

ICP-MS DETERMINATION OF TRACE ELEMENT IN VEGETABLE MODIFICATE FATS

Galya K.Toncheva¹, Deana L.Georgieva², Ginka A. Antova³, Pavel Merdzhinov⁴

¹Department of General and Inorganic Chemistry, University of Plovdiv, 4000 Plovdiv, Bulgaria

²Department of Analytical Chemistry and Computer Chemistry, University of Plovdiv, 4000 Plovdiv, Bulgaria

³Department of Chemical Technology, University of Plovdiv, 4000 Plovdiv, Bulgaria

⁴Department Technology of Vegetable and Essential oils, University of Food Technology,
4000 Plovdiv, Bulgaria

Abstract

Tree types of hydrogenated fats which are used in the industry were tested. The initial trace element content was determined and also its changes during the heat treatment due to short-term or longer stay of the fats in the production container from chrome-nickel steel. Using inductively-coupled plasma-mass spectrometry (ICP MS) the total quantity of the selected metals in the fats was determined: Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ag, Sn and Pb.

Was investigated the migration kinetics of Fe and Cr in coating and filling fats and fatty acid composition of the fats at the beginning and end of the observation. Unacceptable changes were not found in the organoleptic characteristics of the fats, resulting from the migration of metals. The amount of the tested toxic metals does not exceed the limit value. An exception is the increased amount of copper in two and nickel in the tree types of fats.

Key words: ICP-MS, trace element, hydrogenated fats.

1. INTRODUCTION

Vegetable oils are made up mainly of triglycerides of fatty acids, but also contain biologically active components: phospholipids, sterols, vitamins and more. These active components are necessary to build cell membranes, synthesis of numerous proteins, enhancement of protective functions [Iburg, 2004, Ipatova et al., 2009]. Most of the extracted oils are liquid but the industry prefers modified fats. During this technological operation the composition and structure of the oils is changed, there is an improvement of their aroma profile and also permanent and stable parameters are provided, which expands the applicability [Coenen, 1976, Patterson, 1994].

In the food industry hydrogenated fats are used to make pasta, dairy and confectionery. They are preferred in the composition of cocoa coatings because of some technical advantages: good retention of chocolate flavor, non-temper and high crystallization speed, products have good heat resistance [Lonchamp, 2004].

The oil should be melted in a container before they are put in the composition of the coating. This is the stage of the technological process, where the fat is in most prolonged direct contact with the metal surface. During melting, due to catalytic action of metal ions from the walls of the vessel, there may be a change in the organoleptic characteristics of the fat, as well as its elemental composition. After that the melted fat is mixed with the other components of the coating, passes through the stages of the production technology and after the coating process becomes a part of the composition of the final product.

The quality of the modified fats can be assessed by determining their micronutrient composition. Trace elements in modified fats can be categorized as essential - Fe, Co, Cr, Cu, Mn, Zn and those that have potentially toxic effects as Pb, Sn, Ag and Ni. [WHO, 1996, Ochiai, 2011].

The monitoring of the trace element composition of hydrogenated fats is important for manufacturers in order to achieve a deeper understanding of food composition and to enable them to be adapted to the requirements of international standards, quality and safety [Ybañez, 1996, Capar et al., 2007]. The presence of non-essential and toxic elements does not necessarily mean that consumption of these vegetable oils is a health risk prior to the risk assessment [FAO/WHO, 2009; FAO/WHO, 2005].

Quantification of trace elements in modified fats is of great interest because some essential micronutrients (such as Fe, Mn, Zn and Cu) can be toxic above certain limit levels [Ochiai, 2011; WHO, 1996; CR(EG)1881/2006; R BG-31, 2004]. The determination of the micronutrient composition in fats is important because of the presence of variable valence metals in them which can affect the overall quality of

these products (color, taste, stability). Data in the literature on the amount of trace elements in hydrogenated fats are limited [Nach,1983; Benzo,2002], but there is more information about oleaginous foods, including vegetable oils, oleaginous raw materials, nuts, etc.[Liovent – Martinez,2011; Souza et al.,2005; Nardi et al.,2009; Voica et al., 2011; Ataro et al.,2008; Kakalova, 2012; Rivero Martino et al.,2000; Chen,2002; Ochu et al.,2012].

The determination of micro metals in vegetable modified fats is a difficult task because their concentration is very low ($\mu\text{g.g}^{-1}$ or $\mu\text{g.kg}^{-1}$) [Nach, 1983; Olejko, 1976]. So far furnace atomic absorption spectrometry is used (GFAAS) [Nach,1983]. Various techniques are developed to detect trace elements in vegetable oils such as: atomic emission spectrometry (ICP-AES) [Anthemidis,2005], atomic absorption spectrometry (AAS) [Canario,2005], and also (HR-CS FAAS) [Nunes, 2011]. Massspectrometry with inductively-coupled plasma (ICP-MS) combines the extraordinary features of atomization and ionization of samples with the sensitivity and selectivity of mass spectrometry. ICP-MS analysis provides a rapid analysis and possibility for simultaneous multi-element analysis and therefore is used for elemental and isotope analysis of vegetable oils [Borisova, 2009; Liovent – Martinez,2011; Souza et al.,2005].

The purpose of this article is to investigate main quality characteristics of three types of modified fats used in industrial practice, to carry out ICP-MS monitoring of their initial trace element composition and its changes during the heat treatment due to remaining of the fats in a collector for melting, as a part of the overall technological scheme of production.

3. MATERIALS AND METHODS

2.1. Food samples

Three types of hydrogenated fats were examined: type 1 - fat for coating produced in the European Union; type 2 - fat for coating produced in Malaysia and type 3 - fat for filling produced in the European Union. Data from analysis certificate of the manufacturers were compared. They show that at $t=20^\circ\text{C}$ solid fat content is max 80% (type 2), max 95% (type 1) and max 35% (type 3). The fats are suitable and can be used for the production of coated biscuits and fillings. In the analysis certificate of type 1 there is (C18:1)–0.05% and in type 2 - (C16:1)–0.07%, free fatty acids.

The three fats were examined in the production process. Each fat was melted at $t = 45\text{--}50^\circ\text{C}$ in a production collector, equipped with a water mantle and made of chrome-nickel steel. On the bottom of the storage collectors have details of zinc-coated steel. In the normal technological schedule fats stay in it for 1h. In order to monitor the migration of metals, the remaining time in the collector were extended to 16h at the indicated temperature.

During the observation of fats sample were taken. After sample collection fats were cooled and then were heated again on the next day. The data are presented as the mean of three replicates $\pm$ standard deviation.

For the quality control of all samples acid value [ISO 660:2000] and peroxide value [ISO 3960:2001] were determined, which indicate set in oxidation during storage of the fats. The organoleptic properties such as color, taste and smell was analyzed [Gray, 1978].

2.2. Analytical determination of fatty acids

The total fatty acid composition of the oil was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C [ISO 5509, 2000]. Fatty acid methyl esters (FAME) were purified by TLC on 20 cm x 20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane: diethyl ether, 97 : 3 (by volume). Determination was performed on a gas chromatograph equipped with a 60 m x 0.25 mm x 25 μm (I.D.) capillary DB - 23 column (Hewlett Packard GmbH, Vienna, Austria) and a flame ionization detector. The column temperature was programmed from 130°C (hold 1 min), at $6.5^\circ\text{C}/\text{min}$ to 170°C (hold 3 min), at $3^\circ\text{C}/\text{min}$ to 215°C (hold 9 min), at 40°C (hold 1 min); injector and detector temperatures were 270°C and 280°C . Hydrogen was the carrier gas at a flow rate 0.8 ml/min; split was 50:1. Identification was performed by comparison of retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions [ISO 5508, 2004].

2.3. Analytical determination of trace elements

Sample preparation was carried out to determine the content of trace elements in the fats [Fiket et al., 2007; Graças et al., 2008; Dugo et al., 2012]. Fat samples (0.5 g) were mineralized with 5ml of cold concentrated nitric acid HNO_3 (Merck, Darmstadt, Germany). Then the sample was heated in plate for 60 min. To each sample after 20 min was added additionally 1ml concentrated nitric acid HNO_3 . After cooling was added 3ml 30% H_2O_2 (Chimtex Ltd, Dimitrovgrad, Bulgaria) and the sample is thermally treated further 50 min. Furthermore it was cooled and quantitatively transferred into a flask of 50 ml and was dilute up with ultrapure water.

An ICP-MS system was used for simultaneous multi-element detection of titanium Ti, chromium Cr, manganese Mn, iron Fe, cobalt Co, nickel Ni, copper Cu, zinc Zn, silver Ag, tin Sn, and lead Pb. Quadrupole mass spectrometer was used with an inductively coupled plasma ICP-MS Agilent 7700 (Tokyo, Japan) Oktopole Reaction System (ORS) and helium as a collision gas. Operating conditions for ICP mass spectrometer are shown in Table 1.

Table 1 Operating conditions for ICP-MS research

Plasma conditions		
1	RF power	1.5 kW
2	Argon plasma gas flow	15 L.min-1
3	Auxiliary gas flow	0.9 L. min-1
4	Nebulizer gas flow	0.95 L. min-1
5	Sample flow rate	0.34 mL. min-1
6	Nebulizer type	Micromist (Glass Expansion)
7	Spray chamber	Scott double pass, Peltier cooled 20C
8	Interface cones	Ni
9	Collision gas	He
	Collision gas flow	3.5 mL.min-1
10	Mass spectrometer settings	
11	Resolution	Normal
12	Acquisition mode	Peak hoping
13	Channels per mass	1
14	Dwell time	100 ms
15	Replicate	5

For the purpose of the analysis external calibration was made against aqueous multi-element standard solutions in the concentration range 10-1000 µg L-1, prepared after appropriate dilution of the ICP multi-element standard solution VI (110580 Merck, Darmstadt, Germany). Only for the element Sn separate series of calibration standards is prepared from monoelement standard of Sn (CPAchem Bulgaria). To control any nonspectroscopic multiplicative obstacles, monitoring signal Rh (CPAchem Bulgaria) was included, introduced as an internal standard for all test and standard solutions.

3. RESULTS AND DISCUSSION

3.1. Quality and organoleptic parameters of fats

The observed fats take part in the coating and filling of cookies. The filling and coating are chemical nature and are a solution of solvent modified fat and additives (sugar, cocoa mass, cocoa powder, milk powder). To produce the glaze or the filling, fat have to melt. This process takes place in a collector of chrome-nickel steel.

Table 2 Organoleptic properties of hydrogenated fats

	Indicator	Fat blank			Fat after 1h			Fat after 16h		
		type 1	type 2	type 3	type 1	type 2	type 3	type 1	type 2	type 3
1	Appearance, consistency	Solid fat, homogeneous	Solid fat, homogeneous	Soft fat, homogeneous	Solid fat, homogeneous	Solid fat, homogeneous	Soft fat, homogeneous	Solid fat, homogeneous	Solid fat, homogeneous	Soft fat, homogeneous
2	Color	very light yellow	light yellow	light yellow	light yellow	yellow	light yellow	light yellow, gray tinge	yellow, gray tinge	yellow, gray tinge
3	Smell and taste	neutral	neutral	neutral	Neutral with no aftertaste	Neutral with no aftertaste	Neutral with no aftertaste	Neutral with no aftertaste	slight metallic taste	slight metallic taste

Analysis of the organoleptic characteristics of the three fats samples (Table 2) is performed. Fats which remained for 1h in the collector of chrome-nickel steel, have no changes in the organoleptic indexes. In type 2 and type 3 changes in the color and taste of the fat are observed after 16h of contact with the metal surface. Due to the contact of the molten fat with the vessel walls, heavy metal ions pass into the fat. These ions can have an catalytic effect on the fat and change its organoleptic and physico-chemical parameters, which is found in vegetable oils by other authors [Maneva,1984; Razgovorov, 2007].

Peroxide value of the three types in time of observation is in the range of 2.34-3.09 mmol O₂ kg⁻¹. The acid value in the reference period for the type 1 is within the range of 0.18-0.37 mg KOH g⁻¹, for type 2 – 0.24-0.42 mg KOH g⁻¹ and for type 3 – 0.20 - 0.25 mg KOH g⁻¹.

Monitoring data shows that the time of heat treatment leads to rise in acid value for type 1 – from 0.18 to 0.28 mg KOH g⁻¹ and for type 2 – from 0.24 to 0.42 mg KOH g⁻¹. For the peroxide value slight increase is observed in sample 1 which remained 2h – to 3.09 mmol O₂ kg⁻¹. The results of the organoleptic and quality characteristics the three types of fat shows that there are no unacceptable changes in them.

3.1. Fatty acids of fats

It was found (see Table 2) that as a result of heat treatment and several times heating and cooling of the fats in a collector for melting, there can be a insignificant change of certain fatty acids.

3.2. Monitoring of trace element content in fats

During the thermal treatment of the fat, due to the contact of the fat with the walls of the container, there is migration of metal ions to the modified fat. Determining the amount of essential metals and those with potentially toxic effects is of interest. In the three fats total quantity of the following metals was observed: Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ag, Sn and Pb (Table 4).

It is known that small amounts of metals such as Fe, Zn, Cu, Mn, Cr and Co are essential for human health. The absence of any of them in the diet produces functional and structural abnormalities that are associated with or result in specific changes that can be canceled by the presence of metal (WHO, 1996; Goyer, 1998; Protasova, 1998). Iron is a component of hemoglobin and myoglobin. It is stored in the tissues and is used by the body but its absence can cause anemia. Zinc is involved in the composition of more than 300 enzymes and its deficiency results in impaired cell growth. Copper is an element of the elastic tissues, certain copper-containing proteins and enzymes. Manganese activates several enzyme systems and is a major component of two important mitochondrial enzymes. Chromium is required for maintenance of normal glucose metabolism. Cobalt as a part of vitamin B12 and is required for the formation and the function of cells (Ochiai, 2011; WHO, 1996; FAO/WHO, 2009; FAO/WHO, 2005).

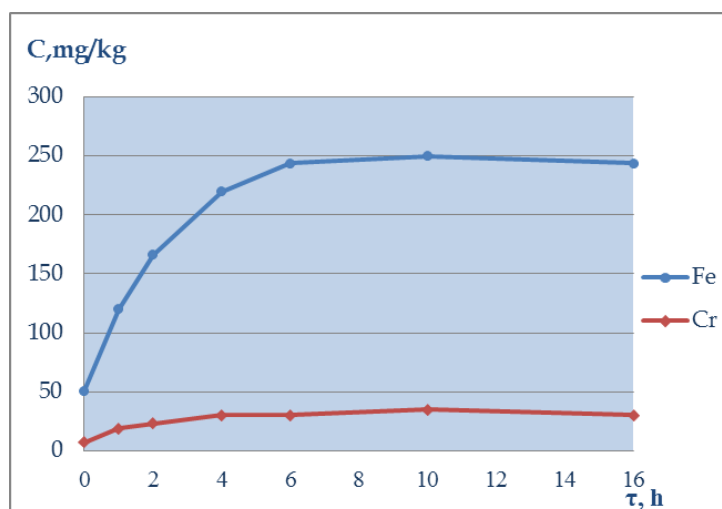
Table 3. Fatty acids of hydrogenated fats

Fatty acids, %	type 1 sample 0h	type 1 sample 16h	type 2 sample 0h	type 2 sample 16h	type 3 sample 0h	type 3 sample 16h
C8:0 – Caprylic acid	-	-	0.1	-	0.2	-
C10:0 – Capric acid	0.1	0.1	0.1	0.1	0.1	-
C12:0 – Lauric acid	1.0	1.0	0.9	0.9	1.1	0.3
C14:0 – Myristic acid	1.6	1.7	1.7	1.6	1.7	1.4
C14:1 – Myristolenic acid	-	-	-	0.1	-	-
C15:0 – Pentadecanoic acid	0.1	0.1	0.1	0.1	0.1	0.1
C16:0 – Palmitic acid	47.0	48.8	57.1	61.0	54.4	55.2
C16:1 – Palmitoleic acid	-	-	0.1	-	0.1	0.1
C17:0 – Heptadecanoic acid	0.1	0.1	0.2	0.2	0.1	0.1
C18:0 – Stearic acid	0.6	0.6	5.1	4.4	4.4	0.3
C18:1 – Oleic acid	49.5	47.6	34.1	30.8	36.2	42.1
C18:2 – Linoleic acid	-	-	-	-	1.1	0.4
C20:0 – Arachidic acid	-	-	0.4	0.8	0.3	-
C20:1 – Gadoleic acid	-	-	0.1	-	0.2	-

Because of the fact that the melting collector is made of chrome-nickel steel, it is expected that iron and chromium will migrate the most (Figure 1, 2 and Figure 3). Observation of type 1 in the entire time range, shows that until the second hour a very slight increase in the total amount of iron passed into the fat. Organoleptic analysis of the fat shows that there is no change in its appearance and color. Variation in chromium is similar - its quantity in the first hours rises steadily, but after the second hour almost does not change.

In fat type 2, an increase of total quantity of iron and chromium is observed, which passed in the fat for the entire time of observation. This sample shows lower absolute values of the migrated iron and chromium in the first hours, but there is increase in their value at the end of the reference period.

According to the requirements of European legislation [CR (EC) №1881, 2006], the amount of iron and chromium in vegetable fats is not subject to control, but it would be correct to compare the results to the FAO/WHO regulated standards for maximum tolerable weekly intake of these two metals.

**Figure 1.** Effect of heat treatment time on the amount of iron Fe and chromium Cr migration in the fat from type

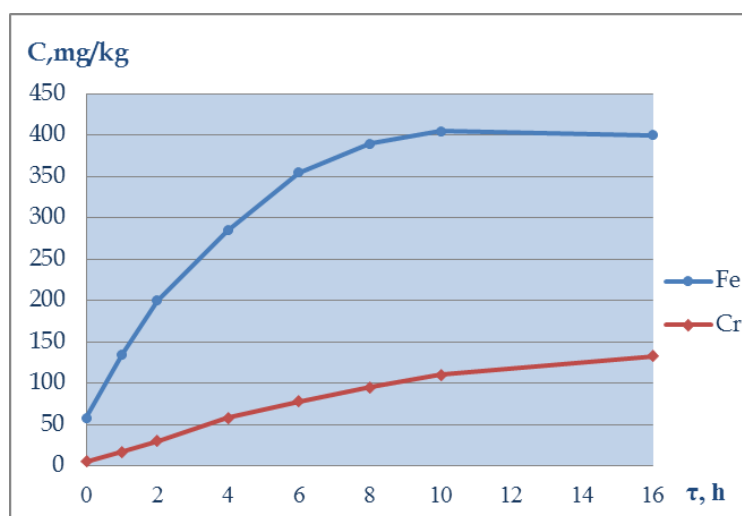


Figure 2. Effect of heat treatment time on the amount of iron Fe and chromium Cr migration in the fat type 2

The observed modified fat form up 30% in weight of coating and the coating participates with 20% in the final product. Therefore the share of fat in the final product is 6%. When the fat is used in production, it melts at $t = 45-50$ °C for about 1h in the production collector and then passes through the production facilities to be included in the composition of the coating. To calculate the intake of iron and chromium, we take the quantity data of the two metals in the fat which stayed in the production collector for 1h.

As established by the FAO/WHO norms, maximum daily intake of inorganic iron (PTWI) is 0.8 mg/kg bw/day and respectively 5.6 mg/kg bw/ week [FAO/WHO, 2005]. In sample 1 after 1h stay the obtained iron is 120 mg/kg in the fat i.e. 7.2 mg /kg in the final product. If a person weighing 70kg consumes 50g per day of this product, he/she will take up to 5.1 µg/kg bw /day of Fe and 36.0 µg/kg bw /week, i.e. 0.6% of the tolerable weekly intake. PTWI of inorganic chromium is 23.3 µg/kg bw/ week [FAO/WHO, 2007]. For sample 1 after 1h stay is determined chromium of 19 mg/kg in the fat i.e. 1.1 mg/kg in the final product. If a person weighing 70 kg consumes 50g of this product per day, he/she will take up to 5.7 µg/kg bw/week of Cr, i.e. 24% of the tolerable weekly intake. Similar limits of Fe and Cr was also established in foods by other authors [Ochu et al., 2012; Kakalova, 2012].

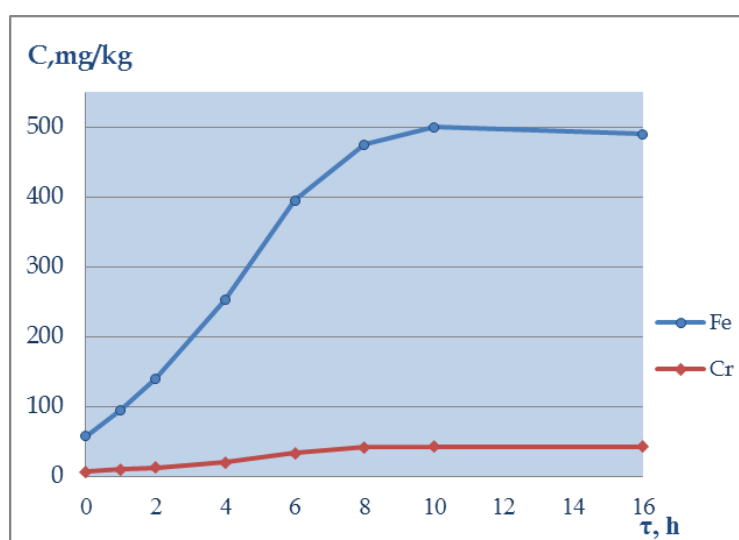


Figure 3. Effect of heat treatment time on the amount of iron Fe and chromium Cr migration in the fat from type 3.

In sample 2 after 1h stay the obtained iron content in the fat is 135 mg/kg, i.e. 8.1 mg/kg in the final product. In this case person weighing 70 kg consumes Fe 5.7 µg /kg bw / day or 40.5 µg/ kg bw / week, i.e. 0.7% of the tolerable weekly intake. The amount of chromium, which a person weighing 70 kg would take per week if you consume this product is Cr 5.1 µg/ kg bw /week, i.e. 15% of the tolerable weekly intake.

The observed filling fat (type 3) form up 30% in weight of filling and the filling participates with 30% in the final product. Therefore the share of fat in the final product is 9%. After 1h stay the obtained iron content in the fat is 80 mg/kg, i.e. 7.2 mg/kg in the final product. In this case person weighing 70 kg consumes Fe 5.1 µg/kg bw/day or 36 µg/kg bw/week, i.e. 0.6% of the tolerable weekly intake. The amount of chromium, which a person weighing 70 kg would take per week if you consume this product is Cr 4.5µg/ kg bw/week, i.e. 19% of the tolerable weekly intake.

Table 4. Range and mean concentration of essential and toxic elements in researched hydrogenated fats

Sample Name	Ti		Cr		Mn		Fe		Co		Ni		Cu		Zn		Ag		Sn		Pb	
	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U	mg/kg	±U
Type 1 control sample	0.9	0.1	7.4	0.3	1.4	0.2	51	4	0.1	0.02	4.5	0.3	1.1	0.2	2.1	0.3	0.1	0.03	0.1	0	0.3	0.1
Type 1 -1h	4.8	0.5	19	1	2.9	0.2	120	14	0.21	0.02	12	1	7.2	0.6	2.1	0.4	<0.1	2	0.2	0	1.9	0.3
Type 1 -2h	1.4	0.2	23	1	2.5	0.2	166	19	0.22	0.02	12	1	1.0	0.1	2.1	0.4	0.26	-	0.6	0.1	0.8	0.1
Type 1 -10h	1.6	0.2	30	3	3.0	0.3	244	9	0.27	0.02	16	2	1.2	0.2	4.1	0.4	13	0.03	1	0.2	1.2	0.2
Type 2 control sample	0.6	0.1	5.2	0.8	0.2	0.1	58	6	<0.1	-	3.1	0.2	0.2	0.06	4.5	0.3	<0.1	-	0.4	0	<0.4	-
Type 2 -1h	1.1	0.2	17	1	1.7	0.2	135	11	0.15	0.02	10	1	0.6	0.1	4.2	0.8	<0.1	-	0.4	0.1	0.6	0.1
Type 2 -2h	1.4	0.2	30	4	2.7	0.4	206	27	0.25	0.02	16	3	0.9	0.1	4.7	0.8	0.12	0.02	0.6	0.1	1.9	0.3
Type 2 -10h	4.8	0.5	133	13	10	1	400	37	1.1	0.1	68	8	4.2	0.7	14	2	0.43	0.06	1.6	0.2	3.8	0.6
Type 3 control sample	0.5	0.1	6.3	0.9	0.2	0.1	57	2	<0.1	-	3.9	0.3	0.3	0.03	5.6	0.7	2.1	0.2	0.3	0.1	<0.4	-
Type 3 -1h	0.7	0.1	10	1	0.5	0.1	80	5	<0.1	-	5.7	0.7	0.2	0.1	6.9	0.3	<0.1	-	0.3	0.1	<0.4	-
Type 3 -2h	2.1	0.4	33	7	3.7	0.3	252	20	0.35	0.04	18	4	2.3	0.3	2	0.3	3.4	0.3	1.4	0.2	0.5	0.1
Type 3 -10h	1.6	0.3	41	5	3.0	0.4	500	43	0.3	0.04	22	3	1.2	0.3	16	2	<0.1	-	150	13	0.4	0.1

In order to fully clarify microelement composition of the test samples other essential elements such as Zn, Mn, and Co (Table 4) were examined. The content of Ti has also been determined. The analysis of the results shows that if the fats are retained longer in the collector, amounts of these minerals increases steadily.

The obtained values were compared with the standards of FAO/WHO for the quantity of Zn and Mn which must be in the diet [FAO/WHO, 2007; Nasreddine, 2010]. If a person consumes 50g/den of a product produced by either of the two fats, he/she will take 6.3 µg Zn (0.6% PMTDI) if it is produced with the first fat and 12.6 µg Zn (1.3% PMTDI) with the second one. The amount of Mn which is taken at similar consumption is 8.7 µg Mn (0.4% RDA) if it is produced with the first fat or 5.1 µg Mn (0.3% RDA) with the second one. If a person consumes 50g/day of a product produced by filling fats –type 3, he/she will take 3.1 µg Zn (0.3% PMTDI) and 0.2 µg Mn (<0.1% RDA).

Data on the quantity of the titanium indicate that it uniformly grows and the sample from type 1 shows higher value in the first hour of observation. Except as an additive in food and pharmaceutical products, titanium is biocompatible and is widely used in medicine [Brunette, 2001; ITA,1999]. Titanium is not toxic and its compounds are adsorbed slightly. Having in mind that percentage size of the fat in the final product (6%), the amount of Ti in the final product is insignificant (<14µg kg-1).

Lead enters the human body through food, water or inhalation of aerosol particles from the air. Damage from lead is expressed in high blood pressure and abnormal activity of the nervous system, liver and kidneys [Zheng et al., 2007; Wang et al., 2007]. The maximum level of lead in fats according to European legislation is 0.1 mg kg-1 [CR (EC) №1881, 2006]. Increased levels of lead in both control fats were observed. The recalculated total Pb content in the final product is less than 70 µg kg-1 (the basis for the calculation is the quantity data of lead in both fats which stay for 1h in the collector).

Elevated quantity of nickel and copper in the control specimens of all the three fats was observed, which may be explained by residual amounts of catalyst for the hydrogenation of fats and is also found by other authors [Nach, 1983; Liorent – Martinez, 2011; Razgovorov, 2007]. From the results obtained it is also visible, that in the process of melting and heat treatment, the amount of the two metals can be increased considerably, especially by long term heat treatment of type 2.

In the production conditions the heat treatment continues for 1h. The amount of nickel, which migrates up to that moment (see Table 4), i.e. in the final product this amount is 0.72 mg kg⁻¹, 0.6 mg kg⁻¹ and 0.3 mg kg⁻¹ respectively for fats type 1, 2 and 3. There is exceeded limit of 0.2 mg kg⁻¹ [CR (EC) №1881,2006] for the nickel content in all the final products with three hydrogenated fats, which was found by other authors in chocolate [Ochu et al., 2012; Sager, 2012].

Copper is a necessary trace element for humans, but this metal can have a toxic effect caused by its accumulation. The amount of copper which migrates in the observed fats after 1h in fats type 1, 2 and 3 (Table 4) corresponds to respectively 0.43 mg kg⁻¹, 0.036 mg.kg⁻¹ and 0.012 mg.kg⁻¹ in the final product produced with these fats. There is exceeded limit of 0.1 mg kg⁻¹ [CR (EC) №1881,2006] for the copper content in coating fats.

After 1h stay in the vessel made of chrome-nickel steel, the amounts of silver and tin (Table 4) in the modified fats are within acceptable limits [CR (EC) №1881,2006].

4. CONCLUSION

1. Using ISP mass spectrometry three kinds of modified fats were observed and the total amount of following metals was evaluated: Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ag, Sn and Pb. It is found that as a result of migration of metals in normal time the technological process, there no change of organoleptic properties and no significant change in the fatty acids.

2. The maximum weekly intake of inorganic metals was calculated if the consumed products are made with the observed fats (stay for 1h in the observed production volume). Consumption in 50 g/day of product with these fats satisfies the recommended daily intake for Fe, Cr, Mn and Zn, which is important for the adequate trace element supply by nutrition.

3. The amount of the tested toxic metals does not exceed permissible limits. The exception is the increased amount of copper in two and nickel in the three type fats.

ACKNOWLEDGMENTS

The authors would like to thank the Research Fund of the Plovdiv University for its financial support (contract NI13-HF006).

REFERENCES

- Anthemidis, A.N., Arvanitidis, V. and Stratis, J.A. (2005). "On-line emulsion formation and multi-element analysis of edible oils by inductively coupled plasma atomic emission spectrometry". *Analytica Chimica Acta*, 537(1–2).
- Ataro, A. McCrindle, R.I., Botha, B.M., C. McCrindle, M.E. and P.P. Ndibewu., (2008). "Quantification of trace elements in raw cow's milk by inductively coupled plasma mass spectrometry (ICP-MS)". *Food Chemistry*, 111(1), 243–248.
- Benzo, Z., Marcano, E., Gómez, C.; Ruiz, F., Salas, J., Quintal, M., Garaboto, A., (2002). "Simultaneous determination of Nickel, Iron, and Copper in Margarine by Inductively Coupled Plasma–Atomic Emission Spectroscopy after Sample Emulsification". *J. of AOAC International*, 85(4), 967–970.
- Borisova, R., (2009). "Fundamentals of chemical analysis". Vodoley, Sofia.
- Brunette, D.M., Tengvall, P., (2001). "Titanium in medicine". Springer.
- Capar, St. G., Mindak, W. R., Cheng J., (2007). "Analysis of food for toxic elements", *Analytical and Bioanalytical Chemistry*, vol. 389, Issue 1, pp. 159–169.
- Canário, C.M., and Katskov, A. D., (2005). "Direct determination of Cd and Pb in edible oils by atomic absorption spectrometry with transverse heated filter atomizer". *J. of Analytical Atomic Spectrometry*, 20.
- Coenen, J.W. (1976). "Fats and oils modification. Hydrogenation of edible oils". *J. Am. Oil Chemist's soc.*, 53.
- Chen, Kuei-Lan and Jiang, S.J. (2002). "Determination of calcium, iron and zinc in milk powder by reaction cell inductively coupled plasma mass spectrometry". *Analyt. Chem. Acta*, 470(2), 223–228.
- Commission regulation (EC) № 1881/2006 of 19 december 2006 setting maximum levels of certain contaminants of food stuffs.

Dugo, G., Di Bella, G., Rando, R. and Saitta, M., 2012. "Sample Preparation for the Determination of Metals in Food Samples". *Anal. Techn. Scientists*, 4, 495–519; Extraction Techniques and Applications: Food and Beverage.

FAO/WHO, (2005). "International Program for chemical Safety. Iron". (accessed march 2013).

FAO/WHO, (2005). "A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances": Report of a Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment.

FAO/WHO, (2007), "Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956–2007) (first through 68th meetings)". FAO of the United Nations and the WHO, ILSI Press International Life Sciences Institute, Washington, DC, (accessed march 2013).

FAO/WHO, (2009), Expert Committee of Food Additives, "Safety evaluation of certain food additives, 71st meeting of the Joint FAO/WHO Expert Committee of Food Additives (WHO technical report series:956)", Geneva.

Fiket, Z., Roje, V., Mikac, N. G., and Kniewald., (2007). "Determination of Arsenic and Other Trace Elements in Bottled Waters by High Resolution Inductively Coupled Plasma Mass Spectrometry". *Croatica Chem. Acta*, CACAA 80 (1) 91, 100.

Gray, J. I., (1978). "Measurement of lipid oxidation: A review". *J. Am. Oil Chemist's soc.*, 55(6).

Graças, M., da Boa Morte, E. S., Batista dos Santos, D. M. and Castro, J.T., (2008). "Sample Preparation for the Determination of Metals in Food Samples Using Spectroanalytical Methods— A Review". *Applied Spectroscopy Reviews*, 43(2).

Goyer, R. A. (1998). "Which metals are essential for human health". *European association of metals*.

Ipatova, L.G., Kochetkova A. A., Nechaev A. P., Tutelian V. A., (2009). "Fatty foods for a healthy diet. The modern view", *De le Print*, 396, Moscow, (in Russian).

Iburg, A. (2004). "Du Monts kleines Lexikon Essig & Öl". Nebel (DuMont), Köln.

International Titanium association, (1999). "Why is titanium the metal of choice for medical application from head to toe".

Kakalova, M., Bekiarov, G., (2012). "Determining the migration level of Cr, Mn, Fe and Sn from metal cans". *Scientific works of UHT, Food science, engineering and technologies*. LIX-2012.

Lioyent – Martinez, E.J., Ortega-Barral, P., Fernández-de Córdova, M.L. A., Ruiz-Medina, A., (2011). "Investigation by ICP-MS of trace element levels in vegetable edible oils produced in Spain", *Food Chemistry*, 127 (3), 1257–1262.

Lonchampt, P., Hartel, R. W., (2004). "Fat bloom in chocolate and compound coatings". *European J. of Lipid Science and Technology*, 106.

Maneva, D., Georgiev, and Ivanov, E. K. (1984). "On the changes in lavender oil during storage in the presence of the heavy metal". *Scientific work of UHT Plovdiv*.

Nach, A.M., Mounts, T.L., Kwolek, W.F., (1983). "Determination of Ultratrace Metals in Hydrogenated Vegetable Oils and Fats". *J. Am. Oil Chemist's soc.*, 80.

Nardi, E. P., Evangelista, F. S., Tormen, L., Saint-Pierre, T. D., Curtius, A. J. and Barbosa, F., (2009). "The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples". *Food Chemistry*, 112(3), 1,727–732.

Nasreddine, L., Nashalian, O., Naja F. and Itani, L. (2010). "Dietary exposure to essential and toxic trace elements from a Total diet study in an adult Lebanese urban population". *Food and Chemical Toxicology*, 48.

Nunes, L.S., Barbosaa, J.T.P., Fernandes, A. P., Lemos, V. A., L. dos Santos, Korna, W.N. and Teixeira, A.L. (2011). "Multi-element determination of Cu, Fe, Ni and Zn content in vegetable oils samples by high-resolution continuum source atomic absorption spectrometry and microemulsion sample preparation". *Food Chemistry*, 127(2), 780–783.

Ochiai, E., (2011). "Chemicals for Life and Living". Springer, Berlin.

Ochu, J. O., Uzairu, A., Kagbu, J. A., Gimba, C. E. and Okunola, O. J., (2012). Evaluation of Some Heavy Metals in Imported Chocolate and Candies Sold in Nigeria. *J. of Food Research*, 1(3).

Olejko J.T., (1976). "Determination of Fe, Cu, Ni, and Mn in fats and oils by flameless atomic absorption spectroscopy". J. of the American Oil Chemists Society, 53(7), 480-484.

Patterson, H.B.W.(1994). "Hydrogenation of fats and oils: theory and practice". AOCS Press, Champaign, Ill..

Protasova, N. A. (1998), "Trace elements: biological role, distribution in soils, effect of human and animal diseases distribution", J. Sorosovski, 12, (in Russian).

Razgovorov, R.B., Smirnov, K.B., (2007). "Removing metals from vegetable oils". Conf. Russia, b.2, University Altay (in Russian).

Regulation BG-31 of 29 July 2004. "Maximum contaminants in food".

Rivero Martino, F. A., Fernández Sánchez, M L. and Medel., A. S., (2000). "Total determination of essential and toxic elements in milk whey by double focusing ICP-MS", J. Anal. At. Spectrom, 163-168.

Sager, M., (2012). „Chocolate and Cocoa Products as A Source of Essential Elements in Nutrition“, J. of nutrition & food science, 2(3).

Souza, R.M., Mathias, B.M., Aucelio, R.Q., (2005). "Inductively coupled plasma optical emission spectro-metry for trace multi-element determination in vegetable oils, margarine and butter after stabilization with propan-1-ol and water". Spectrochimica Acta Part B, 60.

Voica, C., Dehelean, A. and Kovac, M. H., (2011). The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. AIP Conf. Proc. 1425, 110-113; Cluj Napoca, Romania.

WHO, (1996). "Trace Elements in Human Nutrition and Health" World Health Organization, Geneva, 1–361, (accessed March 2013).

Ybañez,N., Montoroa R., Carolib, S. (1996). "Trace element food toxicology: An old and ever-growing discipline". Critical Reviews in Food Science and Nutrition, 36(4).

Zheng, Q.N., Wang, D. Z., (2007). Sci. Tot. Environment, 383.

Zheng,N., Wang,Q., Zhang, X., Zheng, D., Zhang, Z.and Zhang, S. (2007).