorganic species in olive oil after saponification and different UV photolysis times

Inorganic species	% Recovery after					
	30 min	45 min	60 min	75 min	90 min	120 min
Cl^-	85	98	100	101	102	100
PO_4^{3-}	75	87	98	101	100	103
SO_4^{2-}	78	94	102	100	103	101
Pb(II)	78	92	102	101	102	100
Cd(II)	83	96	100	103	99	101
Fe(III)	72	91	99	100	102	101
Cu(II)	84	95	102	100	101	103
Ni(II)	73	92	99	101	100	98
Zn(II)	87	95	101	100	98	102
Co(II)	71	91	99	100	103	101

Olive oil sample: 1000 mg; saponification step: 30 min at 50°C; mean of the values obtained for ten replicates; ion chromatographic conditions as reported in Table 2.

agent additions, followed by the simultaneous determination of the most important parameters.

A range of techniques have been employed in the determination of trace metals and anions in edible oils and fats, including atomic absorption spectrometry (AAS) [1–3,5,6,12–14], inductively coupled plasma atomic emission spectrometry [4,15], voltammetry [16] and neutron activation analysis [17]. Most of these techniques are based on sample pretreatments involving the addition of one or more reagents, wet digestion or dry ashing [9], separations or solvent extractions prior to analysis, while others require very expensive equipment and highly skilled personnel. An alternative to the cited pretreatment techniques, microwave oven digestion [10], has become popular due to the shorter time and low temperature required for sample treatment, but the decomposition of significant quantities of organic matrices is delicate and can cause some problem if precautions are not strictly considered.

Ion chromatography (IC), even if still not applied to oil or fat analysis, seems one of the most effective and simple techniques to determine both anionic as well as cationic species owing to its high sensitivity, rapidity and ease of operation coupled with the advantage of simultaneous determinations.

Table 2
Ion chromatographic conditions

	Anions	Fe, Cu, Ni, Zn, Co	Pb, Cd
Column	IonPac AG9+AS9	IonPac CG5+CS5	IonPac CG5+ CS5
Eluent	2.0 m*M* Na_2CO_3 0.75 m*M* $NaHCO_3$	6 m*M* PDCA 50 m*M* CH_3COOH 40 m*M* CH_3COONa (pH 4.6)	50 m*M* oxalic acid 95 m*M* LiOH (pH 4.8)
Eluent flow-rate	1.0 ml/min	1.0 ml/min	1.0 ml/min
Injection volume	100 μl	150 μl	150 μl
Detection	Suppressed conductivity	Visible absorbance	Visible absorbance
Suppressor	ASRS	–	–
Controller	Position 2 (100 m*A*)	–	–
Post-column reagent	–	0.3 m*M* PAR 1 *M* 2-dimethylaminoethanol 0.5 *M* NH_4OH 0.5 *M* $NaHCO_3$	0.3 m*M* PAR 1 *M* 2-dimethylaminoethanol 0.5 *M* NH_4OH 0.5 *M* $NaHCO_3$
PCR flow-rate	–	0.5 ml/min	0.5 ml/min
Wavelength	–	520 nm	520 nm

Table 3
Detection limits and linearity range for inorganic species determined in edible vegetable oils and fats, after sample pretreatment

Element	Limit of detection[1] (μg/kg)	Linearity range[2] (μg/kg)
Cl	10	20–2000
P-PO_4	35	50–1500
S-SO_4	30	40–2000
Pb	50	100–1000
Cd	100	150–1500
Fe	20	50–2000
Cu	10	20–2500
Ni	50	100–2000
Zn	25	50–1500
Co	50	100–2000

[1] LOD calculated as 3δ + average noise.
[2] $r > 0.995$.

The aim of the present work is to present a simple and accurate procedure for oils and fats pretreatment based on their saponification, followed by oxidative UV photolysis for the complete removal of their organic matrix and an ion chromatographic method that permits the simultaneous conductimetric evaluation of total chloride, phosphate and sulphate (carbonate–hydrogencarbonate eluent), and, with post-column spectrophotometric detection, of iron, copper, nickel, zinc and cobalt [pyridine-2,6-dicarboxylic acid (PDCA) eluent] and lead and cadmium (oxalate eluent).

2. Preliminary studies

2.1. Sample pretreatment

For making sample pretreatment amenable to the widest range of samples, edible vegetable oils and fats of different origin and variable constitution were chosen and the effect of the matrix saponification and UV photolysis step over various anions and cations has been investigated.

Standard oil without anionic or cationic impurities as well as vegetable oil and fat samples, both

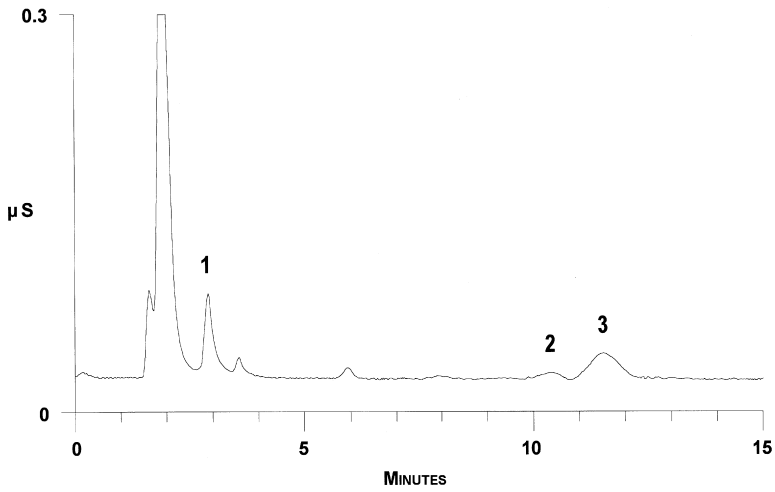

Fig. 1. Determination of anions in a peanut oil sample. Sample pretreatment as described in Section 2.1. Peaks: 1=Cl^- 189 μg/kg; 2=PO_4^{3-} 52 μg/kg; 3=SO_4^{2-} 408 μg/kg. Eluent: 2.0 m*M* Na_2CO_3–0.75 m*M* $NaHCO_3$. Column: IonPac AG9+AS9. Flow-rate: 1.0 ml/min. Detection: suppressed conductivity.

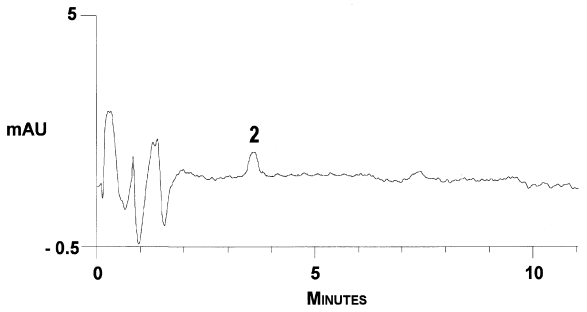

Fig. 2. Determination of cations, using oxalic acid eluent, in a peanut oil sample. Sample pretreatment as described in Section 2.1. Peak: 2=Cu^{+} 48 μg/kg. Column: IonPac CG5+CS5. Eluent flow-rate: 1.0 ml/min. PCR PAR 0.5 ml/min. Detection: visible absorbance.

unspiked and spiked with varying amounts of chloride, phosphate, sulphate and iron(III), copper(II), nickel(II), zinc(II), cobalt(II), lead(II) and cadmium(II) have been subjected to saponification for 1 h and UV photolysis for 2 h prior to instrumental analysis as reported in Section 3. It has been found that chloride, phosphate and sulphate and iron(III), copper(II), nickel(II), zinc(II), cobalt(II), lead(II) and cadmium(II) are not affected by saponification and UV photolysis and the recovery of these species is between 97% and 103%.

Data summarized in Table 1 evidence the effectiveness of the saponification–photolytic pretreatment of the sample in the case of olive oil. The purpose of using hydrogen peroxide in the course of photolysis is to supply extra OH· radicals which accelerate the decomposition of the organic components still present in the matrix at the end of the saponification step. In addition, the decomposition products of this reagent are water and oxygen which do not interfere in the course of analysis.

Saponification and UV photolysis have been combined to reduce the time required by UV photolysis alone for completely destroying any long-chain compound present in vegetable oils and fats.

3. Experimental

3.1. Reagents and standards

Sodium carbonate, sodium hydrogencarbonate, oxalic acid, lithium hydroxide, 2-dimethylaminoethanol, 4-(2-pyridylazo)-resorcinol monosodium salt (PAR) and pyridine-2,6-dicarboxylic acid (PDCA) were chromatographic grade (Novachimica, Milan, Italy), hydrogen peroxide (30% m/m, without stabilizer), ammonium hydroxide (30%), sodium hydroxide, glacial acetic acid and nitric acid (70%) were Erbatron electronic grade reagents (Carlo Erba, Milan, Italy), potassium hydroxide monohydrate was Suprapur reagent (E. Merck, Darmstadt, Germany), dimethylglyoxime and ethanol (95°) were analytical-reagent grade (Carlo Erba). 2 *M* ammonium acetate (pH=5.5) was chelation grade (Dionex, Sunnyvale, CA, USA). Ultrapure water with conductivity <0.1 μS (DI water) was obtained from a Milli-Q (Millipore, Bedford, MA, USA) four-bowl deionization system.

Working standards were prepared daily by diluting E. Merck standards [1.000 g/l (aqueous) or g/kg

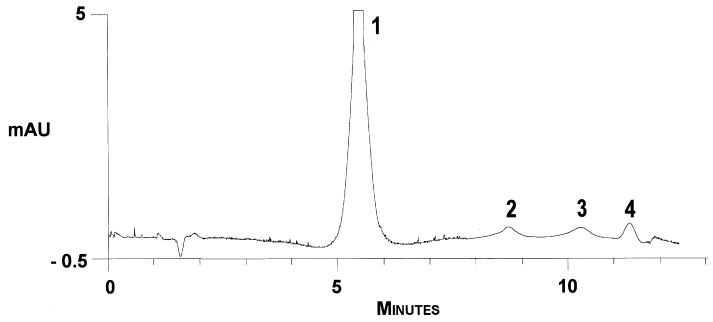

Fig. 3. Determination of cations, using PDCA eluent, in a peanut oil sample. Sample pretreatment as described in Section 2.1. Peaks: 1=Fe^{3+} 1260 μg/kg; 2=Cu^{2+} 51 μg/kg; 3=Ni^{2+} 130 μg/kg; 4=Zn^{2+} 125 μg/kg. Column: IonPac CG5+CS5. Eluent flow-rate: 1.0 ml/min. PCR PAR 0.5 ml/min. Detection: visible absorbance.

Table 4
Comparison of results for edible vegetable oils and fats analysed by ion chromatography and voltammetry (DPASV/DPCSV)

Element	Test[1]	Edible vegetable oil or fat[2]									
		Olive oil		Peanut oil		Soybean oil		Sunflower oil		Margarine	
		μg/kg	s^3	μg/kg	s^3	μg/kg	s^3	μg/kg	s^3	μg/kg	s^3
Pb	IC	<50		<50		<50		<50		<50	
	DPASV	<50		<50		<50		<50		<50	
Cd	IC	<100		<100		<100		<100		<100	
	DPASV	<50		<50		<50		<50		<50	
Fe	IC	392	±5	1257	±30	2675	± 55	575	±6	378	± 8
Cu	IC	12.7	±0.3	48.6	± 2.5	25.5	± 0.5	18.3	±0.5	48	± 1
	DPASV	12.8	±0.3	50.5	± 3.6	25.9	± 0.4	18.6	±0.4	49	± 0.8
Ni	IC	<50		130	± 4	42	± 1	35	±1	50.5	± 1.5
	DPCSV	15.5	±0.3	128	± 4	41	± 1	36	±1	51	± 2
Zn	IC	<25		125	± 3	<25		<25		64	± 1
	DPASV	<50		127	± 6	<50		<50		68	± 2
Co	IC	<50		<50		<50		<50		<50	
	DPCSV	<50		<50		<50		<50		<50	
Cl	IC	35.8	±0.9	189	± 2	35	± 1	58.5	±1.3	795 000	±1300
P	IC	<10		13	± 0.5	530 250	±2350	107	±2	12 938	± 107
S	IC	<10		137	± 2	92	± 2	159	±2	250	± 2

[1] Ion chromatographic conditions as reported in Table 2; voltammetric conditions as in [18].
[2] Sample pretreatment as described in Section 3.4.
[3] Average of five replicates.

(oil)] with DI water or oil [standard oil (E. Merck) without anionic and cationic impurities] as required.

Quartz test tubes and all glassware were cleaned in concentrated nitric acid and carefully washed with DI water. Normal precautions for trace analysis were observed throughout.

3.2. Eluent and post-column reagent solutions

A 2.0 m*M* sodium carbonate and 0.75 m*M* sodium hydrogencarbonate solution was used as chromatographic eluent for anions.

For the analysis of iron(III), copper(II), nickel(II), zinc(II) and cobalt(II), a mixture of 6 m*M* PDCA, 90 m*M* acetic acid and 40 m*M* sodium hydroxide (pH= 4.6) was used as the eluent. A solution of 50 m*M* oxalic acid and 95 m*M* lithium hydroxide (pH=4.8) was employed for the determination of lead(II) and cadmium(II). A mixture of 0.3 m*M* PAR, 1 *M* 2-dimethylaminoethanol, 0.5 *M* ammonium hydroxide and 0.5 *M* sodium hydrogen was used as the post-column reagent for cationic analysis using both the eluents.

3.3. Instrumentation

Oil and fat samples were saponified in quartz test tubes fitted with PTFE stoppers that were then subjected to UV photolysis in a Metrohm (Herisau, Switzerland) 705 UV digester equipped with 500 W high-pressure mercury lamp. The temperature of the sample was maintained at 85±5°C with the help of a combined air/water cooling system.

Chromatographic analyses were performed on a

metal-free Dionex DX-300 ion chromatograph equipped with an AGP gradient pump, an IonPac AG9 guard column and an IonPac AS9 anion separator column, an ASRS anion self-regenerating suppressor, an IonPac CG5 guard column and an IonPac CS5 cation separator column, a post-column pneumatic controller for post-column reagent addition, a CDM-III conductivity detector and a DSA UV–Vis multiple-wavelength detector. All the chromatographic conditions are listed in Table 2.

All measurements were made at room temperature and in all cases, injection of the sample was done at least in triplicate. All the samples were filtered through 0.45 μm filter.

Data manipulation and the operation of all components in the system were controlled by AI-450 Dionex chromatographic software interfaced via an ACI-2 Advanced Computer Interface to a 80386 based computer (Epson, Sesto S. Giovanni, Italy).

A Metrohm 646 VA processor, equipped with a Model 647 VA stand, a Model 675 VA sample changer, a Model 677 drive unit and Dosimat 665 automatic addition burettes, was used for comparison purposes. A multi-mode electrode (MME) was used as working electrode, while the Ag/AgCl (3 *M* KNO_3) was used as a reference electrode and a 6.5 cm long platinum wire was used as an auxiliary electrode.

3.4. Sample preparation

1000 mg of oil or fat was mixed with 2 ml of ethanol (95°), 2 ml of DI water and 0.5 g of KOH·H_2O. The sample was saponified for 30 min at 50°C, closed with its proper conical PTFE stopper tapered to a point. The stopper acted as cooling finger, prevented solution losses and also protected samples against contamination. Then 100 μl of H_2O_2 (30%) was added to the sample and was subjected to UV photolysis at 85±5°C for 30–60 min. During this period, at 10 min intervals, 100-μl aliquots of hydrogen peroxide were added. Later, the sample was cooled, diluted to 10 ml with DI water, and analysed by IC. When the PDCA eluent was used, before making up the volume with DI water, 500 μl of 2 *M* ammonium acetate was added to keep the sample in the 5–6 pH range.

For comparison purposes, voltammetry was applied to the same photolysed samples. The equipment operations must be programmed for a 300 μl addition of 2 *M* ammonium acetate followed by the determination of zinc, lead, cadmium and copper, a successive addition of 300 μl of 5 *M* ammonium hydroxide–ammonium acetate buffer (pH 9.5) and 50 μl of 0.043 *M* dimethylglyoxime solution in ethanol for the determination of nickel and cobalt [18].

4. Results and discussion

Because edible vegetable oil or fat standards for inorganic species do not exist, the detection limits of chloride, phosphate and sulphate and of iron(III), copper(II), nickel(II), zinc(II), cobalt(II), lead(II) and cadmium(II) have been determined by spiking commercial olive oil and margarine with varying amounts of different anions and cations, and subjecting them to saponification for 30 min and oxidative UV photolysis for 1 h, followed by ion chromatographic analysis.

Detection limits and concentration ranges, in which calibration curves are linear, with correlation coefficients greater than 0.995 are summarised in Table 3. It is evident that the proposed pretreatment technique is highly suitable for the determination of very low amounts of iron(III), copper(II), nickel(II), zinc(II), cobalt(II), lead(II) and cadmium(II) and of total chloride, phosphate and sulphate. An excess over 1:1000 of sodium, potassium, calcium, magnesium was proven not to interfere in their determination.

Figs. 1–3 illustrate the chromatographic behaviour of the inorganic anions and metal cations.

Chlorine, phosphorus and sulphur, present in different organic compounds, can be quantitized as total amounts only, without speciation.

Lead(II) and cadmium(II) cannot be determined with PDCA eluent because these ions are so strongly bound to PDCA that they are not detected by PAR. Similarly, iron cannot be determined with oxalate eluent, while copper(II), nickel(II), zinc(II) and cobalt(II) can be determined by either of the two eluents. For convenience, oxalate eluent was used only for lead(II) and cadmium(II), all the other cations were determined by PDCA eluent.

The proposed procedure was applied to determine impurities and the effectiveness of metals scavenging industrial processes in different vegetable oil and fat samples; for comparison purposes the same samples were analysed for copper, cadmium, lead, zinc, nickel and cobalt by voltammetric techniques and the results were found to be consistent. (±3%).

Table 4 lists some results which highlight that, even if the method still suffers from a time-consuming sample pretreatment, it requires minimum reagent additions and is fully automated, resulting in a satisfactory reproducibility.

5. Conclusions

Saponification and oxidative UV photolysis of oils and fats followed by IC was found to be very effective for the simultaneous determination of several inorganic anionic as well as cationic impurities with the same apparatus. The proposed method is characterised by very low blanks and has an excellent resolution and precision if compared to the traditional methods.

Acknowledgements

One of the authors (J.L.S.) acknowledges the financial support from the International Centre for Theoretical Physics (Trieste, Italy).

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