ESSENTIAL AND TOXIC TRACE ELEMENTS IN CHOCOLATE: OCCURRENCE DATA, ASSESSMENT OF NUTRITIONAL MERITS AND RISK EVALUATION

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Abstract- The aim of the study was the assessment of the balance of essential and toxic elements in chocolate in order to evaluate its quality and its nutritional merits. For this purpose, 85 samples of the mostly consumed chocolates in France of different types: dark and milk chocolates, powdered chocolates in addition to cocoa beans were collected from regular markets and analysed in terms of essential (Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Se and Zn), non-essential (B, Ba, Co, Ni, Sr, Ti and V) as well as toxic elements (Al, As, Cd, Sb and Pb). Cocoa beans are found to have higher contents of the measured elements followed by dark or powdered chocolates then by milk chocolates excepted for Ca and Na which are higher in milk chocolates. The latter results show a negative correlation between the cocoa content and Ca and Na concentrations, which can be attributed to the presence of these elements in milk hence leading to an increase of their levels in the milk-chocolate type compared to cocoa solely.
Among the toxic elements, the highest mean levels were found for Al (43.1 mg kg^{-1}) and Cd (0.227 mg kg^{-1}) in powdered chocolates and cocoa beans respectively, whereas the levels of other toxic elements were very low (generally $\leq$ 0.01 – 0.07 mg kg^{-1}). It is worth to note that significant difference ($p<0.05$) was observed between dark and milk chocolates for most elements while no differences were found between bio and regular dark chocolates except for B, V, Fe, Zn, Se, Mo and Cd ($p<0.05$). Nevertheless, it is difficult to directly link these findings to the practices of organic farming. Finally, Cd content was significantly higher in bio (0.378 mg kg-1) than regular chocolate (0.114 mg kg-1). The exposure assessment was estimated and compared to the second French total diet study (TDS2), which was undertaken by the French Food Safety Agency in 2006. The exposition was quite similar in terms of toxic elements excepted from Cd which was ≈4 and 5 times higher than that from TDS2 for children and adults, respectively. The same observation was made for the intakes estimation (adults and children) concerning others elements with comparable results, excepted for Ca, Cr, K, Mg, Mn which were ≈ 2 times lower than those of TDS2 and for Na (≈4 times). No differences were found for Mg and Mn intakes for the adults.

Keywords: *chocolate; occurrence data; essential & toxic elements; exposure assessment.*

1. INTRODUCTION

It is nowadays largely accepted that a better understanding of the impact of food chemical contamination on human health is of utmost importance as food represents one of the most significant pathways of humans' exposure to chemical hazards. This is extremely challenging since such a task requires the development and validation of novel analytical approaches capable to cope with a large panel of chemical contaminants present at trace and ultra-trace levels in a big variety of environmental, food and biological matrices. It is worth to note that inductively coupled plasma-mass spectrometry (ICP-MS) provides nowadays the features of an ideal analytical technique for simultaneous determination of trace and major elements in foodstuff due to its low limits of detection, multielement capability and high sensitivity and selectivity.

One of the foodstuffs whose consumption has considerably grown in the last years is the chocolate in its various forms. Apart from its appreciation as a dessert worldwide, the chocolate is also a source of essential elements such as Se, Cu, Mg, Fe, etc. Nevertheless, it is also a contributor to dietary exposure of several toxic metals such as Cd, especially for infant and young children [1]. This is confirmed by the recent European regulation (to be applied starting from January 2019) which set maximum levels of Cd in chocolate, according to the percentage of dry cocoa solids. In addition, the levels of essential and toxic elements may vary according to the type and origin of cocoa and hence it could play an economical role in terms of trade.

In order to assess the quality and nutritional merits of chocolate, 85 chocolate samples from regular French markets were analysed in terms of 23 elements. Additionally, a preliminary exposure assessment and a nutritional intake assessment were carried out.

2. EXPERIMENTAL

2.1. Sampling

85 chocolate types (various brands) mostly consumed by the French population [2] were collected from the markets according to their availability.The samples were categorized first by their cocoa content (13 – 100%), their type, such as milk (n=24, with 5 bio), dark (n=46, with 16 bio) or powdered chocolate (n=10). Five samples of cocoa beans were also analysed to obtain specific data related to the main ingredient of chocolate.

2.2. Materials and methods

The analyses were carried out by ICP-MS (7700 Series x Agilent Technologies), equipped with a third generation Octopole Reaction System (ORS3) using
helium (He) as collision gas). The method was accredited by the French accreditation body (COFRAC) and the analytical procedure was described elsewhere [3]. Briefly, 0.2–0.4 g of sample was weighed precisely in a quartz digestion vessel and then wet-oxidised with a mixture of 3 mL of ultra-pure water and 3 mL of ultra-pure HNO3 (67% v/v) in a closed microwave digestion system. After cooling to room temperature, the digested samples were transferred into 50-mL polyethylene tubes and then a solution of a mixture of internal standards (In, Y, Bi, Sc, Re) at 2 µg L-1 was added and the tube was made up with ultrapure water to the final volume. A certified reference material SRM 2384 (chocolate) from the National Institute of Standards (NIST, USA) purchased from LGC Standards (Molsheim, France) was used for the internal quality control.

2.3. Food consumption data

For the general population, consumption data were provided by the National Individual Dietary Consumption Survey (INCA2) conducted in 2006 and 2007 in eight regions of France [2]. This survey was designed as a 7-day food diary, supplemented with questionnaires on cooking habits, socio-economic and anthropometric factors. A total of 2624 adults (18 years and over) and 1455 children and adolescents (3-17 years) were included.

3. RESULTS AND DISCUSSION

3.1. General

The experimental data were processed by StatGraphics Centurion XVI 16.1.17, SAS software package, version 9.4. and Microsoft Excel 2010 softwares.For the exposure assessment, the overall censoring rate (values below the LOD or the LOQ) of the data entries was less than 60%. According to this rate the censored data were replaced by a median or middle bound (MB) assumption: concentrations below the LOD were replaced by LOD/2, those below the LOQ but above the LOD by LOQ/2 [4].The total daily exposure (E_i) of individual *i* was
calculated by combining the daily intake (C_i) of chocolate (g/d) with the average
concentration (L) of the element in chocolate (mg kg^{-1}).

For each individual *i*, and only for the contaminants, this value was divided by the body weight (BW) of individual *i* (kg) (1).

$$E_i = \frac{C_i \times L}{BW_i} \qquad (1)$$

The exposure and the nutritional intakes were calculated for adults (>17 years) and children (3-17 years) separately.

3.2. Occurrence data

The range and average concentrations of the elements assessed in this study for the 85 chocolate samples are given in Table 1. All the elements were quantified and their concentration range is quite variable for the chocolate types analysed. When the concentration data are grouped according to their cocoa contents (%), the results show a linear correlations between cocoa contents and the different elements ($P<0.05$) excepted for Sb and Ti. The concentration for these elements is fairly comparable regardless the cocoa content: 0.004 to 0.009 mg kg^{-1} for Sb and 0.851 to 1.75 mg kg^{-1} for Ti. The results show a negative correlation with Ca and Na concentrations, which can be attributed to the presence of Ca-Na in milk added to chocolate hence leading to a more consistent levels of these elements in milk-chocolate compared to cocoa solely [5].

In term of toxic elements, the highest mean concentration was found for Al (24.1 mg kg^{-1}) followed by Cd. In the latter case, highest mean levels were found in cocoa beans and in cocoa powder, respectively. Concentration ranges for As, Pb and Sb were overlapping and were quite low (≤ 0.01 – 0.07 mg kg^{-1}). Apart from a relatively high As content measured in a sample of cocoa bean (0.131 mg kg^{-1}), As levels ranged from < 0.002 to 0.062 mg kg^{-1} with a mean level of 0.014 mg kg^{-1}. The highest level of Pb was found in a dark chocolate (74% cocoa) and the Sb levels were quite similar in all of the samples and below 0.016 mg kg^{-1}, except a sample of dark chocolate (90% cocoa) where Sb was at 0.040 mg kg^{-1}.

Table 1:Concentrations of elements in chocolate samples (n=85)

Analyte	**Mean (mg kg^{-1})**	**Range (mg kg^{-1})**	**>LOQ (%)**	**correlation with cocoa content[a] (R[b])**
Al	24.1	1.66 - 97	100	0.9211
As	0.021	<0.002 - 0.131	89	0.977
Cd	0.200	0.005 - 2.337	100	0.9461
Sb	0.006	<0.001 - 0.040	67	0.5484
Pb	0.044	<0.003 - 0.160	93	0.9091
Ca	1051	224 - 2798	100	-0.6857
Cr	0.789	0.064 - 3.54	100	0.886
Cu	11.4	1.029 - 34.6	100	0.9906
Fe	93.9	5.95 - 312	100	0.8927
K	7027	2996 - 44283	100	0.7576
Mg	1551	314 - 4502	100	0.9873
Mn	13.2	1.04 - 47.2	100	0.9543
Mo	0.185	0.042 - 0.861	100	0.9389
Na	404	<5.0 - 2287	86	-0.8913
Se	0.102	<0.010 - 0.897	94	0.8923
Zn	24.6	5.29 - 122	100	0.9333
B	6,00	0.694 - 21	100	0.9881
Ba	4.11	0.468 - 15.4	100	0.9874
Co	0.271	0.032 - 0.969	100	0.9761
Ni	2.82	0.260 - 8.50	100	0.9978
Sr	4.54	0.902 - 16.9	100	0.9485
Ti	1.28	<0.100 - 5.64	98	0.7935
V	0.085	0.004 - 0.337	100	0.9453

[a] cocoa content is expressed in percentage (%)
[b] R is the correlation coefficient

Amongthe toxic elements, Cd solely is regulated by the European commission (EC) in 2014 (amending Regulation 1881/2006), which set maximum levels (ML) of Cd in foodstuffs, in specific cocoa and chocolate products according to the percentage of dry cocoa solids (to be applied starting from January 2019) [6]. It is worth to underline that Cd concentrations measured in the chocolate samples in this study were below the maximum levels regulated by EC.In terms of essential elements, the highest mean levels were found for K, Mg, Ca and Na followed by Fe, Zn, Mn, Cu and Cr, Mo, Se. This indicates that chocolate(especially dark type)can be considered as a good source for these elements. A variation of the concentration of the non-essential elements in different types of chocolate is also reported in Fig. 1.

As can be seen, cocoa beans contain higher levels of these elements being followed by dark or powdered chocolates types. It is worth to note that, again, the milk chocolates contain lower levels of the non-essential elements, which is in general agreement with the behaviour of the toxic and essential elements.

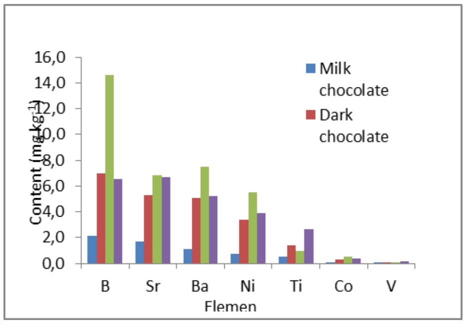

Figure 1: Element contents in different types of chocolate

When comparing the elements contents, we observed significant differences ($p<0.05$) between the dark and the milk chocolate samples, except for Sb (0.006 *vs* 0.003 mg kg^{-1}), while the elements contents of bio dark chocolates (n=16) were significantly different ($p<0.05$) compared with dark regular chocolates (n=30) only for for B, V, Fe, Zn, Se, Mo and Cd. It is difficult to explain this difference, as these elements are not directly involved in organic farming. It can also be noted that Cd level was three times higher in bio chocolates comparing to regular ones (0.378 *vs* 0.114 mg kg^{-1}).

More studies should be pursuit by analysing a larger number of chocolate samples (of each type) in order to obtain an exhaustive comparison between various chocolate types including that obtained in bio-farming in terms of essential and toxic trace elements.

3.3. Exposure assessment and nutritional intakes

The results for adults and children are presented in Table 2. These results were calculated by using the occurrence data of the chocolate samples excluding powdered chocolate and cocoa beans.

3.3.1. Exposure assessment to chemical contaminants

The exposure to chemicals linked to the consumption of chocolate is very close to the French previous estimations of the second total diet study (TDS2) or sometimes less (as As and Sb by a factor 5 to 8 approximatively), except for Cd. In fact, the exposure to Cd (mean: 0.010 µg/kg bw/d for adults and 0.020 µg/kg bw/d for children) is higher than it was estimated previously: 0.002 µg/kg bw/d for adults and 0.006 µg/kg bw/d for children).

3.3.2. Nutritional intakes

In general, the present estimations are very similar to those from TDS2. However, intakes of Ca, Cr, K and Na provided by chocolate are lower for both adults and children (factor ≈ 2 to 4 depending to the element) and for Mg and Mn (factor 1.5) for children only.

Table 2: Exposure assessment and nutritional intakes in adults and children

Mean daily exposure to toxic elements (µg/kg bw/d) as well as nutritional intake of essential elements (µg/d)*		
	Adults >17 yrs	Children (3-17)
Al	1.23	3.09
As	0.001	0.003
Cd	0.010	0.020
Sb	0,000	0,000
Pb	0.002	0.006
Ca*	5805	5805
Cr*	2.73	3.77
Cu*	38.4	53.2
Fe*	330	456
K*	21998	30451
Mg*	5245	7260
Mn*	46,0	63.7
Mo*	0.691	0.957
Na*	1277	1768
Se*	0.307	0.425
Zn*	78.2	108
B	0.310	0.780
Ba	0.220	0.550
Co	0.014	0.040
Ni	0.150	0.360
Sr	0.240	0.590
Ti	0.060	0.160
V	0.004	0.010

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