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Determination of metal contents in various chocolate samples

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Abstract: Seven metals (Mn, Cu, Zn, Cd, Cr, Fe, and Pb) were determined in 17 different samples of chocolate and commonly consumed cocoa products using atomic absorption spectrometry, flame technique (F-AAS). Samples were prepared by wet digestion with concentrated nitric acid. Concentrations of metals (Mn, Fe, Cu and Zn), with exception of Cr, Pb and Cd, were found to be the highest in cocoa powder and black chocolate with a high content of cocoa. Concentrations of Cu, Cd and Pb were found to be below permissible levels prescribed by national Regulation of maximum permitted quantities of certain contaminants in food B&H in all samples.

INTRODUCTION

Chocolate is one of the most commonly consumed dessert worldwide made of cocoa as the main raw material.

The term ‘cocoa’ is a corruption of the word ‘cacao’ that is taken directly from Mayan and Aztec languages. Chocolate is derived from cocoa beans, central to the fruit of cocoa tree, *Theobroma cacao*, which is indigenous to South America and believed to have originated from the Amazon and Orinoco valleys (Afoakwa, 2010).

Chocolate is a food obtained by mixing sugar with two products derived from the industrial processing of cocoa beans (cocoa butter and paste), and for some types of chocolate, by adding milk or other ingredients. As it is mainly composed of cocoa butter and sugar, chocolate has been classified as a sweet snack, a dessert or, depending on the sophistication of the processing methods or the genetic origin of the cocoa, as a luxury or gourmet food. Nevertheless, the health benefits of chocolate have been demonstrated by a number of studies which have shown that apart from its nutritional value, the bio-active components it contains can act to prevent some pathologies (Sira, 2015). Chocolate is composed of solid particles, whose normal solid concentration is about 60 to 70% sugar, cocoa and milk.

Beside these main solid constituents, other ingredients like hydrogenated vegetable oil, salts, buffering agents, permitted emulsifier and cocoa butter could be source of metals (Ciurea and Lipka, 1992; Rehman and Husnain, 2012).

Farming of cocoa and it's treatment with pesticides and fungicides can affect composition of chocolate products and their metal contamination as cocoa is the main raw material. The metal content of chocolate products can be also affected with industrial processes and metals which are used for making vessels and containers to be used in production process of making chocolate products.

Some trace metals aren't toxic and are even essential if their intake is below permissible levels prescribed by national and international legislations, but could be toxic in higher concentrations. Essential trace metals are important for the various biochemical functions and are playing crucial role in metabolic processes as parts of many enzymes. From 7 metals which were determined in this research (Zn, Cr, Mn, Fe, Cu, Cd and Pb), with exception of Cd and Pb, all the others are in a cathegory of essential elements.

The aim of this work was the assessment of metal contents in some samples of cocoa powder, chocolate for cooking, milk chocolate, black chocolate with various content of cocoa and white chocolate using wet digestion and flame atomic absorption spectrometry method.

EXPERIMENTAL

Sampling

Samples were randomly collected as they were available in the markets of the city of Sarajevo in the period from the first of January to the first of February, 2015. Samples were taken according to the content of cocoa. The following samples were taken: 3 samples of cocoa powder, 3 samples of chocolate for cooking, 3 samples of milk chocolate, 5 samples of black chocolate with various content of cocoa and 3 samples of white chocolate.

Sample Preparation Procedure

Samples were crumbled and prepared by wet digestion where weighed mass of around 2.0000 g (± 0.01 mg) (Mettler Toledo, New Classic MF-TYPE) was heated with concentrated nitric acid for 30 minutes at temperatures 70-80 °C. The solution was filtered (blue filtered paper), then the clear solution was transferred in volumetric flask (50 mL) and diluted to mark with distilled water. Digestion of each sample was done in triplicate.

Determination of the metals

The metal (Mn, Cu, Zn, Cd, Cr, Fe, and Pb) content was determined by atomic absorption spectrometry (SpectraAA-10, Varian), flame technique (FAAS). The concentrated nitric acid (Panreac, Spain) was of analytical grade. Glassware was properly cleaned. Standard solutions (Merck; Germany, CertiPUR 1000 ppm) of Mn, Cu, Zn, Cd, Cr, Fe, and Pb were used in preparing subsequent calibration curves after serial dilutions. The sample analyses were done in triplicate. The obtained metal contents were interpreted on the basis of the limit values specified by the Regulation of maximum permitted quantities of certain contaminants in food B&H (Sl. glasnik BiH, br. 68/14).

RESULTS AND DISCUSSION

The metal (Mn, Cu, Zn, Cd, Cr, Fe, and Pb) contents after wet digestion determined by atomic absorption spectrometry, flame technique (FAAS) are shown in Table 1 and Table 2. Results are shown as c (μg/g) ± SD (the average metal concentration of three parallels ± standard deviation).

Table 1 and Table 2 are showing the average concentrations of metals in the aforementioned categories of samples with their respective ingredients.

Table 1: Concentrations of Mn, Cu, Zn, Cd, Cr, Fe, and Pb in analyzed chocolate samples of different types

Sample	c(μg/g) ± SD		
	Mn	Cu	Zn
	Cocoa powder		
K1	11.5±1.06	32.37±1.58	80.02±2.76
K2	45.87±1.25	30.87±0.88	77.78±1.50
K3	36.75±0.50	38.17±0.29	36.38±1.59
	Chocolate for cooking		
JIK1	7.08±0.38	1.25±0.00	12.38±0.18
JIK2	10.42±0.95	9.42±0.63	10.88±0.53
JIK3	7.00±0.25	6.75±0.00	6.25±0.71
	Milk chocolate		
M1	3.13±0.18	2.75±0.00	9.88±0.18
M2	2.75±0.25	2.75±0.00	10.17±0.76
M3	3.08±0.13	3.38±0.18	4.38±0.18
	black chocolate with various content of cocoa		
32%K	2.33±0.29	2.63±0.17	4.5±0.35
52%K	9.83±0.38	7.58±0.38	18.58±0.38
65%K	13.67±0.63	11.42±0.63	27.50±1.14
75%K1	17.5±1.14	14.83±0.76	13.88±1.94
75%K2	22.17±0.38	16.38±0.53	38.13±0.73
	White chocolate		
B1	0.67±0.29	0.63±0.18	7.08±0.88
B2	ND	0.92±0.14	2.00±0.00
B3	0.33±0.14	0.75±0.25	2.25±0.00

ND*- non detected

Table 2: Concentrations of, Cd, Cr, Fe, and Pb in analyzed chocolate samples of different types

Sample	c(μg/g) ± SD			
	Cd	Cr	Fe	Pb
	Cocoa powder			
K1	ND	5.37±0.61	144.36±0.92	4.87±0.11
K2	0.50±0.00	1.41±0.04	86.79±0.02	2.60±0.00
K3	ND	ND	57.07±0.35	4.38±0.36
	Chocolate for cooking			
JIK1	ND	4.90±2.39	21.10±0.82	0.94±0.22
JIK2	ND	ND	39.16±3.05	1.13±0.05
JIK3	ND	ND	38.56±0.81	1.62±0.73
	Milk chocolate			
M1	ND	3.85±0.48	19.29±1.13	ND
M2	ND	2.75±0.01	18.64±0.21	ND
M3	0.25±0.00	ND	20.44±0.32	2.45±0.62
	black chocolate with various content of cocoa			
32%K	ND	ND	8.00±1.64	ND
52%K	ND	ND	20.42±1.42	ND
65%K	ND	ND	38.65±0.89	ND
75%K1	ND	0.3±0.07	74.85±1.00	ND
75%K2	0.50±0.00	ND	65.85±0.00	ND
	White chocolate			
B1	ND	5.05±0.57	3.59±0.88	1.52±0.42
B2	ND	1.94±0.29	8.28±1.42	ND
B3	0.50±0.00	1.31±0.47	14.37±0.00	0.67±0.04

ND*- non detected

The lowest concentration of Mn was observed in a white chocolate sample marked with B2, which concentration was ND (non detected). The highest concentration of Mn (45.87 μg/g) was detected in a cocoa powder marked with K2. As can be seen on the Figure 1, the concentration of

Mn grows with the higher cocoa contents in samples. In national Regulation values of permissible levels of Mn for chocolate and chocolate products aren't given. Similar analysis was made in Serbia on white and milk chocolate samples, where concentration of Mn was in a range of 0.07 – 2.98 µg/g and 1.44 – 15.26 µg/g, respectively (Cvetković, 2013). Concentration of Mn in chocolate samples published in some other countries in the world: Brazil (43.1 – 52.2 mg/kg), Pakistan (1.4 – 8.1 mg/kg) (Rehman and Husnain, 2012).

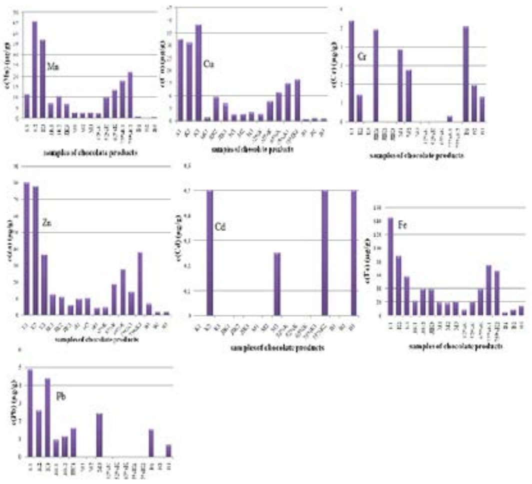

Figure 1: The metal (Mn, Cu, Zn, Cd, Cr, Fe, and Pb) contents in chocolate samples

The concentration of Cu was found to be the lowest in a white chocolate sample marked with B1, which concentration was 0.63 µg/g, whereas concentration in a cocoa powder sample marked with K3 was found to be the highest with value of 38.17 µg/g. All of the concentrations of Cu which were determined are below permissible levels (chocolate and chocolate products – 15 mg/kg, sugar-free chocolate – 30 mg/kg, and cocoa powder – 50 mg/kg) prescribed by national Regulation (Sl. glasnik BiH, br. 68/14) in all samples. According to the research made in Serbia, content of Cu in white chocolate and milk chocolate samples was in a range of ND (non detected) – 1.37 µg/g and 1.64 – 14.23 µg/g, respectively (Cvetković, 2013). Concentration of Cu in chocolate samples published in some other countries in the world: Turkey (5.7 – 10.6 mg/kg), Brazil (26.6 – 31.2 mg/kg), Malaysia (2.9 – 6.3 mg/kg), Pakistan (1.4 – 4.4 mg/kg) (Rehman and Husnain, 2012).

The content of Zn was in a range of 2.00 – 80.02 µg/g, where the lowest concentration was found in a white chocolate sample marked with B2, and the highest concentration was found in cocoa powder sample marked with K1. For Zn similar as for the other two mentioned metals (Mn and Cu), trend of growing concentration by growth of the cocoa content in the samples was found. In national Regulation values of permissible levels of Zn for chocolate and chocolate products aren't given. According to research made in Serbia content of Zn in white chocolate and milk chocolate samples was in a range of ND – 10.17 µg/g and 4.76 – 21.4 µg/g, respectively (Cvetković, 2013). Concentration of Zn in chocolate samples published in some other countries in the world: Turkey (12.1 – 16.7 mg/kg), Brazil (7.5 – 23.3 mg/kg), Pakistan (3.2 – 29.3 mg/kg) (Rehman and Husnain, 2012).

The concentration of Cd was determined in 4 samples, whereas in all the other samples concentration of Cd was not detected. Cadmium was detected in the following samples: K2 (0.5 µg/g), M3 (0.25 µg/g), 75% K2 (0.5 µg/g) and B3 (0.5 µg/g). All of the concentrations of Cd which were determined are below permissible levels (for the food supplements 1.00 mg/kg) prescribed by national Regulation (Sl. glasnik BiH, br. 68/14) in all samples.

The content of Cr was in a range of ND – 5.37 µg/g, where concentration of Cr for 8 samples was not detected, whereas the highest concentration belongs to cocoa powder sample marked with K. In national Regulation values of permissible levels of Cr for chocolate and chocolate products aren't given. According to the research made in Serbia, content of Cr in white chocolate and milk chocolate samples was in a range of 0.12 – 0.38 µg/g and ND – 0.38 µg/g, respectively (Cvetković, 2013).

Iron was present in all samples of chocolate products, with higher values of concentrations detected in cocoa powder and black chocolate with various content of cocoa. Similar as for some of the other mentioned metals (Mn, Cu, Zn) trend of growing of the concentration by growth of the content of cocoa in samples of different types was found. The content of Fe was in a range of 3.59 – 144.36 µg/g, where the lowest concentration was found in white chocolate sample marked with B1, and the highest concentration was found in cocoa powder sample marked with K1. In national Regulation values of permissible levels of Fe for chocolate and chocolate products aren't given, with exception of cocoa butter – 2.00 mg/kg. According to the research made in Serbia, content of Fe in white chocolate and milk chocolate samples was in a range of 1.19 – 6.39 µg/g and 5.90 – 37.90 µg/g, respectively (Cvetković, 2013). Concentration of Fe in chocolate samples published in some other countries in the world: Turkey (2.5 – 3.7 mg/kg), Brazil (1.2 – 140.8 mg/kg), Pakistan (3.2 – 59.6 mg/kg) (Rehman and Husnain, 2012).

The content of Pb in 8 samples was not detected. The highest content of Pb was determined in cocoa powder samples, whereas content of Pb in all samples of black chocolate with various content of cocoa were not detected. The concentration of Pb was in a range of ND – 4.87 µg/g, where the highest concentration belongs to cocoa powder sample marked with K1. All of the concentrations of Pb which were determined are below permissible levels (for the food supplements 3.00 mg/kg) prescribed by national Regulation (Sl. glasnik BiH, br. 68/14) in all samples. According to the research made in Serbia, content of Pb was detected in one white chocolate sample with concentration of 0.41 µg/g, and in milk chocolate samples content of Pb was in a range of ND – 0.78 µg/g and µg/g (Cvetković, 2013). Concentration of Pb in chocolate samples published in some other countries in the world: Turkey (30 – 40 µg/kg), India (49 – 8040 µg/kg), USA (11.9 – 69.8 µg/kg), Malaysia (1100 – 1940 µg/kg), Pakistan (28.6 – 1400 µg/kg) (Rehman and Husnain, 2012).

CONCLUSIONS

Trend of growing of the concentration by the growth of the content of cocoa in samples of different types was found for the most of determined metals, with exception of Cr, Pb and Cd. It can be concluded that the concentrations of metals (Mn, Fe, Cu and Zn), with exception of Cr, Pb and Cd, were found to be the highest in cocoa powder and black chocolate with a high content of cocoa. Toxic heavy metals such as Pb and Cd were found to be below permissible levels prescribed by Regulation of maximum permitted quantities of certain contaminants in food B&H (Sl. glasnik BiH, br. 68/14) in all samples. The metal contents of determined metals in this research which are considered as essential, the most abundant is Fe followed by the Zn, Mn and Cu, whereas the concentration of Cr in some samples was not detected. It can be concluded that all the concentrations of the metals which were determined in this research were found to be below permissible levels prescribed by national Regulation of maximum permitted quantities of certain contaminants in food B&H (Sl. glasnik BiH, br. 68/14) in all samples.

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Summary/Sažetak

Sedam metala (Mn, Cu, Zn, Cd, Cr, Fe, i Pb) su određeni u 17 različitih uzoraka čokolade i najčešće konzumiranih kakao proizvoda korištenjem atomske apsorpcione spektrometrije, plamena tehnika (F-AAS). Uzorci su pripremljeni primjenom mokre digestije sa koncentrovanom nitratnom kiselinom. Može se zaključiti da su koncentracije metala (Mn, Fe, Cu i Zn), izuzev Cr, Pb i Cd, bile najveće u uzorcima kakao praha i uzoraka crne čokolade sa različitim sadržajem kakaoa. Koncentracije Cu, Cd i Pb su u svim uzorcima bile ispod maksimalno dozvoljenih vrijednosti propisanih od strane državnog Pravilnika (Pravilnik o maksimalno dozvoljenim količinama za određene kontaminante u hrani, (Sl. glasnik BiH, br. 68/14)).