Talanta 119 (2014) 1–4

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Trace elements in cocoa solids and chocolate: An ICPMS study

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ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form
21 October 2013
Accepted 22 October 2013
Available online 28 October 2013

Keywords:
ICPMS
Chocolate
Trace elements
Lead
Chromium

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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http://dx.doi.org/10.1016/j.talanta.2013.10.048

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%.