



Determination and comparison of some trace elements in different chocolate types produced in Turkey

Metin Gldař ^{1*}, Adnan F. Dađdelen ² and Gnnur F. Biricik ²

¹Department of Food Technology, Uludag University, Karacabey Vocational School, Karacabey 16700, Bursa, Turkey;

²Food Control and Central Research Institute, Ministry of Agriculture, Bursa, Turkey.

*e-mail: mguldas@uludag.edu.tr

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Abstract

Different chocolate types belonging to plain chocolate, milk chocolate and chocolate with pistachio that were supplied from different markets in Bursa, Turkey were investigated for trace element contents. Determinations of mercury (Hg), arsenic (As), iron (Fe), copper (Cu) and zinc (Zn) were performed by using Flame Atomic Absorption Spectrometry (FAAS), while the other elements (lead (Pb), cadmium (Cd) and nickel (Ni)) were determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). According to chocolate types, contents ranged from 0.03 to 0.04 (Pb), 0.02 to 0.04 (Hg), 0.02 to 0.03 (Cd), 0.01 to 0.02 (As), 1.29 to 4.78 (Ni), 5.74 to 10.61 (Cu), 2.52 to 3.67 (Fe) and 12.11 to 16.68 mg kg⁻¹ (Zn), respectively. The levels of Cu, Zn and Fe were highest in chocolate with pistachio samples. The contents of Hg and Ni were highest in milk and plain chocolates, respectively. Arsenic (As) was highest in both chocolate types (milk chocolate and chocolate with pistachio). There was no difference among the chocolate types in terms of Pb and Cd contents (p<0.01). The heavy metal contents of the analyzed samples are within the ranges reported by the WHO and Turkish Food Codex regarding to chocolate and other foods.

Key words: Chocolate, microwave digestion, atomic absorption spectrometry, heavy metals, trace elements.

Introduction

Chocolate is a very nutritious food product. It has a high level of cacao butter, fats and sugar. Therefore, it contains high energy value. Additionally according to chocolate type, it comprises proteins, vitamins, minerals and flavonoids in different amounts ¹. Annual production of chocolate and candies are about 400,000 tons in Turkey. There are many chocolate types as plain chocolate, sweet chocolate, bitter chocolate, couverture chocolate, milk chocolate, white chocolate, chocolate with nuts and etc. The most preferred chocolate type is milk chocolate (33%). This is followed by chocolate with pistachio (21%), chocolate with hazelnut (16%), chocolate with caramel (15%) and bitter or plain (12%) ².

The chocolate is obtained from cocoa products and sugars and contains minimum 35% total dry cocoa solids, minimum 18% cocoa butter and minimum 14% of dry non-fat cocoa solids ^{3,4}. In the Turkish Food Codex ⁵, the ratio of total dry cocoa solids in the chocolate definition is slightly different than the definition given above, as 32%. The milk chocolate contain, in addition to the ingredients counted for chocolate minimum 25% of milk or milk products and minimum 3.5% milk fat ^{3,5}. The definition is very close to FDA's definition ⁴. According to FDA ⁴, the nut varieties (including pistachio) either whole or broken, do not exceed 60% of the total weight of the product. On the other hand, the nuts can be added into the chocolate as 20-40% and 15-40% according to FSA ³ with TFC ⁵, respectively.

Chocolate ingredients change depending upon the chocolate type. In addition to cocoa products (cocoa powder and cocoa

butter), the chocolates have number of ingredients such as white sugar, milk powder, vegetable oils (sun flower oil, palm oil and etc.), emulsifier (lecithin), flavorings, dried fruits (raisin, pistachio, hazel nut, almond etc), whey powder etc. ⁶

The importance of heavy metals and other trace elements on human health have been increasing ⁷. Some heavy metals such as Pb, Cd, Hg, As and Ni may be toxic for human body if excessive amounts are consumed. On the other hand, some other elements such as Fe, Cu and Zn play significant role for health ⁸.

Presence of trace elements in foods depends on many reasons. Variety of animal and plant, feeding characteristics of animals, storage and processing conditions and composition of added ingredients are among the factors influencing elemental composition of foods. For example, nickel is an element found in hydrogenised vegetable fats used in chocolate production ⁹. Other factors affecting trace elements content in foods are environmental pollution, fertilization intensity, pesticides and climatic conditions ¹⁰. As an example, copper compounds are extensively used as fungicides in the cacao plantation ¹¹.

Permissible tolerable weekly intakes of Pb, Cd and Hg have been defined as 25 (Pb), 7 and 1.6 µg kg⁻¹ of body weight, respectively ¹². The maximum permitted level of As in foods is 1 mg kg⁻¹ according to WHO ¹² and 0.5 mg kg⁻¹ according to Turkish Food Codex ¹⁴. The dietary contribution of Ni ranges from 200 to 900 µg day⁻¹ and tolerable oral intake of Ni can be changed depending on individual ¹⁵. The maximum permitted levels of Fe and Zn ranges

0.2-25 mg kg⁻¹ and 2-50 mg kg⁻¹ in various foods while the highest level for Cu is 15 mg kg⁻¹ in chocolate according to TFC¹⁴.

FAAS (Flame Atomic Absorption Spectrometry) and GFAAS (Graphite Furnace Atomic Absorption Spectrometry) have been still used largely in many countries for the determination of Cu, Ni, Pb, Cd, Mo and Zn in organic samples^{7, 16}. Prior to FAAS/GFAAS analyses, a digestion process is needed for decomposition of solid samples. Microwave digestion is fast and reliable method for the digestion of organic samples⁹. Furthermore, more attention should be paid for digestion of certain foods having high organic matter content such as chocolate¹⁷. Since the microwave digestion is accomplished in closed vessel and it provides a clean process minimizing contamination from dust.

On the other hand, standard reference material (SRM) is needed to verify whether the method or process is reliable for the analyses of trace elements. Therefore, the results obtained from the digestion procedure are compared with those of obtained from SRM¹⁸.

As it is reported in previous years, there is a need to update the data related with heavy metals and trace elements in chocolate products and foods^{8, 9, 19}. On the other hand, there are limited number of researches on the assessment of heavy metals and trace elements in chocolate^{9, 15, 18}. In this study, some heavy metals (Pb, Cd, As, Hg and Ni) and some minerals (Fe, Zn and Cu) in three different chocolate types (plain, milk and pistachio) belonging to same brand were determined by using microwave digestion with FAAS and GFAAS techniques and compared.

Material and Methods

Sampling and apparatus: Chocolate types mostly preferred by Turkish consumers such as milk chocolate, chocolate (plain) and chocolate with pistachio were selected for comparing elemental differences by using microwave digestion prior to atomic absorption spectrometry analysis. For this reason, twelve chocolate samples belonging to each type chocolates were investigated. The chocolate samples of the same brand were randomly taken from different supermarkets in the Bursa city (Turkey).

Determinations of Ni, Cd and Pb were done by a Perkin-Elmer Analyst 700 (Überlingen, Germany) Graphite Furnace Atomic Absorption Spectrometer (GFAAS). Fe, Cu, Zn, As and Hg were measured by Shimadzu AA-6701F (Kyoto, Japan) Flame Atomic Absorption Spectrometer (FAAS). Shimadzu Hydride Vapour Generator AAS-HGV-1 (Kyoto, Japan) equipped to FAAS (AA-6701F) was used for Hg and As determinations to provide accuracy. The technical specifications for Flame Atomic Absorption Spectrometer (FAAS) and Graphite Furnace Atomic Absorption Spectrometer (GFAAS) are given in Table 1 and 2. All samples were analyzed in triplicate.

Reagents: All reagents were of analytical grade unless otherwise stated. Ultra pure water was supplied by a Milli-Q reagent water system (Millipore Corp., resistivity of 18.2 MΩ cm). Nitric acid (HNO₃) and H₂O₂ were of suprapure quality (Merck, Darmstad, Germany). All laboratory glassware was kept overnight in 10% v/v HNO₃ acid solution and rinsed with deionized water and dried in a dust-free environment prior to use. For the calibration, standard solutions of metal ions were prepared by diluting a stock solution of 1000 mg/L of (CertiPUR). Investigated elements were supplied by Merck (Darmstad, Germany).

Standard reference materials such as BCR-185R (Bovine Liver, Institute for Reference Materials and Measurements, Geel, Belgium), SRM-1577b (Bovine Liver, National Institute of Standards and Technology, USA) and BCR-679 (White Cabbage, Institute for Reference Materials and Measurements, Geel, Belgium) were used to verify microwave digestion process.

Digestion process: The microwave digestion procedure was used for the decomposition of the chocolate samples. For this purpose, a microwave (Berghof Speedway, MVS-3⁺, Eningen, Germany) was used. A mass of 0.25 g of homogenized chocolate sample was weighed and 5 mL of concentrated HNO₃ (65%, v/v) and 2 mL of H₂O₂ (35%, v/v) were added prior to digestion. The samples were digested by using the pressure microwave system according to the procedure recommended by the manufacturer. The chocolate ingredients were also digested in the same manner as the chocolate samples. The operation programme of the microwave digestion system is given below:

Operation steps	I	II	III	IV	V
Pressure (bar)	35	35	35	35	35
Temperature (°C)	150	170	195	100	100
Rise time (min)	5	5	5	1	1
Hold time (min)	10	10	20	1	1
Power (%)	60	75	90	10	10

Statistical evaluation: The one-way analysis of variance (ANOVA) test was used to determine the different means among the chocolate types. Statistical analyses were done by using Minitab 13.30 statistical software for Windows, Minitab Inc., Pennsylvania, USA.

Results and Discussion

To obtain accurate and reliable results, digestion method is verified by using standard reference materials. In our research, BCR-185R Bovine Liver, SRM-1577b Bovine Liver and BCR-679 White Cabbage were used as standard reference materials for the validation. As given in Table 3, the recoveries of microwave digestion method were quite high. The recoveries ranged between 96 with 102%. The standard deviations of the results were lower than 6.56%. From these results, microwave digestion technique was found suitable for digestion of chocolate samples.

The contents of Cu, Zn, Fe, Ni, Pb, Cd, As and Hg of different chocolate types produced in Turkey and the differences among these chocolates were investigated. In terms of nutritious elements (Cu, Zn and Fe), the highest levels were obtained with the pistachio chocolate as 10.61, 16.68 and 3.67 mg/kg, respectively (Fig. 1). Probably, the high levels of these nutritious elements are related to the high pistachio content in pistachio chocolate. According to TFC⁵, the chocolate should contain at least 15% of pistachio. Pistachio is a rich source of trace elements such as Cu, Zn and Fe. The contents of Cu, Fe and Zn have been reported as 8.5-12 µg g⁻¹, 24.0- 36.5 µg g⁻¹ and 17.1- 26.9 µg g⁻¹ in Turkish pistachio by Anderson and Smith²⁰, respectively.

In previous researches, the contents of Cu and Fe were reported ranging from 0.02 to 6.0 and from 1.6 to 2.4 mg kg⁻¹, respectively^{1,7, 11,21}. The contents of Zn and Ni in chocolates were also determined

Table 1. Technical specifications for flame atomic absorption spectrometry (FAAS, Shimadzu AA6701).

	Element				
	Fe	Cu	Zn	As	Hg
Lamp current [mA]	12	6	8	14	4
Wavelength [nm]	248.3	324.8	213.9	193.7	253.7
Slit width [nm]	0.2	0.5	0.5	0.2	0.5
Fuel gas flow [L min ⁻¹]	2.2	1.8	3	2	-
Type of oxidant	Air/acetylene				-
Type of measurement	Flame	Flame	Flame	Flame+hydride vapour	Hydride vapour
Detection limit (mg kg ⁻¹)	0.009	0.004	0.001	0.001	0.001

Table 2. Technical specifications for graphite furnace atomic absorption spectrometry (GFAAS, Perkin-Elmer AAnalyst 700).

	Element														
	Cd					Pb					Ni				
Lamp current [mA]	6					10					30				
Wavelength [nm]	228.8					283.3					232				
Slit width [nm]	0.7					0.7					0.2				
Detection limit [mg kg ⁻¹]	0.001					0.001					0.001				
Matrix modifier	Palladium %1					Palladium %1					-				
Temp. [°C]*	100	140	850	1650	2600	100	140	700	1800	2600	100	140	1400	2500	2600
Ramp[°C s ⁻¹]*	5	15	10	0	1	5	15	10	0	1	5	15	10	0	1
Hold [s]*	20	15	20	5	3	20	15	20	5	3	20	15	20	5	3
Air flow [m min ⁻¹]*	250	250	250	0	250	250	250	250	0	250	250	250	250	0	250

*Atomization stages

as 4.01-11.01 and 0.041-8.29 mg kg⁻¹, respectively ^{7,15,21}. Regarding to Food Codex, the limits have been defined for several foodstuffs: Cu 15 mg kg⁻¹ in chocolate, Zn should be maximum 50 mg kg⁻¹ and Fe 25 mg kg⁻¹ in various foods ¹⁴. As reported by EU ¹⁹, the daily contribution of nickel to diet was reported as 200-900 µg.

There was no difference between milk chocolate and plain chocolate in terms of Cu content, while the content of Zn changed according to chocolate type, (p<0.01). Iron (Fe) content also did not change between plain chocolate and milk chocolate.

The highest Ni content was 4.78 mg kg⁻¹ and obtained from plain chocolate. As given in Table 4, it is probably because of the high content of hydrogenised vegetable oil and cacao butter of pistachio chocolate. Nickel content did not differ between other chocolate types (milk chocolate and pistachio chocolate) (p<0.01).

Some chocolate ingredients were also investigated for toxic heavy metals (Table 4). Ni was detected only in cacao butter and palm oil samples. These two are the two main ingredients used in chocolate productions. The content of As was also high in cacao powder, palm oil, sun flower oil, whey powder and white sugar. Cacao powder was found to contain higher amount of Hg, Cd and Pb as well. Despite the fact that Cd was detected in white sugar,

Table 3. Comparison of certified and measured mean values of trace elements (± standard deviations, N= 5).

Element	Certified Value	Microwave Digestion	Recovery (%)
Cd (mg kg ⁻¹)	0.544 ¹ ±0.012	0.549±0.014	101
As (mg kg ⁻¹)	0.033 ¹ ±0.04	0.032±0.05	97
Cu (mg kg ⁻¹)	277 ¹ ±2.01	274.2±4.76	99
Pb (mg kg ⁻¹)	0.172 ¹ ±0.014	0.175±0.017	102
Zn (mg kg ⁻¹)	138.6 ¹ ±1.55	141.3±5.87	102
Fe (mg kg ⁻¹)	184 ² ±3.19	176.6±6.56	96
Ni (mg kg ⁻¹)	27 ² ±0.71	26.7±1.65	99
Hg (µg kg ⁻¹)	6.3 ³ ±0.63	6.24±0.70	99

¹BCR-185R Bovine Liver (Institute for Reference Materials and Measurements, Geel, Belgium)²SRM- 1577b Bovine Liver (National Institute of Standards and Technology, USA)³BCR-679 White Cabbage (Institute for Reference Materials and Measurements, Geel, Belgium)

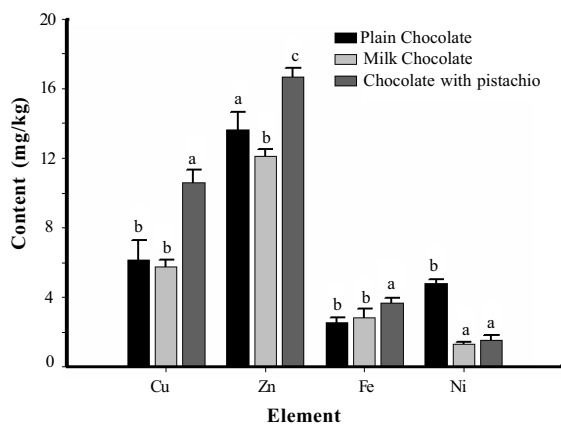


Figure 1. Cu, Zn, Fe and Ni contents in chocolates (bars ± standard deviations with different letters for chocolate type are significantly different, $p < 0.01$).

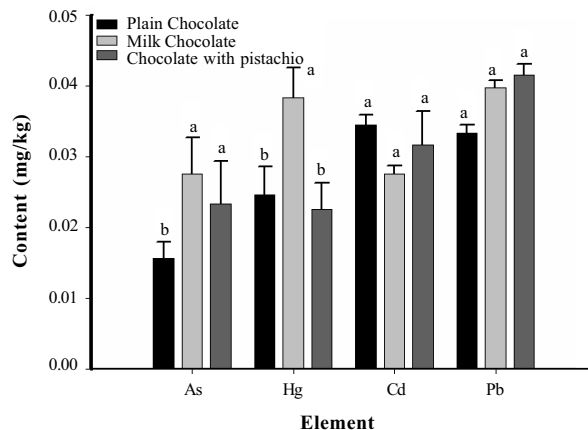


Figure 2. As, Hg, Cd and Pb contents in chocolates (bars ± standard deviations with different letters for each chocolate type are significantly different, $p < 0.01$).

Table 4. Selected heavy metal contents in some chocolate ingredients as mg kg^{-1} (± standard deviations).

Element	Milk powder (32% fat)	Whey powder	Sun flower oil	Palm oil	Cacao butter	Cacao powder	White sugar	Pistachio
As	0.017±0.012	0.038±0.016	0.040±0.026	0.042±0.015	0.008±0.004	0.043±0.025	0.035±0.026	0.016±0.012
Hg	0.007±0.003	0.007±0.005	0.015±0.009	0.013±0.008	0.009±0.004	0.061±0.054	0.009±0.004	<0.001
Cd	0.061±0.022	0.005±0.003	0.012±0.008	0.014±0.007	0.011±0.005	0.181±0.078	0.013±0.006	0.012±0.006
Pb	0.009±0.004	0.009±0.005	0.041±0.008	0.041±0.025	0.008±0.004	0.087±0.032	0.041±0.033	0.006±0.003
Ni	-	-	-	0.041±0.021	0.054±0.028	-	-	-

* Each mean consists of three replicates ± standard deviation.

palm oil, cocoa butter and sun flower oil samples, higher quantities were detected in cacao powder and milk powder, respectively. In Fin. 2 it can be seen that toxic heavy metal contents were high in milk chocolates. Pb content was highest in pistachio chocolate among the chocolate types. There were no differences between milk chocolate and pistachio chocolate for As and between plain chocolate and pistachio chocolate in terms of Hg content. Either Cd or Pb did not differ among all chocolate types ($p < 0.01$). It is thought that investigated heavy metals would contaminate chocolates by many environmental sources such as pesticides used in plantations, transfer of the pesticides and fertilizers to the milking cows near by the roads and etc.

Conclusions

In the literature, the content of Pb was reported to vary between 0.07 and 4.0 mg kg^{-1} in different chocolate types^{7,21,22}. Cd content was also exhibited great variations and changed between 0.001 and 2.73 mg kg^{-1} ^{7,15,21} varying between. The elemental contents of the analyzed samples were lower of the legal limits reported by the legal authorities for chocolate and other foods.

The higher levels of toxic heavy metals observed in the cocoa powder was probably aroused from pesticides and fertilizers used in cocoa plantation and environmental pollution.

It is essential that it is necessary to get clear color after adequate digestion process in the organic products. In addition, digestion of fat in the food matrix is very important to get effective digestion. After the microwave digestion, since we obtained clear solution with light yellow color, we assumed that the fat in the chocolate

can be digested by microwave system used.

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