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Trace elements in cocoa solids and chocolate: An ICPMS study

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ABSTRACT

The concentrations of eight trace elements: lead (Pb), cadmium (Cd), chromium (Cr), manganese (Mn), cobalt (Co), arsenic (As), bismuth (Bi) and molybdenum (Mo), in chocolate, cocoa beans and products were studied by ICPMS. The study examined chocolate samples from different brands and countries with different concentrations of cocoa solids from each brand. The samples were digested and filtered to remove lipids and indium was used as an internal standard to correct matrix effects. A linear correlation was found between the level of several trace elements in chocolate and the cocoa solids content. Significant levels of Bi and As were found in the cocoa bean shells but not in the cocoa bean and chocolate. This may be attributed to environmental contamination. The presence of other elements was attributed to the manufacturing processes of cocoa and chocolate products. Children, who are big consumers of chocolates, may be at risk of exceeding the daily limit of lead; whereas one 10 g cube of dark chocolate may contain as much as 20% of the daily lead oral limit. Moreover chocolate may not be the only source of lead in their nutrition. For adults there is almost no risk of exceeding daily limits for trace metals ingestion because their digestive absorption of metals is very poor.

1. Introduction

Chocolate is produced from cocoa beans – the fruit of the cocoa tree (*Theobroma cacao*). Cocoa is grown principally in West Africa, Central and South America and Asia. The eight largest cocoa-producing countries at present are Côte d'Ivoire, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador and Malaysia. These countries represent 90% of the world cocoa production [1].

There is a common phrase “Dark chocolate is healthy chocolate” and it is today recognized as a contributor to health. Cocoa and chocolate have several beneficial health effects [2] mainly because of their high content of antioxidants (flavonoids, catechins, epicatechin, procyanidins and polyphenols) that decrease the number of free radicals [3] and help prevent infectious and autoimmune diseases [4] and reduce the risk of heart disease [5]. The polyphenols in cocoa help control the nitric oxide level that is critical for blood pressure regulation and blood flow [6], other components (like catechins, phenyl ethylamine, tryptophan, endorphins and antioxidants) are anticancer substances [7], serving as brain stimulators [8] and can lower cholesterol levels in adults [9]. On the other hand, large quantities of any energy-rich food, such as chocolate, may increase the risk of obesity. Studies of elderly women showed that chocolate might enhance osteoporosis [10] and in some individuals chocolate can cause heartburn [11].

One measure of chocolate quality is the content of cocoa solids so that dark chocolate is considered the healthiest type of chocolate and of the highest quality. The cocoa solids contents of commercial dark chocolate bars can range from 47% (sweet dark) to 70%, 75%, or even above 90% for extremely dark bars. It is known that the cocoa may contain trace levels of heavy metals [12–17] that have almost no detrimental effects on adults. On the other hand children are the most vulnerable age group to any kind of heavy metal contamination in food and chocolate is often the favorite food items of children. For example, lead like other heavy metals is removed from the body very slowly, so consumption of food containing lead during a short period of time can cause digestive problems like constipation, vomiting, weight loss, abdominal pain, behavior change, language development delay, anemia and lethargy [18,19]. When accumulated in the body lead interferes with normal neurological functions causing irreversible damage to the child's ability to learn, especially the ability to retain new information and may even cause a decrease in intelligence quotient [20]. In addition this could lead to hyperactivity, headaches, hearing problems, slowed growth, memory and behavior problems and in extreme cases damage to the brain

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and nervous system [18–20]. Adults can suffer from: reproductive problems (in men and women), high blood pressure, nerve disorders, memory and concentration problems, anemia, muscle and joint pain [18,19]. A study indicates that approximately 11% of the ingested lead will be absorbed by the digestive tract of adults while absorption may be as high as 30–75% in children (2- to 6-year-old) [21].

In the present study we examined the levels of some essential and toxic elements in different brands of chocolate containing a broad range of cocoa solids. Inductively coupled plasma mass spectrometry (ICP-MS), which has become the method of choice for determination of trace levels of heavy metals, was used here. After screening several elements, eight elements were selected for this study: lead (Pb), cadmium (Cd), chromium (Cr), manganese (Mn), cobalt (Co), arsenic (As), bismuth (Bi) and molybdenum (Mo). Indium (^{115}In) was used as an internal standard.

2. Experimental

2.1. Instrumentation

All analyses were carried out with a commercial ICP-MS instrument (Elan-6000, Perkin-Elmer/Sciex, Canada) with a flow-injection inlet system (FIAS-400, Perkin-Elmer, Germany) at the Geological Survey for Israel (GSI) in Jerusalem [22].

The Cr^{+6} concentration was determined with an ion chromatograph (Dionex, model DX-500, with a UV detector 540 nm) equipped with a Dionex HPIC-AS7 separator column and a Dionex NG1 guard column.

2.2. Sample collection and preparation

Cocoa bean and cocoa butter samples were received from the importers of the chocolate brands in Israel. In addition, we purchased chocolate bars from various brands and countries (Europe, USA and Israel) with different concentrations of cocoa solids from each brand. To create representative samples from the cocoa beans we separated the shells from the beans and examined each component separately.

Sample preparation and treatment were conducted in a “clean room”. Triplicate samples, 100 mg each, were taken from each chocolate brand. Each sample was digested by a 1:2 mixture of 30% H_2O_2 and concentrated HNO_3 (super pure), respectively, that was heated in a hot water bath. The samples were filtered to remove undigested lipids and to obtain a clear solution. Each sample was spiked with an internal standard of ^{115}In (at 10 μg L^{-1}). The internal standard was added to correct the matrix effect that may arise from the plasma changes that are due to the organic matrix. One milliliter of each sample was diluted by a factor of ten with distilled water (Millipore Milli-Q system with a resistivity of $>$18 MΩ) [23]. Blank samples were prepared by taking the nitric acid and 30% hydrogen peroxide that were spiked with the internal standard, filtered and diluted like the samples. Certified multi-element standard solutions were used for calibration (ICP multi element VI, standard solution, Merck, Germany).

The trace element concentrations (ng mL^{-1}) in the blank samples are summarized in Table 1. The blank measurement for arsenic was below the limit of detection for that element that was 0.25 ng mL^{-1}. The mean relative standard deviation (RSD) from each triplicate lies in the range of 0.5–19%. The interday RSD, measured over four different days, was below 10% for all elements, except chromium that was 26%.

Table 1
Concentrations of the trace metals in different brands of chocolate normalized to 70% cocoa solids. (The blank unit is ng mL^{-1} and the As results in the blank are below the LOD).

	Blank (ng mL^{-1})	Brand A (ng g^{-1})	Brand B (ng g^{-1})	Brand C (ng g^{-1})	Brand D (ng g^{-1})
Cr	34 ± 7	2431 ± 49	475 ± 29	1428 ± 208	1413 ± 81
Mn	10.3 ± 0.4	20248 ± 508	14638 ± 1092	18924 ± 111	16255 ± 464
Co	5.3 ± 0.2	524 ± 12	339 ± 30	451 ± 18	417 ± 14
As	< 0.25	23.1 ± 4.9	12 ± 1.2	28 ± 5.7	20 ± 0.4
Mo	1.24 ± 0.08	153 ± 4.9	168 ± 23.2	305 ± 3.1	227 ± 3.5
Bi	5.7 ± 0.03	< 5.7	< 5.7	< 5.7	< 5.7
Pb	5.3 ± 0.3	86 ± 18	88 ± 4.8	230 ± 87	139 ± 13
Cd	3.1 ± 0.3	65 ± 5.6	141 ± 6.5	131 ± 11	84 ± 5.2

Table 2
Measured lead and chromium concentrations in chocolate with different cocoa solids percentage from different brands.

	Cocoa solids (%)	Pb concentration (ng g^{-1})	Cr concentration (ng g^{-1})
Brand E	27	62.9 ± 2.8	410 ± 96
	30	73.0 ± 11.5	430 ± 59
	40	67.7 ± 6.6	754 ± 55
	47	82.7 ± 1.5	566 ± 37
Brand D	53	103.1 ± 6.4	1026 ± 85
	73	145.2 ± 13.1	1473 ± 85
Brand B	70	87.9 ± 4.8	475 ± 29
	85	100.5 ± 1.0	956 ± 31
Brand A	60	84.1 ± 6.0	2010 ± 258
	72	88.5 ± 18.8	2501 ± 50
	85	86.0 ± 14.0	2913 ± 324

2.3. Isotopic measurements

The concentration of lead was determined by summation of the four isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb) in the standard and the samples. Chromium concentrations were determined by measurement of the ^{53}Cr isotope because of the large isobaric interference from (ArC^{+}) on the most abundant ^{52}Cr isotope. There is an isobaric interference also on ^{53}Cr caused by ^{13}C (that is 1.1% from total carbon) but this interference is 8.8 times smaller than the interference on ^{52}Cr. The concentrations of the other elements were derived from measurement of the following isotopes: ^{111}Cd because of interference of ^{112}Cd from other isobaric ions, ^{55}Mn, ^{59}Co, ^{75}As, ^{98}Mo, ^{209}Bi and ^{115}In. The results were normalized relative to the indium internal standard to correct instrumental fluctuations in sensitivity and experimental error. In the case of chocolate, the signal intensity is attenuated by a “matrix effect” caused by the naturally occurring sugars and lipids in the chocolate specimen. Correction for the matrix effect was made by normalization to the multi element standard solution (Merck multi element ICP-VI) that was also spiked with the internal standard of ^{115}In at 10 μg L^{-1}.

3. Results and discussion

Lead and chromium concentrations in chocolate were found to be correlative to the cocoa solids content in all brands as shown in Table 2. Thus, the concentrations of Pb and Cr are higher in dark chocolate (47–85% cocoa solids concentration) and lower in the milk chocolate (27–30% cocoa solids concentration). Each result represents the average and standard deviation of measurements of three samples from the same batch. The RSD from each triplicate lies in the range of 1–22%.

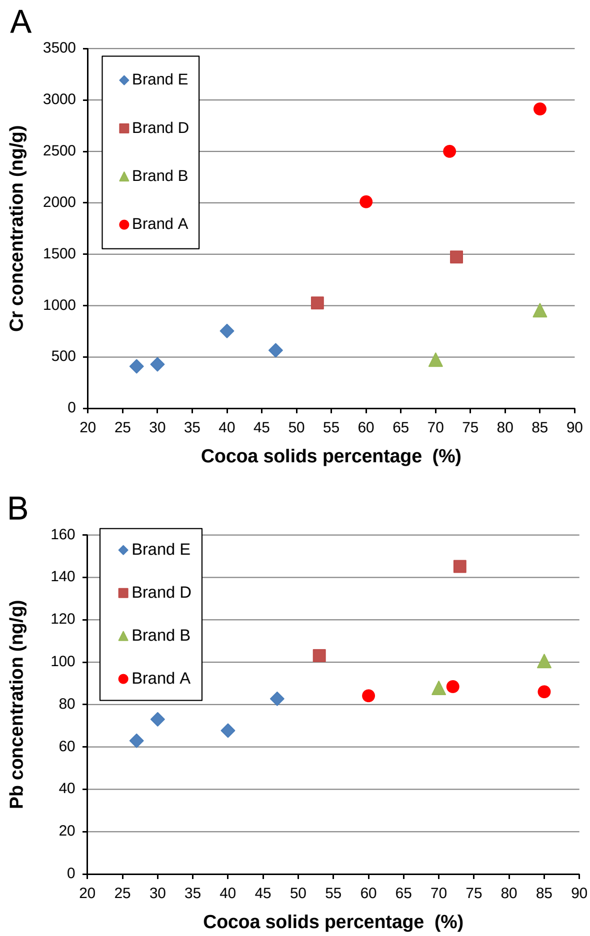

Fig. 1. Plots of chromium (A) and lead (B) concentrations vs. percentage of cocoa solids in different brands of chocolate.

The Pb and Cr concentrations are below the USP (U.S Pharmacopeia convention) oral limits [24] which are <1000 ng g^{-1} and <15000 ng g^{-1} for Pb and Cr, respectively. The correlation between the Pb and Cr concentration and cocoa solids content shows a linear behavior (Fig. 1A and B). Two kinds of chocolate from E brand (30% and 47% cocoa solids) deviate from linearity. This may be due to the different production processes of these chocolates: 30% is the white chocolate and 47% is airy dark chocolate. It is probable that the manufacturing process affects the trace metal concentrations. The other trace metals Mn, Co, Mo and Cd also increase linearly with the cocoa solids content.

Trace metals analyses of different brands from several countries showed quite similar concentrations, as shown in Table 1. The cocoa solids concentration in these brands was either 70% or normalized to 70% where the concentration was close to this value (72%, 73% etc.). The RSD of triplicates lies between 0.5% and 22%. Bismuth concentrations were below the blank values <5.7 ng g^{-1}. Some of the metals are considered as essential and some toxic but their concentrations are well below the USP oral limit [24] (Mn $<700{,}000$ ng g^{-1}, Co $<100{,}000$ ng g^{-1}, As <1500 ng g^{-1}, Mo $<25{,}000$ ng g^{-1} and Cd <2500 ng g^{-1}). The metal concentration results compared with the oral limit of all brands for Cd, Cr and Pb elements (percent of limit) are 3–6%, 3–16% and 9–23%, respectively, for 1 g of chocolate (calculated according to USP oral limit for one gram). For each brand the element with highest concentration is different. Manganese is the most abundant of these elements but is considered as an essential, non-toxic, element. We found that the Pb concentrations were in the range of the results reported by Rankin et al. [16] and Manton [15]. In comparison to Duran et al. [13], we found lower Cr, Cd and Pb levels and higher Mn concentrations.

Cocoa powder and butter are products of processed cocoa beans which are generally composed of a bean core and a shell. The concentrations of the chosen trace metals in the bean core, bean shell, cocoa powder and cocoa butter are presented in Table 3. The Bi concentrations in the cocoa butter, powder and bean are below the limit of detection (<5.7 ng g^{-1}) but in the cocoa bean shells the Bi concentration is 210 ± 47 ng g^{-1}. The cocoa butter appears to have the lowest levels of the trace elements (except Pb). The results show that most of the essential trace metals are found in the cocoa bean core while the highest level of toxic trace metals is found in the cocoa bean shells (Pb, Bi and As). In the production process of the cocoa powder and butter, the bean core is separated from the shell. The shell is then removed (along with Bi and As) and the bean core is processed to produce the cocoa powder and butter. The concentrations of the other elements (Cr, Mn, Co, Mo, Pb and Cd) in the cocoa powder and butter are higher than their concentrations in the cocoa bean core. This indicates that most of the trace metal contaminations in these products are found after the beans are harvested, dried and shipped, namely during the manufacturing of cocoa and chocolate products. This observation supports Rankin et al. [16] that arrived at the same conclusion. Manton [15] suggests that the Pb content in the cocoa powder comes from small amounts of shells that are processed with the beans, but this does not explain the high concentrations of the other elements that are higher than the sum of the shell and the core. The RSD for each triplicate lies in the range of 2–20%. The Pb concentrations we found fall in the same range of the concentrations presented by Rankin et al. [16], Manton [15] and Dahiya et al. [12] (that also found the same range of Cd levels). Guldas et al. [14] report Pb, As and Cd levels that are in the range of the cocoa powder whereas the other results differ (all the results of the butter, except As) from those obtained in the present work.

Although Cr and Pb concentrations in chocolate are below the USP recommended oral limits, the lead concentration should still be considered as a health concern. That is because of the high digestive tract absorption factor, especially for children (30–75% by children 2- to 6-year-old compared to11% absorption by adults) [21]. Chromium has a lower digestive tract absorption 0.5–3% by adults [25] and 10% in children [25]. In Table 4 we present calculations of the number of chocolate cubes (each cube is considered as10 g) that a child may eat until the daily consumption limit of lead is reached [24]. The calculation assumed maximum digestive tract uptake of 75%, to emphasize how easily children can exceed the daily lead oral limit from chocolate only. For adults it is almost impossible to surpass the oral limit because low absorption and higher body weight.

We also tried to determine the valence state of the chromium in the chocolate because Cr^{+6} is a known carcinogen with an oral

Table 3
The distribution of trace metals in cocoa beans components and products. (Some of the Bi, Cd and Mo results are below the limit of detection.)

	Cocoa powder	Cocoa butter	Cocoa bean shells	Cocoa beans
Cr (ng g^{-1})	1310 ± 121	247 ± 43	962 ± 29	497 ± 58
Mn (ng g^{-1})	36081 ± 753	583 ± 32	15091 ± 1410	12228 ± 1445
Co (ng g^{-1})	757 ± 17	30 ± 3	394 ± 43	292 ± 39
As (ng g^{-1})	41 ± 6	12.2 ± 2	160 ± 2	16 ± 4
Mo (ng g^{-1})	297 ± 7	<1.24	291 ± 22	32 ± 5
Bi (ng g^{-1})	<5.67	<5.67	210 ± 47	<5.67
Pb (ng g^{-1})	103 ± 9	67 ± 1.5	1289 ± 193	40 ± 8
Cd (ng g^{-1})	125 ± 11	<3.1	85 ± 1.3	72 ± 1.1

Table 4
The number of chocolate cubes needed to be consumed by a child to reach the daily consumption limit of lead.

	Cocoa solids concentration (%)	Number of chocolate cubes to exceed the daily limit of lead
Brand E	27	22
	30	22
	40	19
	47	13
Brand D	53	13
	73	9
Brand B	70	15
	85	13
Brand A	60	17
	72	15
	85	15

limit $<2\ \mu g\ g^{-1}$ while Cr^{+3} is considered as an essential element with an oral limit [24] of $<15\ \mu g\ g^{-1}$. Chocolate samples were dissolved in different organic solvents, including methylene chloride, dimethyl sulfoxide, methanol, chloroform, ether, toluene and acetone, and heated in a hot water bath. After the solution cooled to room temperature it was mixed with an equal volume of pure water (resistivity of >18 MΩ). After rigorous shaking the mixed solutions were poured into a separation funnel and the clear aqueous phase was analyzed by ion chromatography. Hexavalent chromium was not detected even when a spike from a standard solution containing $10\ \mu g\ g^{-1}$ of Cr^{+6} was added to the chocolate samples. This is probably due to the reduction of Cr^{+6} to Cr^{+3} (which is not detected by this technique) caused by the reducing character of the solution containing the antioxidants in the chocolate. The absence even of the spiked Cr^{+6} implies that there is little chance to find Cr^{+6} in chocolate.

4. Conclusions

This study demonstrates that there is a linear correlation between the level of trace elements in chocolate and the cocoa solids content. No fundamental difference was found in the distribution of heavy metals in different brands with the same concentration of cocoa solids (70%). The difference between the levels found in the cocoa products (butter and powder) to those measured in the cocoa beans indicates that most of the trace metal contamination in those products occurs during the manufacturing of cocoa and chocolate products. Perhaps the fact that the reported levels of some of these elements in the chocolate products [12–14,17] have different concentration ranges indicates that the manufacturing processes influence the metal concentration. Environmental contaminants like Bi and As are found in the cocoa bean shells but most of them do not pass into the cocoa bean and chocolate products. Children, who are big consumers of chocolates, may be at risk of exceeding the daily limit of Pb, due to their low body weight and higher digestive tract uptake. They may be vulnerable to lead exposure from these products – one cube of dark chocolate can contain up to 20% of the lead oral limit; furthermore chocolate may not be the only source of lead in their nutrition thus increasing the risk of exceeding the daily limit. In large quantities it is preferable for children to eat milk or white chocolates than eating dark chocolates. For adults there is almost no risk from trace metals in chocolate because their digestive absorption of metals is poor. Regarding chromium levels in chocolate it appears that there is no real risk as the antioxidants in the chocolate convert Cr^{+6} to the nontoxic form of Cr^{+3}. In summary, these potential risks should be considered against the benefits of the antioxidant content, and concentration of other components that contribute to health and well being of consuming dark chocolate.

References

[1] United Nations Conference on Trade and Development (UNCTAD), Infocomm, Market Information in the Commodities Area. Available from: ⟨http://unctad.org/infocomm/anglais/cocoa/market.htm⟩, (accessed 07.05.13).
[2] R. Kelishadi, ARYA J. 1 (1) (2005) 29–35.
[3] F. Visioli, L. Borsani, C. Galli, Cardiovasc. Res. 47 (3) (2000) 419–425.
[4] C. Sanbongi, Cell Immune 177 (2) (1997) 129–136.
[5] D. Taubert, R. Berkels, R. Roesen, W. Klaus, J. Am. Med. Assoc. 290 (8) (2003) 1029–1030.
[6] R. Corti, A.J. Flammer, N.K. Hollenberg, T.F. Lüscher, Circulation 119 (2009) 1433–1441.
[7] M. Demeule, J. Michaud-Levesque, B. Annabi, D. Gingras, D. Boivin, J. Jodoin, S. Lamy, Y. Bertrand, R Béliveau, Curr. Med. Chem. Anti-Cancer Agents 2 (2002) 441–463.
[8] D.M. Small, R.J. Zatorre, A. Dagher, A.C. Evans, M. Jones-Gotman, Brain 124 (2001) 1720–1733.
[9] J. Mursu, S. Voutilainen, T. Nurmi, T.H. Rissanen, J.K. Virtanen, J. Kaikkonen, K. Nyyssönen, J.T. Salonen, Free Radical Biol. Med. 37 (9) (2004) 1351–1359.
[10] J.M. Hodgson, A. Devine, V. Burke, I.M. Dick, R.L. Prince, Am. J. Clin. Nutr. 87 (2008) 175–180.
[11] D.W. Murphy, D. Castell, Am. J. Gastroentrol. 83 (6) (1988) 633–636.
[12] S. Dahiya, R. karpe, A.G. Hegde, R.M. Sharma, J. Food Compos. Anal. 18 (2005) 517–522.
[13] A. Duran, M. Tuzen, S. Soylak, Environ. Monit. Assess. 149 (2009) 283–289.
[14] M. Guldas, A.F. Dagdelen, G.F. Biricik, J. Food Agric. Environ. 6 (2008) 90–94.
[15] W.I. Manton, J. Agric. Food Chem. 58 (2) (2010) 713–721.
[16] C.W. Rankin, J.O. Nriagu, J.K. Aggarwal, T.A. Arowolo, K. Adebayo, A.R. Flegal, Environ. Health Perspect. 113 (10) (2005) 1344–1348.
[17] C. Voica, A. Dehelean, M.H. Kovacs, AIP Conf. Proc. 1425 (2012) 110–113.
[18] C.D. Klaassen, Casarett and Doull's Toxicology: the Basic Science of Poisons, 5th ed., McGraw-Hill, Blacklick, Ohio, U.S.A. (1995) 699.
[19] H. Needleman, Annu. Rev. Med. 55 (2004) 209–222.
[20] S. Hou, L. Yuan, P. Jin, B. Ding, N. Qin, L. Li, X. Liu, Z. Wu, G. Zhao, Y. Deng, Theor. Biol. Med. Modelling 10 (2013) 13.
[21] D. Farley, FDA Consum. 32 (1) (1998) 16–21.
[22] A. Lorber, Z. Karpas, L. Halicz, Anal. Chim. Acta 334 (1996) 295–301.
[23] R. Gonnen, R. Kol, Y. Laichter, P. Marcus, L. Hailicz, A. Lorber, Z. Karpas, J. Radioanal. Nucl. Chem. 243 (2000) 559–562.
[24] USP Advisory Panel on Metal Impurities. General Chapter on Inorganic Impurities: Heavy Metals. Available from: ⟨http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/2008-04-10InorganicImpuritiesStim.pdf⟩, (accessed 13..05.13).
[25] J.M. O'Heany, Summary of Health Effects of Chromium, 1986. ⟨http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/water-eau/chromium-chrome/chromium-chrome-eng.pdf⟩, (accessed 13.05.13).