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Cadmium voltametric quantification in table chocolate produced in Chiquinquira-Boyaca, Colombia

Cuantificación voltamétrica de Cadmio en chocolate de mesa producido en Chiquinquirá-Boyacá, Colombia

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Abstract

Bioaccumulation of heavy metals such as cadmium has been a major concern in scientific communities and international food organizations, given the great toxicological risk to the consumer, and in many places there is no detailed record of its actual content. In this way, the need arises to carry out a study and registration of the concentration of this metal in products such as table chocolate, of great consumption at regional and national level. Likewise, we seek to have effective quantification tools and a reliable and affordable method to achieve the aim of this research. In this research, Cadmium content in powdered and granulated table chocolate was determined, elaborated and commercialized in the municipality of Chiquinquira, Boyacá-Colombia, using the differential pulse voltammetric method of anodic redisolution (DPVMAR). Previously, the parameters of this method were evaluated, selecting selectivity, linearity, sensitivity, precision and accuracy with satisfactory results as follows: selective at a potential range of 0.54 to 0.64 V, sensitivity in ppb, R^2> 0.95, % CV <10% % R> 80%). Analysis of variance showed no significant statistical differences (P <0.05) between the results. Cadmium quantification in samples of granulated and powder chocolate showed values of concentration between 214 and 260 ppb, with the highest concentrations of powder chocolate. Cadmium level did not exceed the tolerable weekly intake limit for this type of food.

Keywords: Food, cocoa, electrochemistry, heavy metals, validation.

Resumen

La bioacumulación de metales pesados como el Cadmio ha sido una de las principales preocupaciones en comunidades científicas y organizaciones alimentarias a nivel internacional, dado el gran riesgo toxicológico que supone para el consumidor, y que en muchos lugares no se cuenta con un registro detallado de su contenido real. Es de esta manera que surge la necesidad de realizar un estudio y registro de la concentración de este metal en productos como el chocolate de mesa, de gran consumo a nivel regional y nacional. De igual manera, se busca contar con herramientas de cuantificación eficaces y con un método confiable y asequible que permita lograr este objetivo. En este trabajo se determinó el contenido de Cadmio en chocolate de mesa en polvo y granulado, elaborado y comercializado en el municipio de Chiquinquirá, Boyacá-Colombia, empleando el método de voltamperometría diferencial de pulso de redisolución anódica (PDASV). Previamente, se validaron los parámetros de este método, evaluando selectividad, linealidad, sensibilidad, precisión y exactitud con resultados satisfactorios: selectivo a un rango de potencial de 0.54 a 0.64 V, sensibilidad en ppb, R^2 > 0.95, %C.V < 10%, %R > 80%). El análisis de varianza mostró que no existen diferencias estadísticas significativas (P<0.05) entre los resultados. La cuantificación de Cadmio en muestras de chocolate granulado y en polvo mostró valores de concentración entre 214 y 260 ppb, registrando las concentraciones más altas el chocolate en polvo. El nivel de cadmio no superó el límite de ingesta semanal tolerable para este tipo de alimentos.

Palabras clave: alimentos, cacao, electroquímica, metales pesados, validación.

Introduction

Table chocolate is made with cacao beans (*Theobroma cacao* L.), fruit which occupies the third place in the world market of raw materials after the sugar and coffee. This plant species grows mainly in western and central Africa, South America and Asia (Yanus *et al.*, 2014). Colombia is the eighth largest cocoa producer in Latin America, with a production of more than 54000 tons (Presidencia de la República de Colombia, 2016). Its main varieties are stranger, Trinitarian and Creole. Creole cacao is the finest, characterized by its pleasant taste and its exquisite aroma. While the outsider, is the lowest quality. However, it presents other quality conditions for the industry, such as its performance in fat content. Within this type of cocoa are produced in Trinidad and Tobago, Ecuador, West Africa, Asia, and Brazil.

Currently, in Colombia the sowings are being carried out with materials known as clones, which correspond to the combination of Creole and Trinitarian cacao carried out by research programs (Fedecacao, 2015). This food is cultivated in almost all the national territory with more than 160000 planted hectares, but is basically concentrated in the departments of Santander, Arauca, Antioquia, Huila, Tolima and Meta (Fedecacao, 2015). The cocoa chain production produces three goods: cocoa beans, cocoa liquor, butters, cakes and cocoa powder, and final products such as table chocolates and confections. Cocoa beans and chocolate, have several health benefits, including high antioxidants, which reduce the number of free radicals, help prevent infectious and autoimmune diseases, and reduce the risk of heart attack (Kelishadi, 2005).

Natural processes such as volcanic activity or rock erosion, coupled with anthropogenic factors such as excessive waste deposition in different ecosystems, have led to a destabilization in the biological balance. All these factors have increased the presence of compounds and xenobiotic elements with bioaccumulative and toxic properties, among which are heavy metals, which pass directly or indirectly to different foods, finally affecting the human being (Huang *et al.,* 2014). Some heavy metals have chemical properties similar to those of nutrients such as calcium or magnesium and, if available in water or soil, can be absorbed by plants (Kabata, 2000).

Natural geological cycles are being altered as a result of human activities such as mining, excessive emission of gases from cars, operation of the plastics and paint industry, among others. Dumping of heavy metals, including Cadmium, is among one and three times greater than those from natural flows, thus generating a greater availability of these into the environment, causing in addition alterations in different ecosystems arriving to reduce the life quality of the alive beings (Madero & Marrugo, 2011). High-consumption products such as fruits, vegetables, meats, and by-products are vulnerable to the heavy metal bioaccumulation with Cadmium being one of the worldwide major concerns due to its special neoplastic nature and pulmonary, kidney incidence (Chang *et al.*, 2014). This metal is nephrotoxic, causing tubular damage to the kidneys and bones, and increasing the risk of cancer (Jarup & Akesson, 2009).

Cadmium highest concentrations have been found in rice, wheat, oysters, and mussels in the animal renal cortex (Gama *et al.*, 2006). Some of the methods used for Cadmium analysis are atomic absorption spectrometry (Oymak *et al.*, 2009), X-ray fluorescence spectrometry (Martínez *et al.*, 2010), mass spectrometry with inductive coupling plasma (ICP-MS) (Behrooz *et al.*, 2009) and the use of electrodes with copper amalgam (Bi *et al.,* 2013). However, these methods involve sophisticated instruments and maintenance with high-cost inputs. On the contrary, the electrochemical method is one of the most favorable techniques for the determination of contaminants in food, since it offers a non-destructive test, of easy operability which can reach a greater sensitivity (Rosolina *et al.,* 2015). In addition, due to the reagents reduction, it is an environmentally friendly technique. Therefore, this method could be an alternative for the evaluation of heavy metals in food.

According to the above, the present research aims to validate an analytical method which allows the Cadmium quantification in table chocolate from Chiquinquira, Boyaca- Colombia, allowing the metal level determination in this type of food, establishing the degree of exposure to which consumers in the sector are currently subject.

Materials and methods

Equipments

The equipment used in this research, includes a BAS CV 50W polarograph equipped with a voltammetric analyzer and a CGME electrochemical station, consisting of a mercury drop electrode and an Ag/AgCl reference electrode. In addition, a sartorius basic analytical balance BA 210S (previously calibrated), Cole Parmer pHmeter WD35624-34 with resolution 0.01 and a YSI 63 conductivity meter of resolution 0.1 were used. All equipment had their calibration certificates.

The results comparison for the validation against the spectrophotometric method by atomic absorption, was carried out in acetylene-air flame spectrophotometer (Mark Shimadzu) with previous wet sample digestion according to SM 3030E method (APHA, 2012).

Reactives

All reagents used were analytical grade: Sodium hydroxide Panreac 99% purity, Acetic acid JT Baker 99.8%, Boric acid JT Baker 99%, Phosphoric acid Panreac 85% purity, Sodium chloride 99% JT Baker and solution 1000 ppm Cadmium JT Baker standard 99% purity. All solutions were prepared with deionized water (System Milli-Di™ Simplicity), with conductivity less than 0.05 uS.

Solutions

From a Cadmium stock solution of 1000 ppm (JT Baker 99 % of purity), two standard solutions of 100 ppm and 30 ppm. The electrochemical measurements were carried out in buffer Britton Robinson solution as support electrolyte. It was added to a 100 mL, 0.618 g of boric acid, 0.56 mL of acetic acid and 0.48 mL of phosphoric acid, was adjusted to pH of 4.8 with NaOH 0.1N, and deforested with deionized water.

Determination of general parameters for measures implementation

Cadmium quantification was carried out by the technique of Differential Pulse Voltammetry of Anodic Redisolution (DPVAR). A saturated solution of NaCl and deionized H_2O to clean the electrode system, and working electrode was verified obtaining satisfactory results for several runs of the support electrolyte, where no interference with the polarographic method or instrumental noise was evidenced. An initial potential of -400 mV and a final potential of -800 mV, a sample deoxygenation time of 200 s (Nitrogen bubble), a scanning rate of 20 $mV.s^{-1}$, a deposition time of 90 s, and a dwell time of 10 s.

Sample treatment

The analysis performed on table chocolate was carried out in triplicate, weighing approximately 10 g of sample of each presentation: table chocolate, Sugar-free granules and table-top powdered chocolate with flour of seven grains, which are two commercial products of two companies of the Municipality of Chiquinquira, Boyaca-Colombia. These samples were deposited in previously tared crucibles, at a temperature of 400°C for 8 h. The ashes obtained were subjected to an acid digestion process with nitric acid, hydrochloric acid and concentrated sulfuric acid. They were taken to dryness and dissolved with HCl concentrated, filtered and washed with hot water to a volume of 10 mL.

Cadmium polarographic determination

4 mL of Britton-Robinson buffer solution pH=4.8 (electrolite support) and 3 mL of each table chocolate to the electrochemical cell. After the first test, an addition of 35 µL of working solution, nitrogen deoxygenation was carried out over a period of 200s, and the run was repeated, repeating this procedure three times. The potential and time of deposition were determined according to the preliminary results.

Linearity and sensibility

To determine the method linearity, a calibration curve was carried out with seven concentration solutions of 150, 250 and 350 ppb, from a standard of 30 ppm and 450, 550, 650 and 750 ppb, from a standard of 100 ppm and deionized water as white. 4 mL of Britton-Robinson solution and 3 mL of deionized water were added.

To generate the calibration curve, the current (signal or response) was plotted at -0.599V as a function of metal concentration, and the statistical data treatment was determined by determining its slope (sensitivity) and correlation coefficient. The sensitivity of the voltammetric method, was established by calculating the slope of the calibration curve.

Method validation

The main attributes of the method were determined: limit of detection (DL), limit of quantification (QL), sensitivity (Eurachem, 2005), precision (with standard deviation and coefficient of variation) and accuracy (with recovery percentage). This was achieved by evaluating six batches of solutions, consisting of one blank (Britton Robinson solution), one standard (150 ppb), two natural samples (one sample of powder and granulated chocolate) for each presentation, carrying out the evaluation by two analysts. The results were compared with those obtained in counter samples, by the technique of atomic absorption spectroscopy.

Cadmium determination in table chocolate samples

Once the method for the Cadmium determination was validated, it was applied to samples of table chocolate obtained from two factories in Chiquinquira, Boyaca, Colombia. Sampling was carried out for two weeks by randomly taking a sample of two different batches for each week and

for each factory, respectively. These samples were prepared and analyzed in triplicate as explained in previous sections in the Laboratory of Research Group in Environmental Chemistry (GIQUA) of the Universidad Pedagógica y Tecnológica de Colombia, Boyaca-Tunja, Colombia.

Experimental design

Validation was carried out throughout an experimental 26 factorial design, where the variables were as follows: analysts (2) and solution lots (6). Samples were randomized in triplicate, giving a total of 64 assays. The data statistical treatment was performed with the SPSS 2012 ™ package.

Results and discussion

Linearity method

Figure 1, shows the corresponding polarograms to the standards used for the calibration curve elaboration in Figure 2. The linearity of the method is verified by means of the run of these patterns, the correlation value coefficient of generated curve, indicates the method is linear. The sensitivity is low, taking into account the slope value, resulting in small changes in concentration, do not significantly affect the value of the generated intensity.

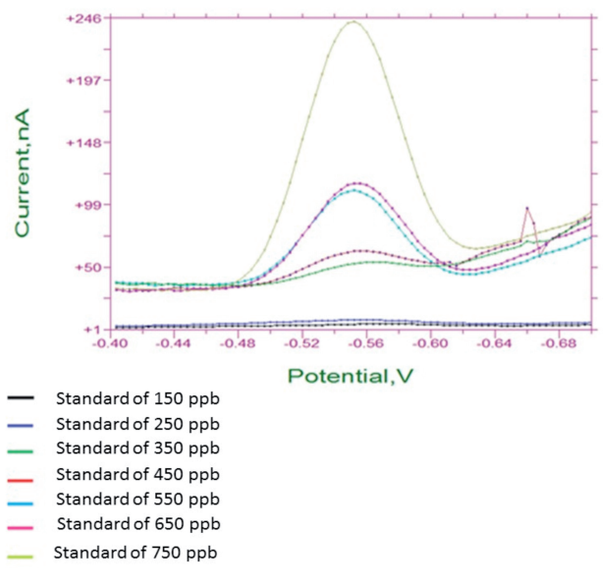

Figure 1. Polarogram of the cadmium calibration curve

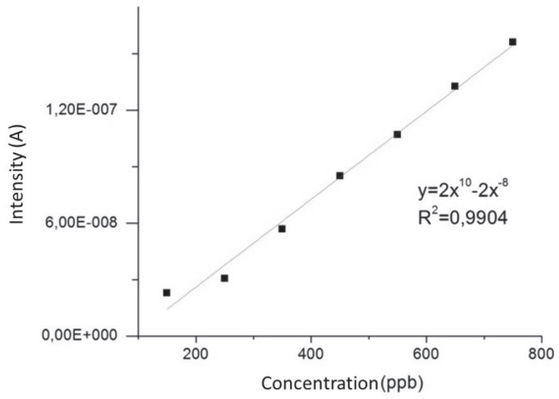

Figure 2. Cadmium linearity

Validation method

Validation attributes (Table 1), indicates the accuracy is above 82%. The coefficients of variation are below 10%, which shows the good precision. The limits of detection and quantification are within the results obtained in other investigations (Wang *et al.*, 2016; Yavuz *et al.,* 2016; Wang *et al.,* 2015).

Table 1. Validation parameters for cadmium quantification

Attribute	Cadmium
Number of standards	7
Lineal range (ppb)	150 - 750
Lineal regression	
Correlation coefficient (R^2)	0.9904
Confidence level (%)	95
Detection limit (ppb)	50
Quantification limit (ppb)	100
Sensibility (ppb)	
Added recovery (%)	82-90
Accuracy (reproducibility) (%)	80

Analysis of variance (Table 2), indicated there was no statistically significant difference between the lots and between the analysts since the value of F calculated does not exceed the critical F value. The counter samples analyzed by atomic absorption spectrophotometry did not present statistical differences (P <0.05) in the Cadmium content.

Table 2. Analysis of variance for the method validation

	Low standard		Natural sample 1		Natural sample 2		MN1+ Addition of standard		MN2+ Addition of standard	
Variation source	F calculated	Critical value for F	F calculated	Critical value for F	F calculated	Critical value for F	F calculated	Critical value for F	F calculated	Critical value for F
Samples	0.498	5.050	2.831	5.050	0.684	5.050	0.498	5.050	0.840	5.050
Analysts	0.865	6.608	0.073	6.608	0.053	6.608	0.865	6.608	1.614	6.608

Table 3. Cadmium quantification results in samples of chocolate powder and granules

		Lot 1			Lot 2		
	Sample	Concentration (ppb)	Mean (ppb)	C.V (%)	Concentration (ppb)	Mean (ppb)	C.V (%)
Week 1	Powder Chocolate	227.895	232.036	1.699	231.671	235.517	1.610
		232.469			235.625		
		235.743			239.254		
	Granulated chocolate	209.873	214.251	2.017	213.703	212.604	1.266
		214.365			214.574		
		218.514			209.536		
Week 2 Semana 2	Powder Chocolate	232.717	235.411	2.350	263.767	262.437	2.360
		231.741			255.687		
		241.774			267.858		
	Granulated chocolate	229.296	232.257	1.338	226.021	227.024	0.536
		231.983			226.673		
		235.493			228.377		

C.V: Coefficient of variation

Cadmium quantification in chocolate table

In Table 3, the results obtained for Cadmium quantification in two batches of powder and granulate chocolate samples produced in 2 weeks, are shown.

For each week and batch, Cadmium concentrations in chocolate powder were higher than granulated chocolate, possibly due to the fact the moisture of the product may generate a greater or lesser matrix dilution. The results (from 214 to 263 ppb) are higher than those found by Yanus *et al.* (2014), for different chocolate brands (84 - 141 ppb), and for Dahiya *et al.* (2005), in chocolates and Candy shop (0.001 a 2.73 ppb). Which can be caused by contamination from planting, harvesting and fruit transporting during the manufacture of the products when get in touch with water, equipment or supplies which have this metal. In addition, it can be related to soil contamination, which occurs in cacao crops in Ecuador (Chavéz *et al.*, 2015).

Taking into account the tolerable weekly Cadmium intake value established by OMS (2014), (7 μg.kg^{-1} of body weight), the equivalent value (μg) was calculated for a weight of 30 kg (210 μg), 50 kg (350 μg) and 70 kg (490 μg). According to this estimate, and assuming a daily diet of 10 g of table chocolate, consumption levels (μg) exhibited in a week ranging from 2.1 to 2.5 μg can be obtained (Table 4).

Table 4. Weekly intake (μg of Cadmium), according to the results obtained in the quantification.

	Sample	Average concentration (μg.K^{-1})	μg in 10 g of sample (up to date)	μg weekly
Week 1	Powder Chocolate	233.777	2.338	16.364
	Granulated chocolate	213.428	2.134	14.940
Week 2	Powder Chocolate	248.924	2.489	17.423
	Granulated chocolate	229.641	2.296	16.072

It is pertinent to conclude the mean concentrations found in each of chocolate samples do not exceed the limit established by the World Health Organization (WHO, 2006) for any of the populations within the ranges of body weight shown, but it is recommended to expand the samples number for future investigations, as this was an exploratory study.

Chocolate has been part of the people daily diet in different ages and conditions, with abundance of products of several varieties and presentations. Martínez *et al.* (2010), show the Cadmium accumulation impact in these products, can cause in this population. Although no report exceeded the limits set by the World Health Organization (WHO, 2006), it is argued that excessive consumption of these contaminated foods can affect the population in short or long term. Dahiya *et al.* (2005), concluded that Cadmium content is usually higher in cocoa-based chocolate candies than in milk-based

and sugar-only ones, possibly due to the soil contamination where cocoa was cultivated.

At national level, and in Boyaca department, the elaboration of cacao-based foods has increased. However, studies reporting the Cadmium levels in these products are incipient. Thus, it is necessary the analysis protocol standardization which allows to quantify the possible Cadmium content in alimentary matrices, objective to which, this research contributed significantly.

Conclusion

Anodic Redisolution Pulse Differential Voltammetry (ARPDV) was validated for Cadmium quantification, and the content of this metal in table chocolate produced in the Municipality of Chiquinquira-Boyaca, Colombia, was evaluated. The values between 230 and 263 ppb, were found in powder chocolate samples and between 214 and 227 ppb in samples of granulated chocolate. The values obtained are below from the maximum tolerable weekly limit intake established by World Organization Health. However, are high compared to other studies, possibly due to contamination from planting, harvesting, and fruit transporting during the manufacture of the products. This research, performs an analysis alternative method for heavy metals determination, such as Cadmium, in food from the family basket, which allows greater sensitivity, selectivity and robustness compared to the traditional methodology of Atomic Absorption Spectrophotometry.

References

APHA. American Public Health Association, American Water Works Association, Water Pollution Control Federation. (2012). Standard methods for the examination of water and wastewater. Edition 22nd. *https://www.standardmethods.org/*

Behrooz, J., Naficeh, S., Mohammad, R.O., Abdol-azim, B., Hossein, K. & Alireza, S. (2009). Simultaneous determination of lead, cadmium, copper and zinc in infant formula by anodic stripping voltammetry. *Iran J Pharm Res,* 8(3), 159-162.*http://ijpr.sbmu.ac.ir/article_805_9827b87582d03cfe86ecfb2f7c25e65b.pdf.*

Bi, Z., Salaun, P., & Van Den Berg, C. (2013). Determination of lead and cadmium in seawater using a vibrating silver amalgam microwire electrode. *Anal Chim Acta,* 769, 56-64. *http://dx.doi.org/10.1016/j.aca.2013.01.049*

Chávez, E., He, Z.L., Stoffella, P.J., Mylavarapu, R.S., Li, Y.C., Moyano, B. & Baligar, V.C. (2015). Concentration of cadmium in cacao beans and its relationship with soil cadmium in southern Ecuador. *Sci Total Environ,* 533, 205-214. *http://dx.doi.org/10.1016/j.scitotenv.2015.06.106*

Dahiya, S. Karpe, R. Hegde, A., & Sharma, R. (2005). Lead, cadmium and nickel in chocolates and candies from suburban areas of Mumbai, India. *J Food Comp Anal,* 18(6), 517-522. *http://dx.doi.org/10.1016/j.jfca.2004.05.002*

Duran, A. Tuzen, M., & Soylak, M. (2009). Trace metal contents in chewing gums and candies marketed in Turkey. *Environ Monit Assess,* 149(1-4), 283-289. *http://dx.doi.org/10.1007/s10661-008-0202-0*

Eurachem. (2005). Métodos analíticos adecuados a su propósito. Guía de Laboratorio para la validación de métodos y temas relacionados. Segunda Edición. México, Mexico D.F. p. 69.

Fedecacao. (2015). Producción Nacional registrada de cacao en grano por departamentos de 2002 a 2014. *http://www.fedecacao.com.co/portal/index.php/es/2015-02-12-17-20-59/nacionales.*

Gama, E. M., Da Silva, L. A., & Lemos, V. A. (2006). Preconcentration system for cadmium and lead determination in environmental samples using polyurethane foam/Me-BTANC. *J Hazard Mater,* 136(3), 757–762. *http://dx.doi.org/10.1016/j.jhazmat.2006.01.015*

Huang, Z., Pan, X.D., Wu, P.G.; Han, J.L., & Chen, Q. (2014). Heavy metals in vegetables and the health risk to population in Zhejiang, China. *Food Control,* 36(1), 248-252. *http://dx.doi.org/10.1016/j.foodcont.2013.08.036*

Jarup, L., & Akesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol*, 238(3), 201–208. *http://dx.doi.org/10.1016/j.taap.2009.04.020*

Kelishadi, R. (2005). Cacao to cocoa to chocolate: healthy food? *Arya Journal,* 1(1), 29-35. *https://sacredchocolate.com/docs/sacredpdf/cacao-cocoa-chocolate-arya-journal.pdf.*

Madero, A., & Marrugo, J. (2011). Detección de metales pesados en bovinos, en los valles de los ríos Sinú y San Jorge, departamento de Córdoba, Colombia. *Rev MVZ Cordoba,* 16(1), 2391-2401.

Martínez, T., Zarazua, G., Ávila-Pérez, P., Navarrete, M., & Tejaeda, S. (2010). Total reflection X-ray fluorescence analysis of trace-elements in candies marketed in Mexico. *Spectrochim Acta B,* 65(6), 499-503. *http://dx.doi.org/10.1016/j.sab.2010.04.002*

OMS. (2014). Organización Mundial de la Salud. Niveles máximos para el cadmio en el chocolate y productos derivados de cacao. *ftp://ftp.fao.org/codex/meetings/cccf/cccf9/cf09_06s.pdf*

Oymak, T., Tokalioglu, S., Yilmaz, V., Kartal, S. Y., & Aydin, D. (2009). Determination of lead and cadmium in food samples by the coprecipitation method. *Food Chem,* 113(4), 1314-1317. *http://dx.doi.org/10.1016/j.foodchem.2008.08.064*

Presidencia de la República de Colombia. (2016). Producción de cacao aumenta en 2015 y se consolida como cultivo clave en el posconflicto. *http://es.presidencia.gov.co/noticia/Produccion-de-cacao-aumenta-en-2015-y-se-consolida-como-cultivo-clave-en-el-pos-conflicto.*

Rosolina, S.M., Chambers, J.Q., Lee, C.W., & Xue, Z.L. (2015). Direct determination of cadmium and lead in pharmaceutical ingredients using anodic stripping voltammetry in aqueous and DMSO/water solutions. *Anal Chim Acta,* 893, 25-33. *http://dx.doi.org/10.1016/j.aca.2015.07.010*

WHO. World Health Organization. (2006). WHO Child Growth Standards. United Nations Children's Fund. p.300. *http://www.who.int/childgrowth/en/*.

Yanus, R.L., Sela, H., Borojovich, E.J.C., Zakon, Y., Saphier, M., Nikolski, A., Lorber, A. & Karpas, Z. (2014). Trace elements in cocoa solids and chocolate: An ICPMS study. *Talanta,* 119, 1-4. *http://dx.doi.org/10.1016/j.talanta.2013.10.048*