

Improved Extraction Method for the Determination of Fe, Cu, Zn, and Ni in Fat Samples Using Atomic Absorption Spectrophotometer

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Summary: The present work dealt with a rapid, ultrasonically assisted acid extractive method applicable for the determination of iron, copper, nickel and zinc in margarine and shortening samples using the atomic absorption spectrophotometer as analytical tool. The percent recovery of Cu, Fe, Ni and Zn was found to be 96.45–97.08, 96.5–97.85, 95.8–97.5 and 96.0–97.8 % as compared with the conventional extraction method of 80.52, 81.94, 84.09 and 85.68% respectively, in the margarine and shortening samples which were fortified with 0.0, 0.125, 0.25, 0.5, 1.0 and 2.0 $\mu\text{g/gm}$ of each metal ion using the standard addition method. The use of ultrasonic intensification in the present method reduced the conventional acid extraction time from 180 to 10 minutes only, with detection limit achieved down to nanomole level.

Most of the investigated samples of different brands were found contaminated with notable amount of iron and nickel ranged 0.52 to 2.38 $\mu\text{g/g}$ and 0.041 to 3.40 $\mu\text{g/g}$ respectively. The magnitude of occurrence for copper and zinc was low within the range of 0.03 - 0.08 $\mu\text{g/g}$. Results of the proposed method were also found in complete agreement to those of wet digestion method and showed very good statistical correlation with coefficient of variation below 2.2 %.

Introduction

The amount of some trace metals in different varieties of crude oil is very low but the potential exists for increasing the level due to oil treatment and contamination during processing i.e. nickel from hydrogenation and Cu, Zn and Fe from processing equipments. Iron and copper are strong prooxidants and exhibit noticeable oxidative effect at concentration as low as 0.005, 0.03 $\mu\text{g/g}$ [1,2], and thus dramatically reduce the oxidative stability and effect shelf life of edible oils [3,4]. The manifestations of oxidation are deterioration of flavor, odor, color and essential fatty acids, which may lead to organoleptic failure and decrease of nutritive value of oil and fat products [4,5].

It is difficult to remove some metallic compounds completely during processing with out incorporating post treatments, particularly, if the diameters of the particles are less than 2 μm [7, 8]. As the occurrence of trace metals is at the cutting edge both for oil quality and human health [9-11], so, their analysis is of significance.

Several atomic absorption spectrophotometric methods have been developed over the years for the determination of trace metal content in edible oil and

fats [12-14]. Most of the procedures involved time consuming and tedious digestion and ashing of the oil and fat samples before their analysis. There is no universal ashing procedure for all metals because of their reactivity and volatility [7]. Currently, acid-extraction of various trace metals from oil and ghee matrix and then analysis of all the metals in matrix by atomic absorption methods, without their isolation, has become an important tool for quality control of vegetable oils and fats [13,15]. Some of the reported acid-extraction and dispersion sample preparation techniques [1, 7, 16, 17], in view of their analytical accuracy and applicability, still have some limitations and need further improvement.

In the present study an attempt has been made to establish a precise and improved sample preparation method that could be used for the estimation of various trace metals in different margarine and shortening samples having wide range of application. The experimental parameters such as time, volume and dilution factor have been optimized, and a number of samples of each brand investigated for iron, copper, nickel and zinc content by atomic absorption spectrophotometer, Model Hitachi 180-50, using Air-Acetylene flame.

Results and Discussion

Atomic absorption spectrophotometer has gained wide acceptance and is routinely used for the analysis of trace metals in vegetable oils and fats. The atomic absorption methods require destroying of the organic matter by digestion with mineral acids, ashing or extraction of the samples before analysis of different metals. Conventional acid-extraction that involves simple shaking of the samples could not intensively break and separate the molecules of fat matrix to let the bounded metal ions free, to aqueous phase. It is suggested that a portion of these metals is either tightly bound to the constituents of oils, or else existed in a form, such as organometallic compounds, which has a high affinity for the oil phase [16].

In our proposed sample preparation method various experimental parameters such as volume ratio, concentration of the reactants, extraction period and equilibration time were optimized, that maximized the percent recovery of the metals. Pre-treatment of the samples by 2ml conc. nitric acid, prior to the addition of 10 ml CCl₄ and 10ml 2N nitric acid was found to behave as a better digestive and extractive media. The use of ultrasonic intensifications with vigorous speed and high intensity, in the present analysis, instead of conventional shaking, proved quite effective in breaking of oil matrixes.

Our findings revealed that an equilibrium stage that permits the transfer of encountered metals from the organic matrix to aqueous phase is an important factor in this procedure. This analytical equilibrium was earlier attained by the improved proposed conditions and only 10 minutes time was enough for complete extraction of metals from oil to aqueous phase when compared with normal acid-extraction that needed 180 minutes (13).

It was also investigated that after the metals were extracted in aqueous phase, an equilibration of 20 minutes is enough for satisfactory data and results. Over night stayed samples show no significantly

varied results. Instead of equilibration in separating flask, use of centrifuge reduced the time period from 20 to 2 minute only.

The data for the percent recovery test (standard addition method) for iron, copper, nickel and zinc by different sample preparation methods is given in the table 6. The percent recovery for iron, copper, nickel and zinc i.e. 96.5-97.5%, 96.45-97.08%, 95.8-97.5% and 96.0-97.8% respectively as got by the proposed acid-extractive method are in close agreement to those by digestion method. Table 1. shows statistical data for the calibration of standards solutions (concentration range 0.0-0.5 ppm) of the investigated metals with a very good correlation of coefficient ranged 0.9970-0.9990.

Table 2. showed the amount of iron determined by different sample preparation methods in various samples of margarines and shortenings. The level of iron was ranged between 0.52 - 2.38 ppm. Most of the samples were found to be high in their iron content which could be attributed to the rusting of black iron equipment used in the processing of vegetable oils and fats.

Table 3. showed the amount of copper in various brands of investigated margarines and shortenings. Copper content was ranged 0.028-0.076ppm. Presence of copper may be the result of the degradation of the copper alloys probably used in the processing equipment. It is noteworthy that iron and copper are strong prooxidants and for the oil's best stability the level of iron and copper should, be below 0.1, 0.02 ppm respectively [18]. A high level of iron and copper in most of the investigated samples reflects ill controlled operation and poor maintenance of processing equipment in the industries of this sector.

Table 4. showed the amount of nickel present in various brands of margarine and shortening. The content of nickel was ranged 0.41-3.40ppm. It was

Table 1. Slopes and intercepts with errors of linear regression concentration versus absorption data of standard solutions of metals.

Elements	Conc. Range (ppm)	Absorbance range	Statistical data		
			r	m	c
Iron	0.00-0.5	0.0-0.047	0.999	0.090	0.0015
Copper	0.0-0.25	0.0-0.023	0.998	0.0861	0.0009
Zinc	0.0-0.5	0.0-0.098	0.998	0.196	0.0008
Nickel	0.0-0.5	0.0-0.065	0.997	0.0627	0.003

Table 2: Comparison of sample preparation method for analysis of iron in various margarine and shortenings samples

No	Sample	Digestion Method	Conventional Extraction	Improved Extraction
1	RB/Sh*	1.44 ± 0.08	1.15 ± 0.06	1.38 ± 0.09
2	RN/ Sh	0.57 ± 0.09	0.42 ± 0.05	0.54 ± 0.10
3	TI/ Sh	2.38 ± 0.07	1.83 ± 0.08	2.32 ± 0.14
4	BP/ Sh	0.59 ± 0.06	0.43 ± 0.07	0.54 ± 0.08
5	BB/Sh	1.71 ± 0.06	1.25 ± 0.09	1.62 ± 0.16
6	BC/Sh	0.52 ± 0.10	0.37 ± 0.09	0.49 ± 0.1
7	BL/ Ma [@]	0.68 ± 0.07	0.52 ± 0.06	0.60 ± 0.09
8	PB/ Ma.	0.60 ± 0.06	0.42 ± 0.08	0.56 ± 0.09
9	Ba/ Ma.	1.46 ± 0.08	1.20 ± 0.07	1.4 ± 0.16
10	Pa/ Ma.	0.82 ± 0.09	0.63 ± 0.06	0.79 ± 0.08

Key: * Shortening
@ Margarine

Table3: Comparison of sample preparation method for analysis of copper on margarines and shortenings

No	Sample	Digestion Method	Conventional Extraction	Improved Extraction
1	RB/Sh	0.038 ± 0.007	0.028 ± 0.005	0.034 ± 0.009
2	RN/ Sh	0.038 ± 0.006	0.032 ± 0.008	0.036 ± 0.008
3	TI/ Sh	0.057 ± 0.005	0.048 ± 0.007	0.052 ± 0.006
4	BP/ Sh	0.045 ± 0.006	0.038 ± 0.006	0.042 ± 0.009
5	B B/Sh	0.028 ± 0.004	0.019 ± 0.005	0.024 ± 0.003
6	BC/Sh	0.046 ± 0.009	0.038 ± 0.005	0.043 ± 0.008
7	BB/Ma.	0.058 ± 0.008	0.03 ± 0.009	0.056 ± 0.007
8	PB/Ma	0.039 ± 0.009	0.029 ± 0.008	0.035 ± 0.005
9	Ba/ Ma	0.037 ± 0.005	0.030 ± 0.007	0.035 ± 0.008
10	Pa/ Ma	0.076 ± 0.005	0.05 ± 0.008	0.06 ± 0.007

Table 4: Comparison of sample preparation method for analysis of nickel in margarines and shortenings

No	Sample	Digestion Method	Conventional Extraction	Improved Extraction
1	RB/ Sh	0.56 ± 0.08	0.45 ± 0.09	0.53 ± 0.09
2	RN/ Sh	0.89 ± 0.10	0.75 ± 0.06	0.82 ± 0.14
3	TI/ Sh	0.54 ± 0.06	0.44 ± 0.08	0.50 ± 0.08
4	BP/ Sh	2.01 ± 0.11	1.84 ± 0.10	1.94 ± 0.18
5	BB/Sh	3.40 ± 0.14	3.25 ± 0.08	3.36 ± 0.28
6	BC/Sh	0.55 ± 0.09	0.48 ± 0.07	0.51 ± 0.08
7	B B/ Ma	1.16 ± 0.04	0.92 ± 0.05	1.1 ± 0.14
8	PB/ Ma	0.41 ± 0.08	0.35 ± 0.06	0.40 ± 0.07
9	Ba/ Ma	0.51 ± 0.09	0.39 ± 0.08	0.46 ± 0.09
10	Pa/ Ma	0.87 ± 0.06	0.65 ± 0.07	0.83 ± 0.08

observed that most of the samples of margarine and shortening have high amount of nickel. Nickel is used as a catalyst during hydrogenation of vegetable oils. Hydrogenated oils require a special post-treatment to remove the traces of residual nickel prior to final filtration. Alarming high content of nickel in majority of the investigated margarine and shortening samples showed that most of the industries in this segment do not follow PSI (Pakistan Standard Institute) standards i.e. 0.5ppm, permissible legal limit [19].

Table 5: Comparison of sample preparation methods for the analysis of zinc in various oil and fat samples ($\mu\text{g/g}$)

No.	Sample	Digestion Method	Conventional Extraction	Improved Extraction
1	RB/SH	0.057 ± 0.008	0.039 ± 0.006	0.053 ± 0.008
2.	RN/Sh	0.072 ± 0.008	0.059 ± 0.005	0.068 ± 0.01
3.	TI/Sh	0.078 ± 0.005	0.062 ± 0.008	0.074 ± 0.008
4.	BP/Sh	0.080 ± 0.006	0.061 ± 0.005	0.076 ± 0.007
5.	BB/Sh	0.077 ± 0.005	0.063 ± 0.007	0.074 ± 0.004
6.	BC/Sh	0.043 ± 0.007	0.034 ± 0.007	0.039 ± 0.009
7.	BL/Ma	0.068 ± 0.006	0.059 ± 0.008	0.065 ± 0.009
8.	PB/Ma	0.069 ± 0.007	0.057 ± 0.006	0.063 ± 0.01
9.	Ba/Ma	0.044 ± 0.005	0.037 ± 0.007	0.045 ± 0.008
10.	Pa/Ma	0.046 ± 0.004	0.036 ± 0.005	0.042 ± 0.007

Table 5. showed the amount of zinc present in various samples of margarine and shortening. It was noted in the results that most of the investigated samples were found to have zinc content with in the range of their copper content. Various alloys of copper and iron may have zinc in certain ratio, and their deterioration may be the source of zinc contamination and traces of it may present naturally in different vegetable oils, extracted from the seeds along with their protoplasm [20].

Experimental

Materials

Samples of different brands of shortenings namely; Royal blue (RB), Royal brown (Rn), Tullio industrial (TI), Backman bisco(Bb), Backman cremo(BC) and margarines namely; Blue band (BB), Pak band (PB), Bakery (B) , Pak (P) were collected from the open market and local industries over a period of six months. Six samples of each Brand /company with different code/batch numbers were taken and preserved in clean Teflon bottles before analysis.

Chemicals

Sulphuric acid, nitric acid, carbon tetrachloride and hydrogen peroxide used were of E. Merk analytical reagent grade.

Standard solutions of iron, zinc, copper and nickel were prepared from certified standard solution of 1000 ppm (Fluka kamica) of corresponding metal ions. Acid washed glassware and deionized water was used through out the analysis for the preparation of standard and stock solutions.

Instrumentation

A Hitachi Ltd. (Tokyo, Japan), Model 180-50, S.N. 5721-2, Atomic Absorption Spectrophotometer with a D₂ lamp for background correction with linear (least square) mode, equipped with a Model, 056 Hitachi recorder was used for recording the analytical data for the metals under investigation.

Preparation of Samples

Samples of oil and ghee were prepared for analysis by two methods.

(a) Digestion (b) Extraction

(a) Digestion Method

Triplicate samples of margarine and shortening were weighed in separate flasks and treated with concentrated nitric acid to oxidize the organic matter [21]. Then added sulphuric acid and hydrogen per oxide and heated to decompose completely the organic matter, till clear solutions were obtained. Evaporated all the contents of flasks, and dissolved semidried materials in small amount of deionized water. The contents of the flasks were filtered through whatman filter paper No. # 42. and made up to volume with deionized water.

(b) Conventional Extraction Method

Triplicate samples of margarine and shortenings (1.0g) were taken in 100-ml conical flasks, heated on hot plate and then added 10 ml of carbon tetrachloride and 10 ml 2N nitric acid. The conical flasks were stoppered and the contents were shaken on Gallenkamp flask shaker for 45 minutes. The resultant mixtures were then poured in separating funnels and allowed to equilibrate for 2-3 hour. The upper aqueous layers was collected and aspirated directly in flame for the determination of the metals; iron, copper and nickel and zinc by Atomic Absorption Spectrophotometer.

Improved Extraction Method

Triplicate samples of margarine and shortenings (1.0g) were taken in flasks; added 2 ml concentrated nitric acid and heated for five minutes. Then added 10 ml of carbon tetrachloride and 10 ml 2N nitric acid. The contents of the flasks were fed to ultrasonic bath and intensified continuously for different time periods of 5, 10, 15, 20 minutes, and

one hour at 30 ± 1 °C. The resultant mixtures were then poured in separating funnels and allowed to equilibrate for 10, 15, 25 and 60 minutes, and one of the samples to over night stay. Duplicate samples processed side by side after extraction were subjected to centrifugation to reduce the time period consumed in equilibration. The upper aqueous layers was collected and aspirated directly in flame for the determination of iron, copper, zinc and nickel by Atomic Absorption Spectrophotometer Hitachi Model, 180-50 using air acetylene flame.

For the above methods, flasks containing all reagents used for digestion and extraction except samples and standards, blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Standard Calibration Curves for Iron, Copper, Nickel and Zinc

A series of standard solutions of each cation in the range of the absorbance noted for unknown samples were simultaneously run on the atomic absorption spectrophotometer. Standard calibration curves were obtained for concentration verses absorbance data that was statistically analysed using fitting of straight line by least square method mentioned in table 1.

Percentage Recovery Test

The margarine and shortening samples were spiked with 0.0, 0.125, 0.25, 0.50, 1.0, 2.0 µg /g of each metal ion using the standard addition method and subjected to extraction and digestion as described above. The values are given in table 6.

Table 6: Percentage recovery test

Elements	Conventional Extraction	Improved Extraction	Digestion method
Iron	72.80-81.94	96.5-97.5	98.4-99.0
Copper	74.48-80.52	96.45-97.08	97.85-99.4
Nickel	78.00-84.09	95.80-97.50	98.74-99.0
Zinc	80.00-85.68	96.00-97.8	96.95-98.84

Conclusions

The trace quantities of some metals are present in plants, seeds and crude oil extracted from them. As the biological pattern of cultivation and the process equipment technology vary from unit to unit. So, it is very hard to conclude in absolute terms (what

would be) the exact level of such metallic contents in raw and end-use products. However, such metallic contamination may be kept to a minimum by proper treatment and handling during storage and processing of oil and fats. It is impossible to eliminate oil contact with iron because much of the industry uses black equipment. The use of stainless steel, 304 S.S, 316 S.S sheets is recommended for fabrication of process equipment to keep iron contamination as low as possible. To get rid of strongest prooxidant copper, the use of copper alloys in oil processing units and fabrication should be avoided.

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