



Determination of Toxic Metals in Different Brand of Chocolates and Candies, Marketed in Pakistan

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Abstract

In present study three toxic metals, cadmium (Cd), nickel (Ni) and lead (Pb) were determined in chocolates and candy samples available in local markets of Hyderabad, Pakistan. Concentrations of understudy toxic metals (TMs) were determined by electrothermal atomic absorption spectrometry (ETAAS) prior to microwave assisted acid digestion. Validation of the methodology was performed by standard addition method and conventional acid digestion on electric hot plate to obtained TMs concentration, for comparative purpose to obtain results within the 95% confidence level. No significant differences were observed for TMs obtained from both methods ($P = 0.05$). The concentration of Cd, Ni and Pb were observed in chocolates and candy samples is ranged as of 0.099 - 0.353, 1.45 - 4.33 and 1.11 - 2.48 $\mu\text{g/g}$, respectively. The results indicated that cocoa-based chocolates have higher contents of TMs than milk- based chocolates and candies.

Keywords: Toxic metals, Chocolates, Candies, Atomic absorption spectrometry

Introduction

Chocolate is an ingredient usually used in the preparation of several foods (cakes, cookies, breads, ice creams, etc.), which are consumed mainly by children. Thus, the mineral content of this ingredient has great value for nutritional control of the foods. However, the determination of metals in such samples involves a difficult step of digestion, considering that its matrix contains high contents of organic compounds [1]. Chocolate is a vastly nutritious energy source, with a fast metabolism and good digestibility. The presence of cocoa, milk and sugar in its composition can be the warranty of proper ingestion of proteins, carbohydrates, fats, minerals and vitamins [2]. It is therefore necessary to monitor human exposure to TMs present in the food chain [3].

Toxicity of Cd came in the headlines after the Itai-Itai disease was found to be caused by high intake of Cd in Japan. When Cd is ingested in excess amounts, it induces toxicity symptoms like gastrointestinal pains, nausea, respiratory distress, diarrhoea, impaired reproductively, kidney damage and hypertension [4-6].

Lead contamination in chocolates and candies is a very old problem that has evolved with time. Since the middle of the 19th century, various measures including regulations and public education were implemented to minimize the contamination of chocolates and candies from such sources [7]. Nowadays, industrial activities dominate the global flux of lead in the environment [8, 9] and have become the predominant sources of lead in many food items, including candies [10].

Nickel occurs naturally more in vegetables than in animal flesh [11]. However, nickel toxicity in humans is not a very common occurrence because the intestinal absorption of nickel is very low [12]. Apart from environmental contamination sources of nickel in foods, this metal may also be derived in foods from processing activities such as drying, cooking and canning in nickel-containing vessels [13].

Different complex matrices of the analytical sample require prior mineralization for most analytical methods, and this step is critical in the whole analytical

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procedure for the determination of metal concentration. Analysis of metals in food samples has conventionally been performed by atomic absorption spectrometry [14, 15]. Sample digestion techniques, such as microwave, and conventional acid digestion method (CDM) for TMs determination have been used widely for the dissolution of target elemental analytes. These digestion techniques, however, require the use of concentrated mineral acids and high temperatures whereas high pressures are required in the case of microwave applications [16].

The present study reports concentration of Pb, Cd and Ni in the chocolates and candies available in different brands available in Hyderabad, Pakistan. Chocolates and candies/toffees are the favorite food items of children and they are the most susceptible age group to toxic metals intake from such type of food items. In view of the complex matrixes of chocolates and candies having high sugar and organic contents, proper acids mixtures were selected for decomposition of samples. Conventional wet acid digestion on an electric hot plate method was also used to compare the results with those obtained by microwave assisted digestion. The understudy toxic metals were analysed by electrothermal atomic absorption spectrometry.

Materials and Methods

Reagents

Ultrapure water obtained from ELGA labwater system (Bucks, UK), was used throughout the work. All reagents used were of analytical reagent-grade. Concentrated Nitric acid (65%), and hydrogen peroxide (30%), were spectroscopic grades (Merck, Darmstadt,

Germany). Certified standards of Cd, Ni and Pb (1000 ppm) were obtained from Fluka (Buchs SG, Switzerland). Chemical modifiers, Mg (NO₃)₂ stock standard solution, 2.00 g L⁻¹, was prepared from Mg (NO₃)₂ (Merck) and Pd stock standard solution, 3.00 g L⁻¹, was prepared from Pd 99.999% Sigma Aldrich (Milwaukee, WI, USA).

Laboratory glass wares was kept overnight in 10% (v/v) nitric acid solution and washed with distilled water and finally with ultrapure water before use.

Apparatus

Atomic absorption spectrometer of Hitachi Ltd., Model 180-50, S.N.5721-2, equipped with graphite furnace G-03 was used. The instrumental parameters are shown in Table 1. A PEL domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900W, was used for total digestion of samples. For graphite furnace measurements, argon was used as inert gas.

Sampling

A total of 40 different brands of chocolate and candies were collected from the different markets of Hyderabad city. Different batches on different dates of same brand and type of samples (chocolates and candies) were purchased directly from food vendors to observe the variation in the elemental contamination levels of the products. The collected samples of different batches were dried at 70 °C for 2 h separately. The dried samples were ground and stored in plastic bottle at -4 °C.

Table 1. Measurement conditions for electrothermal atomization AAS*.

Parameter	Cd			Pb			Ni		
Lamp current (mA)	7.5			7.5			10		
Wave length (nm)	228.8			283.3			232.0		
Slit-width (nm)	1.3			1.3			0.2		
Cuvette	Tube			Tube			Tube		
Chemical Modifier	Mg(NO ₃) ₂ +Pd(NO ₃) ₂			Mg(NO ₃) ₂			Mg(NO ₃) ₂		
Temperature Programme									
Drying	80	120	30 Sec	80	120	30 Sec	80	120	30 Sec
Ashing	300	300	30	400	400	30	700	700	30
Atomization	1500	1500	10	200	200	10	2700	2700	10
Cleaning	1800	1800	3.0	2400	2400	3.0	2800	2800	3.0

*Common parameters

Sample volume = 10 µL analyte + 10 µL corresponding modifiers

Background correction : D² lamp

Carrier gas Argon = 200(mL/min)

Procedure

Microwave acid digestion (MAD)

A microwave-assisted acid digestion procedure was carried out in order to achieve a shorter digestion time and eliminate matrix effects. About 0.2 g of duplicate samples of each chocolates and candies were taken in digestion flasks, added 2 mL of mixture of HNO_3 : H_2O_2 (1:1, v/v), and kept for 10 min at room temperature, then the flasks were placed in a PTFE container. This was then heated following a one-stage digestion programme at 80% of total power (900 W) for 3 - 5 min. After cooling the digestion flask, the resulting solutions were evaporated to semidried mass to remove excess acid, and then diluted to 10.0 mL in volumetric flasks with 0.2 M HNO_3 .

Conventional digestion method (CDM)

About 0.5 gm of five replicates samples were taken separately in pyrex flasks (50 mL in capacity), the contents of flasks were treated with 2 mL mixture of acid and oxidant (65% HNO_3 and 30% H_2O_2) in the ratio of (1:1, v/v) for decomposition of organic matter. The contents of flasks were heated on electric hotplate at 80 °C, for 2–3 hrs, till clear. The resulting solutions were evaporated to semidried mass and then final solutions were made up to 25 mL with 0.2 M HNO_3 , for the determination of Cd, Ni and Pb by ETAAS. Blanks (without sample) were carried through the complete procedure of both methods. The concentrations were obtained directly from calibration graphs after correction of the absorbance for the signal from an appropriate reagent blank. All experiments were conducted at room temperature (30°-35°C) following well-established laboratory protocols. The blanks were always prepared in same manner as samples. The digests obtained by both methods were subjected to ETAAS.

Analytical criteria

Calibration and standard addition graphs were obtained for TMs. The linear range of the calibration curve reached from the detection limit up to 10, 50, and 100 $\mu\text{g/L}$, for Cd, Pb and Ni, respectively. The detection limit (LOD) was defined as $3s/m$, where s is the standard deviation corresponding to 10 blank injections and m is the slope of the calibration graph. The LODs of 0.05, 0.15 and 0.55 $\mu\text{g/L}$ were calculated for Cd, Pb and Ni, respectively.

A recovery of the spiked metals is close to (97-99%) would show quantitative recovery of Cd, Ni and Pb by proposed method as shown in Table 2. Comparison of results obtained by microwave digestion

with conventional wet digestion for analysis of TMs in different understudy samples revealed that difference was significant $p > 0.05$ (paired t- test), when comparing the average values of TMs were used.

Table 2. Standard Addition/ recover for Cd, Ni and Pb determination in chocolate samples (Cocoa-based) by microwave assisted digestion (MAD) and Conventional digestion methods (CDM) ($\mu\text{g/g}$).

Toxic metals	MAD	% recovery	CDM	% recovery	$t_{\text{crit}}=2.306$
Cadmium					
0.0	0.353 ± 0.025	0.353 ± 0.025	...	0.443
1.0	1.35 ± 0.114	99.7	1.35 ± 0.116	99.6	2.01
2.0	2.35 ± 0.211	99.8	2.36 ± 0.234	100	0.483
3.0	3.353 ± 0.252	100	3.355 ± 0.353	100	1.13
Nickel					
0.0	4.33 ± 0.27	4.33 ± 0.27	0.15461
2.0	6.18 ± 0.455	97.6	6.19 ± 0.457	97.8	0.47851
4.0	8.2 ± 0.812	98.4	8.22 ± 0.836	98.7	0.503731
8.0	12.15 ± 1.01	98.5	12.2 ± 1.05	98.9	1.599347
Lead					
0.0	2.48 ± 0.19	--	2.48 ± 0.19	--	0.153393
2.0	4.40 ± 0.341	98.2	4.48 ± 0.347	98.6	3.340267
4.0	6.41 ± 0.471	98.7	6.47 ± 0.483	98.2	1.987767
8.0	8.35 ± 0.812	98.5	8.45 ± 0.845	98.7	4.297584

Statistical analyses

All experimental data processing was done with Minitab 13.2 (Minitab Inc., State College, PA) and Microsoft Excel 2000.

Results and Discussion

The microwave digestion program typically took 30–35 min. to complete digestion of chocolate and candy samples to allow vessels to cool for safe handling, while the conventional wet digestion procedure required 3-4 hrs for the decomposition of matrix. Using a simple microwave digestion the recoveries of fortifications of toxic metals in chocolate and candies samples were comparable as those obtained from conventional digestion method. Although the microwave digestion procedure is free from contamination risk and has low time and chemical consumption.

Cocoa is a main ingredient in chocolate, besides milk and sugar. The analytical results are given in Table 3. The mean and standard deviation of the TMs concentrations in different chocolates and candies are given in Table 3. The concentrations of Cd found in cocoa, milk-based and sugar based candies ranged as (0.099-0.353), Pb (1.11-2.48) and Ni (1.45-4.33) $\mu\text{g/g}$. The toxic metals concentration was found higher in cocoa based chocolates as compared to milk and sugar - based chocolates and candies.

Table 3. Determined concentration ($\mu\text{g/g}$) of Cd, Ni and Pb in Chocolates and candies.

Chocolate/ candies samples	Sample (n*)	Cd	Ni	Pb
Cocoa based chocolates	20	0.353	4.33	2.48
		± 0.025	± 0.25	± 0.19
Milk based chocolates	12	0.132	3.55	2.25
		± 0.012	± 0.22	± 0.13
Sugar-based candies	8	0.099	1.45	1.11
		± 0.0051	± 0.13	± 0.12

In general children can absorb lead more easily than adults. The proportion of Pb absorbed from gastrointestinal tract is about 10% in adults, where as level of 40-50% have been reported for infant [17]. The mean concentrations of Pb in understudy samples are comparable with the lowest reported concentrations of lead [18, 19].

The susceptibility of infants and young children towards the adverse effects of toxic metals is higher than those of adults; metal contaminations should seriously be taken into concern [20, 21]. Therefore, concentrations of Cd and Pb, in these products and raw materials used for their production are a matter of significant public concern [22, 23]. There are no well-defined limits for these metals in chocolate and candy in most of the countries except a few. In Poland, the national standard for Pb in chocolates is 0.30 mg/kg and for Cd 0.05 mg/kg [24, 25]. The maximum tolerable levels of Cd in chocolate and in cocoa powder have been set to 0.4 mg/kg in Germany, 0.5 mg/kg in Finland and Central European countries and 1.0 mg/kg in Malaysia [26]. Accordingly, safe daily levels of Cd intake should be kept below 30 μg per person. An individual variation in Cd absorption and sensitivity to toxicity predicts that a dietary Cd intake of 30 $\mu\text{g/d}$ may result in a slight renal dysfunction in about 1 % of the adult population [27]. Cadmium accumulates mainly in the kidneys and the liver of human body, having a half-life of several decades. The toxic effects occur in the kidneys and may lead to proteinuria. Provisional tolerable weekly intakes (PTWI) have been established at 7 $\mu\text{g/g}$ of body weight [28].

The mean nickel levels from all categories are 1.45 to 4.33 mg/kg, respectively. The natural Ni content of various food items around the world varies from 0 to 10 mg/kg [29]. The observed values are in good agreement with the reported values of 0.33 to 1.52 mg/kg [30].

From a survey of children residing in different urban areas of Hyderabad city, Pakistan, it was found that cocoa-based chocolates are their first choice first choice of children as well as adult and they eat daily 2–5 chocolates. The weight of chocolates varied from 5 to 20 g, but the majority of the chocolates weigh about 10 g. As these items are not a regular food item, average ingestion of 40 g chocolate/day is assumed which comprises the 5.3-14.5 μg of Cd/person /day. This study confirms that a coca-based chocolate is a notable source of toxic metals as compared to other chocolates.

The sources of contamination of toxic metals in for different chocolates and candies metals were mainly due to raw materials used, environmental and processes of manufacturing, and leaching of these metals from the vessels or wrappers in which they are stored. Thus, the chocolates have a great contribute to dietary intake of TMs, due to the high concentration observed.

Conclusion

The proposed method was used for determination of TMs in chocolate and candies samples collected from the market of Hyderabad City, Pakistan. The microwave assisted digestion method is used for the estimation of TMs in chocolate and candy samples by ETAAS, to avoids the time-consuming sample digestion procedure, that is subject to the risks of analyte loss and or contamination and provides information about mineral nutrients in chocolate flavoured beverages. The agreement between the results obtained by using microwave oven assisted digestion and wet digestion method is quite good, representing the potentiality of the time saving microwave methodology for routine analysis. The information obtained will be useful to know the content of these toxic metals in a kind of food which is consumed by a large proportion of the population of children.

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