



Analytical Methods

Variation in accumulation of heavy metals in different varieties of sunflower seed oil with the aid of multivariate technique

Rehana Ansari^a, Tasneem Gul Kazi^{a,*}, Muhammad Khan Jamali^a, Muhammad Balal Arain^a, Muhammad Dowood Wagan^b, Nusrat Jalbani^c, Hassan Imran Afridi^a, Abdul Qadir Shah^a^a National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan^b PCSIR Laboratories, Jamshoro, Sindh, Pakistan^c PCSIR Laboratories, Karachi, Pakistan

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ABSTRACT

In this work, three heavy metals (HMs) cadmium, lead, and zinc were determined in 16 newly bred varieties of sunflower seed oil, using atomic absorption spectrometry, prior to use of microwave-assisted acid digestion method (MWD). The accuracy of the proposed method was studied by standard addition method and values obtained by conventional wet acid digestion methods (CAM) on same samples. The detection limit obtained from the standard addition curve was 0.327, 3.38, 10.0 ng/ml for Cd, Pb, and Zn, respectively, with high percentage recoveries. Multivariate statistical techniques (cluster and principal component analysis) were applied to modeling classes of different varieties of sunflower seed oils grown in same agricultural plot, on the basis of the analytical data of HMs. The results obtained providing sufficient information on tolerable limit of all three HMs by different varieties of sunflower oil to grow safely.

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1. Introduction

Sunflower oil is a widely consumed product with high nutritional value and significant health benefits. It is known for its superior organoleptic characteristics (taste) and remarkable antioxidant properties; it contains vitamin A, D, and a sufficient amount of vitamin E. Besides anti-oxides, it contains a huge amount of oxidants and also heavy metals (HMs) like Cu, Fe, Ni, Cd, Pb, and Zn. Concerning vegetable oils, its HMs composition is an important decisive factor for the assessment of their quality; it is known that the HMs affect their rate of oxidation, nutritional value, keeping properties, and storability, so their determinations in vegetable oils could be important (Murillo et al., 1999).

A great deal of research has been devoted to discover why fats and oils undergo changes more rapidly than others, what the changes are, what are the causes for these changes and how they can be controlled (Murillo et al., 1999). Edible fats and oils are frequently subjected to processing including refining, bleaching, and

deodorization, which inevitably makes the oils come into contact with metallic surface areas, often at high temperatures (Martin-Polvillo, Albi, & Guinda, 1994). The main sources of HMs to plants are their growth media, like nutrient solution and soils (Tüzen, 2003a, 2003b). The extent, to which plants take up metals, depends on the binding of trace analytes to soil constituents; other sources of HMs may include pesticides and fertilizers. Cadmium and lead are highly toxic elements that accumulate in biological systems and have a long half-life. They are not essential elements in plant nutrition; it is easily transferred from soil to plants, which are increasingly contaminated by these elements from phosphate-based fertilizers (Mena, Cabrera, Lorenzo, & Lopez, 1996). Cadmium and lead can also be present in edible oils and fats, as a result of contamination from the environment, the refining process, the storage tank, or the packing material (e.g., as a colourant or stabilizer in plastics) (Dalen, 1996). Many reports have described the deleterious effects that HMs has on the flavour and oxidative stability of oils (Chen, Chen, Cheng, & Chou, 1999; Murillo et al., 1999).

The accurate analysis of organic liquids for the determination of metal concentrations is still an analytical challenge, owing to their low concentration level and the difficulties that arise due to the characteristics of the matrix. However, atomic absorption spectrometry is still the most cited technique (De Leonardis, Macciola, & De Felice, 2000; Bati & Cesur, 2002). Various articles have been published dealing with the determination of HMs in vegetable oils

* Corresponding author. Tel.: +92 022 2771379; fax: +92 022 2771560.

E-mail addresses: rehana_ansari_57@yahoo.com (R. Ansari), tgkazi@yahoo.com (T.G. Kazi), mkhanjamali@yahoo.com (M.K. Jamali), bilal_ku2004@yahoo.com (M.B. Arain), md_wagan@yahoo.com (M.D. Wagan), nusratjalbani_21@yahoo.com (N. Jalbani), hassanimranafriidi@yahoo.com (H.I. Afridi), aqshah07@yahoo.com (A.Q. Shah).

at sub-ppm levels using different spectroscopic techniques (Brenner, Zander, Kim, & Sholnik, 1996; Kumar & Gangadharan, 1999; Castillo, Jimenez, & Ebdon, 1999; Tüzen, 2003a, 2003b).

Most of the spectroscopic techniques require sample pretreatments. Various methods, including extraction, solubilization, pre-concentration, and dilution, are used to minimise the organic matrixes in vegetable oil (Martin-Polvillo et al., 1994). In most cases, these pretreatment methods are tedious and time consuming with the consequent risk of sample contamination (i.e., due to the atmospheric pollution of the muffle), and analyte loss (due to formation of volatile compounds, etc.) or to an incomplete mineralisation of the organic matrix (Carrilho, Gonzalez, Nogueira, Cruz, & Nobrega, 2002). The microwave digestion system for laboratory applications has offered enormous advantage in mineralisation (Bellido-Milla, Moreno-Perez, & Hernandez-Artiga, 2000; Ibrahim, 1991).

The atomic absorption spectrometry technique in combination with multivariate statistical techniques such as principal component analysis (PCA) and cluster analysis (CA) have been applied successfully to classify and discriminate the oils (Lee, Lee, Kim, Kim, & Kim, 2001; Lee, Noh, Bae, & Kim, 1998). Chemometric methods have been commonly applied for matching, discrimination, classification and prediction in assessing the HMs levels in different varieties of sunflower seed oils (SFSO). Mineral seem to be good candidate for a classification system because they are stable and their content depends on the type of soil in which the oil seeds were grown.

The main aim of the present work was to evaluate the HMs in 16 newly breaded varieties of SFSO, by using multivariate technique (PCA and CA). The oil samples were oxidised by microwave-assisted acid digestion method. The accuracy of digestion method was checked by standard addition method and with those values obtained from conventional wet acid digestion method. For HMs analysis, the pretreated oil samples were subjected to atomic absorption spectrometry, Zn was determined by flame atomic absorption spectrometry (FAAS) using air acetylene flame, whereas Cd and Pb were determined by electrothermal atomic absorption spectrometry (ETAAS). Pattern recognition techniques (PCA and CA) were applied within HMs descriptors to characterise and differentiate the all varieties of sunflower seed oil, grown in same agricultural plot. On the bases of result to gain information about the possibility of selection of those varieties which one is more resistant to uptake of these HMs.

2. Material and methods

2.1. Apparatus

A PEL (PMO23) domestic microwave oven (900 W) was used to digest the samples. The analysis was carried out using a Perkin–Elmer model A-Analyst 700 (Norwalk, CT, USA) atomic absorption spectrometer and GF 3000 with deuterium background correction, equipped with a graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, autosampler AS-800 and interfaced to a personal computer. Hollow cathode lamps (Perkin–Elmer) were used as radiation sources. The operational parameters are shown in (Table 1).

2.2. Reagents and glassware

Ultra pure water obtained from ELGA lab water system (Bucks, UK), was used throughout the work. All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). A mixture of nitric acid (65%) and H₂O₂ 30% (2:1 ratio) were used for wet acid digestions. 1000 mg/l standard solutions of Cd, Pb, and

Zn were prepared by dilution of certified Standard solutions, Fluka kamika (GmbH CH-9471, Buchs, Switzerland). The chemical modifiers, Pd (NO₃)₂ was used for Cd, and NH₄H₂PO₄ was used for Pb (Merck Ltd., Poole, Dorset, UK). Acid-washed plastic (polypropylene) vessels were used for preparing and storing solutions. All solutions were stored at –4 °C until needed for analysis. All glassware and plastic materials and the auto sampler cups were treated for 24 h with 5 M analytical grade HNO₃, rinsing with distilled water and Milli-Q water, dried and stored in a class-100 laminar flow hood.

2.3. Sampling

Sixteen samples of different varieties of sunflower seed oil were grown in the same agricultural plot of Sindh Agricultural Research Centre Tando Jam University, Sindh, Pakistan (oil seed section), in the year 2006–2007. The samples of sunflower seed varieties were crushed and extracted with n-hexane separately, on electric hot plate at 65 °C by Soxhlet for about four to five hours. After cooling, filtered the extract through 42 Whatman filter paper. The oil was then separated from solvent (Hexane), and dried on a rotary evaporator at 60 °C for 10 min and finally the clear oil was preserved in clean polyethylene tubes before analyses at 4 °C.

3. Methods of analysis

3.1. Conventional wet acid digestion method

Triplicate samples (0.5 g) of each sunflower oil variety were accurately weighed into separate conical flasks (100 ml). About 10 ml of a freshly prepared mixture of concentrated HNO₃–H₂O₂ (2:1, v/v) was added to each flasks and kept for 10 min at room temperature, then the content of flasks were heated on an electric hot plate at 80 °C, until clear solutions were obtained. The contents of the flasks were evaporated, and the semidried mass was dissolved in 5 ml 0.2 M HNO₃, filtered through Whatman No. 42 filter paper, and made up to final volume of 10 ml in volumetric flasks with ultrapure water and kept as stock sample solution.

3.2. Microwave-assisted acid digestion method

A microwave-assisted digestion procedure was carried out, in order to achieve a shorter digestion time. Triplicate samples of sunflower oil samples, of each variety (0.2 g) were placed in polytetrafluoroethylene (PTFE) flasks (25 ml in capacity). Added 2.0 ml mixture of acid and oxidant, HNO₃ (65%) and H₂O₂ (30%) 2:1 ratio, and kept the flasks at room temperature for 1 h, then placed the flasks in a covered Teflon container, and placed in a domestic microwave oven and heated at 80% of total power (900 W), for 5 min to decompose the organic matter. After cooling the digestion flasks; the resulting solutions were evaporated to semidried mass to remove excess acid, and diluted with 0.2 M nitric acid upto 10 ml in volumetric flasks and kept as stock sample solution.

A blank (without sample) was carried out through the both procedures. The concentrations of understudy HMs were obtained directly from calibration graphs after correction of the absorbance for the signal obtained from an appropriate reagent blank. Blanks and standard solutions were prepared in a similar acid matrix. In both acid digestion methods, analyte addition tests have confirmed the adequacy of aqueous calibration.

The validity and efficiency of the microwave-assisted digestion method was checked by standard addition method, the known amount of standard (0.5 mg/l) was spiked with some varieties, using replicate five samples of each, and with those obtained from CAM. All experiments were conducted at room temperature (30 ± 5 °C). The results obtained are reported in Table 2.

Table 1
Measurement conditions for atomic absorption spectrometer (AAS 700).

Parameters	Pb	Cd	Zn*
Lamp current (mA)	8.0	6.0	10.0
Wave length (nm)	283.3	228.8	214.0
Slit-width (nm)	0.7	0.7	0.7
Background correction	D ₂ lamp	D ₂ lamp	Oxidant (Air) 17.0 l/min
Cuvette	Cup	Cup	
Drying temperature °C/ramp/hold(s)	140/15/5	140/15/5	Fuel (acetylene) 2.0 l/min
Ashing temperature °C /ramp/hold(s)	700/10/20	850/10/20	
Atomization temperature (°C/ramp/hold) (s)	1800/0/5.0	1650/0/5.0	Burner height 7.5 (mm)
Cleaning temperature (°C/ramp/hold) (s)	2600/1/3	2600/1/3	
Chemical modifier	15 µg Mg(NO ₃) ₂	5 µg Pd	

* Zinc was determined by flame atomic absorption spectrometry.

Table 2
Comparative results for the % recovery of Zn, Cd, and Pb by standard addition method with different varieties of sunflower seed oils using conventional (CAM) and microwave acid digestion methods (MWD) (µg/g).

Samples	Standard addition method				Paired <i>t</i> -test ^c <i>t</i> _{Experimental}	% Recovery	
	CAM ($\bar{x} \pm ts/\sqrt{n}$) ^b		MWD ($\bar{x} \pm ts/\sqrt{n}$)				
	Before	After	Before	After			
Zn ^a	HA10	3.26 ± 0.26	3.69 ± 0.23	3.19 ± 0.1	3.61 ± 0.23	0.0174	96.8–98.2
	Res15	4.29 ± 0.35	4.71 ± 0.23	4.2 ± 0.12	4.55 ± 0.23	0.0284	
Cd ^a	HA10	2.25 ± 0.30	2.69 ± 0.17	2.1 ± 0.12	2.45 ± 0.12	0.0164	96.7–98.6
	Res15	2.48 ± 0.51	2.94 ± 0.14	2.2 ± 0.11	2.66 ± 0.16	0.0178	
Pb ^a	HA10	1.08 ± 0.06	1.53 ± 0.11	1.05 ± 0.05	1.52 ± 0.1	0.023	96.9–98.7
	Res15	1.08 ± 0.04	1.56 ± 0.1	1.05 ± 0.03	1.51 ± 0.12	0.025	

*t*_{Critical} at 95% confidence limit = 2.77.

^a 0.5 mg/l standard were added.

^b Average value ± confidence interval (*P* = 0.05).

^c Paired *t*-test between CAM vs. MWD, degree of freedom (*n* – 1) = 4.

3.3. Data treatment by statistical method

All mathematical and statistical computations were made using with the Excel X State computer program (Microsoft Corp., Redmond, WA, USA) and Minitab 13.2 (Minitab Inc., State College, PA, and XL stat (version)). Multivariate analysis of the 16 varieties of seed oils was performed through principal component and cluster analysis techniques (Kazi et al., 2008).

4. Quality control

The linear range of the calibration curve reached from the detection limit up to 0.0–25, 0–100, and 0–500 µg/l for Cd, Pb, and Zn, respectively. The data revealed excellent coefficient of correlation range, $R^2 = 0.999$. The limits of detection (LOD) and limit of quantification (LOQ) for elements were calculated as under, $LOD = 3 \times (s/m)$ and $LOQ = 10 \times (s/m)$, respectively, where “*s*” is the standard deviation of 10 measurements of the blank and “*m*” is the slope of the calibration graph obtained for each case, the LODs; 0.327, 3.38, 10.0 ng/ml and LOQ 1.09, 11.3 and 33.5 ng/ml for Cd, Pb, and Zn, respectively.

The accuracy of the proposed method was studied by standard addition method, and the results obtained with MWD were compared with those obtained from CAM. For the recovery test, laboratory-extracted SFSO from known seed varieties (HA1, HA7, HA10, and HA 15) were spiked with 0.5 µg/ml for, Cd, Pb, and Zn, respectively. Results of the proposed MWD showed the recovery of Cd, Pb, and Zn ranged between 96.7–98.6%, 96.9–98.7%, and 96.8–98.2%, respectively, no significant differences were observed for *p* < 0.05 when comparing the values obtained by both methods (paired *t*-test) (Table 2).

5. Results and discussion

For the estimation of HMs in edible oil samples by AAS have shown better results in terms of percent recovery and time constraints. The exposure time of microwave energy have proved very effective in breaking of oil matrixes within few minutes as compared to those obtained from CAM in 3–4 h. The validity and efficiency of the microwave-assisted digestion method was checked with standard addition method to spike the known amount of standard in seed oil samples of different SFSO varieties, and with those obtained from conventional wet acid digestion method on same samples, as reported in Table 2. These results suggested that the microwave-assisted acid digestion offers a facile alternative for the analysis of a large number of oil samples in a minimum time (Richter, Link, & Kingston, 2001). In addition the final solution obtained can be directly analysed by atomic absorption spectrometer without all the instrumental problems that would occur with the direct analysis of oil or solvent–oil mixture.

5.1. Principal component analysis (PCA) and cluster analysis (CA)

The application of atomic absorption spectrometry in combination with multivariate statistics gave good results in the characterisation of all 16 newly bred varieties of sunflower edible seed oil. Different varieties of sunflower seeds grown in same agricultural plot of Sindh Agricultural Research Centre Tando Jam University, Sindh, Pakistan (oil seed section), and were analysed for Cd, Pb, and Zn contents. Chemometric methods were applied for quantitative discrimination in uptake of Cd, Pb, and Zn contents amongst different varieties of SFSO using CA and PCA (Martinez et al., 2003).

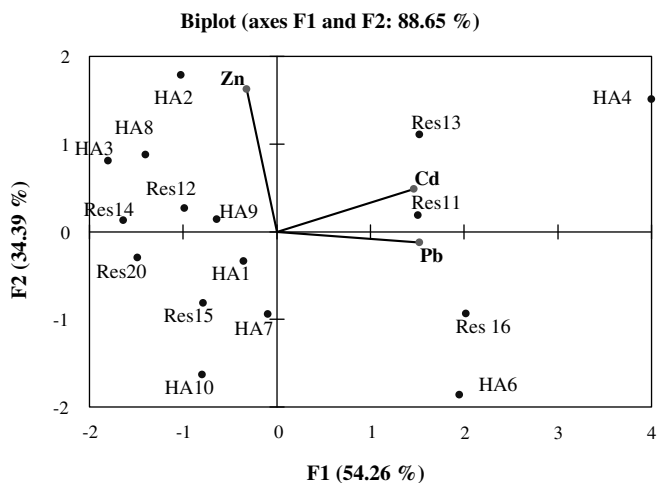


Fig. 1. Biplot of sunflower seed oil samples by using the values from Table 4.

The PCA allowed considerable reduction in a number of variables and the detection of structure in the relationship between metals and different varieties of SFISO that give complimentary

information. All varieties contain different amount of metals as predicted by PC score plot (Fig. 1), although they were grown in same agricultural plot. The full autoselected data matrix consisting 16 different varieties of SFISO were submitted to PCA. For PCA modeling, the 16×3 data matrix was divided into a sample code and different metal data sets. All samples in the different categories were selected randomly. The results for the three HMs (Cd, Pb, and Zn) were determined in SFISO samples. The variability of results for all three metals in 16 varieties are large, so the different categories of SFISO are formed according to the concentration of HMs.

For visualising the data trends and the discriminating efficiency of the metals descriptors, a scatter plot of samples using the first three principal components (PCs) issued from PCA of the data matrix (also called scores plot) was obtained (Fig. 1). As can be seen, there is a neat separation of the 16 new varieties of sunflower seed oil according to the concentration of HMs. The first three principal components, accounting for 88.65% of the total variability were considered to be sufficient for such data (Table 4). In first component that represent 54.3% of the total variability, Cd and Pb are dominant variables. In second component which was 34.4% of total variability is dominant for Zn and Cd; whilst Pb is dominate in the third principal component that represents 11.3% of total variability in understudy varieties of SFISO (Table 4).

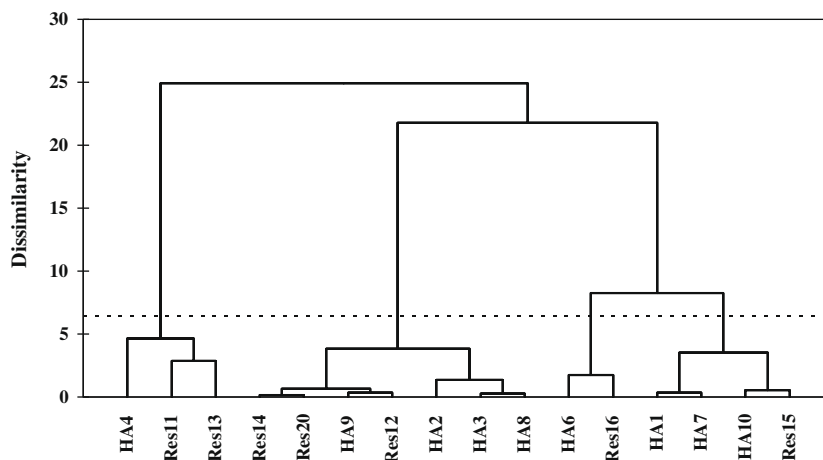


Fig. 2. Dendrogram of cluster analysis for 16 varieties of sunflower seed oils.

Table 3

Total metals detected from 16 varieties of sunflower seed oils ($\mu\text{g/g}$).

Observation	Class	Zn	Cd	Pb
HA1	1	5.27 \pm 0.92 ^a (5.16 \pm 0.2) ^b	1.79 \pm 0.28 (1.59 \pm 0.09)	2.47 \pm 0.12 (2.39 \pm 0.13)
HA2	2	7.69 \pm 0.46 (7.53 \pm 0.31)	2.85 \pm 0.63 (2.53 \pm 0.08)	1.08 \pm 0.04 (1.05 \pm 0.06)
HA3	2	6.66 \pm 0.35 (6.53 \pm 0.17)	1.84 \pm 0.23 (1.64 \pm 0.08)	0.79 \pm 0.03 (0.77 \pm 0.04)
HA4	3	6.55 \pm 0.57 (6.42 \pm 0.2)	6.18 \pm 0.88 (5.49 \pm 0.2)	4.4 \pm 0.21 (4.27 \pm 0.21)
HA6	4	2.73 \pm 0.40 (2.4 \pm 0.13)	3.57 \pm 0.32 (3.17 \pm 0.13)	3.37 \pm 0.11 (3.27 \pm 0.19)
HA7	1	4.44 \pm 0.17 (4.35 \pm 0.17)	1.88 \pm 0.30 (1.67 \pm 0.04)	2.57 \pm 0.11 (2.5 \pm 0.12)
HA8	2	6.86 \pm 0.35 (6.72 \pm 0.31)	1.70 \pm 0.15 (1.51 \pm 0.07)	1.48 \pm 0.04 (1.43 \pm 0.05)
HA9	2	5.68 \pm 2.56 (5.57 \pm 0.33)	2.34 \pm 0.46 (2.08 \pm 0.08)	1.67 \pm 0.04 (1.62 \pm 0.05)
HA10	1	3.26 \pm 0.26 (3.19 \pm 0.1)	2.25 \pm 0.30 (2.1 \pm 0.12)	1.08 \pm 0.06 (1.05 \pm 0.05)
Res11	3	4.91 \pm 0.31 (4.81 \pm 0.12)	5.02 \pm 0.34 (4.46 \pm 0.19)	1.88 \pm 0.09 (1.82 \pm 0.08)
Res12	2	5.68 \pm 0.17 (5.57 \pm 0.2)	2.67 \pm 0.61 (2.37 \pm 0.15)	0.91 \pm 0.04 (0.88 \pm 0.02)
Res13	3	6.60 \pm 0.12 (6.47 \pm 0.17)	3.96 \pm 0.82 (3.52 \pm 0.19)	3.2 \pm 0.12 (3.1 \pm 0.13)
Res14	2	5.73 \pm 0.35 (5.61 \pm 0.3)	1.88 \pm 0.34 (1.67 \pm 0.11)	0.79 \pm 0.04 (0.77 \pm 0.02)
Res15	1	4.29 \pm 0.35 (4.2 \pm 0.12)	2.48 \pm 0.51 (2.2 \pm 0.11)	1.08 \pm 0.04 (1.05 \pm 0.03)
Res 16	4	4.24 \pm 0.38 (4.15 \pm 0.13)	2.99 \pm 0.34 (2.66 \pm 0.12)	4.29 \pm 0.06 (4.16 \pm 0.19)
Res20	2	5.22 \pm 0.20 (5.11 \pm 0.3)	1.74 \pm 0.18 (1.55 \pm 0.06)	1.02 \pm 0.04 (0.99 \pm 0.04)

^a Conventional acid digestion methods (CAM).

^b Microwave digestion methods (MWD).

Table 4

Eigenvector and eigenvalues on the correlation matrixes of heavy metals concentration in 16 varieties of sunflower seed oils.

Parameters	F1	F2	F3
Zn	-0.150	0.955	0.255
Cd	0.684	0.287	-0.671
Pb	0.714	-0.074	0.697
Eigenvalues	1.63	1.03	0.340
Variability (%)	54.3	34.4	11.3
Cumulative %	54.3	88.6	100

Cluster analysis was also used to qualitatively test the class separation (Silva & Rezende, 2002). Hierarchical cluster analysis applied to study the results of the analysis of HMs, in different varieties of sunflower seed oil seems to discriminate between samples even they belong to the same plot used for the cultivation. In (Fig. 2) the resulting dendrogram shows the three main clusters, the first cluster (C1) contain variety HA1, HA7, HA10, and Res15, which show less uptake of all three HMs as compared to other varieties. The second cluster (C2) at 21% dissimilarity have HA2, HA3, HA8, HA9, Res12, Res14, Res 20, these varieties show the uptake of Zn was on higher side (5.22–7.69 mg/kg), whilst the varieties belong to cluster 3 (C3), HA4, Res11, Res13 shows high level of Cd (3.96–6.18 mg/kg) as compared to other varieties at dissimilarity level 25%. These results suggested that the varieties belonging to C1, HA1 HA7, HA10, and Res15 which contain less amount of all three HMs are suitable for the edible oil production.

In principal, this approach provides a very flexible method for assessment of suitable variety of sunflower seed oil from the measured data of Zn, Cd, and Pb. Oils and fats, particularly vegetable oils, are essential for a healthy diet because they provide the body with nutrients and are low in cholesterol, rich in unsaturated fatty acids, aid in the absorption of vitamins, and act as a carrier of flavours.

5.2. Evaluation of metals in sunflower seed oils

Vegetable oils and fats contain trace levels of various metals depending upon many factors, such as species, soil used for the cultivation, irrigation water, variety, and stage of maturity. Trace quantities of some metals are present naturally in the seeds, and the crude oil extracted from them contains these metals in different concentrations. The uptake of metals by plants from soil also depends on plant species; this was proved in our investigation.

The results of HMs in different varieties of SFSO grown in the same environment are shown in Table 3. Most of these samples were found to have high Cd and Pb contents. The maximum amount of Zn (7.69 µg/g) was observed in sunflower species HA2, whilst the minimum level of Zn (2.73 µg/g) was observed in HA6; these values were obtained by using the wet acid digestion method. The amount of Cd in various sunflower oil varieties was found by using the CAM to be in the range of 1.70–6.18 µg/g for HA8 to HA4 varieties. The high amount of Cd 5.02 and 6.18 µg/g were present in Res 11 and Res 4, respectively, whilst the low amount of Cd contained in remaining varieties. The all varieties of sunflower seed oil samples contains Pb in the range of 0.79–4.4 µg/g; the high level of Pb was observed in HA1, HA4, HA6, HA7, Res13, and Res16, whilst remaining contained lower concentration. All restorer (Res) varieties contain high levels of the all three HMs as compared to other varieties, due to high affinity for these HMs present in agricultural soil. These conditions depend on nature or the lack of awareness of growers; about the dangers of the unhygienic conditions caused by use of contaminated wastewater, fertilizers, and high application of pesticides. Because of the biological pattern of cultivation, trace quantities of some metals

are present naturally in oilseeds and crude oil extracted from them. However, such contamination may be kept to a minimum level by using proper conditions of pre-cultivation; the soil should be analysed and certified, the water used should not be contaminated, and the seeds used for the cultivation should be certified for high tolerance to HMs.

6. Conclusions

It was concluded that the digestion of oil samples with microwave oven is a reliable method of sample preparation, for the determination of heavy metals in different varieties of sunflower seed oils by atomic absorption spectrometer. The proposed procedure allows for a reduction in the preparation time and the manipulation of the samples when compared to the conventional wet acid digestion method using electric hot plate.

The 16 new breaded sunflower seed oils were discriminated by PCA and CA in determining heavy metals patterns in all varieties. In general, this approach provides a very flexible method for assessment of product quality. The PCA and CA analysis shows that all varieties of sunflower oil contains notable amounts of Zn, and some varieties shows high accumulation of toxic metals, Cd and Pb, which poses a threat to oil quality and to human health. Whilst the varieties HA1 HA7, HA10, and Res15, which contain less amount of all three HMs are suitable for the edible oil production.

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