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HEALTH RISK APPRAISAL OF SELECTED HEAVY METALS IN SOME IMPORTED CHOCOLATES SOLD IN SOUTHWESTERN, NIGERIA

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ABSTRACT

Consumption of chocolate is common among Nigerian children who live in the cities. In spite of the palatability and nutritional significance of chocolate, it hardly exists without traces of heavy metal contamination. The study was carried out to evaluate the level of selected heavy metals in chocolate and their potential health impacts on children who consume the product on regular bases. Thirty (30) brands of chocolates including 26 milk and 4 dark chocolates were purchased and analyzed. Results showed that Ni ranged between 1.26 and 3.79 $mgkg^{-1}$ while Pb, Cr, Cu, Zn and Fe had concentration range of 0.10-0.47, 2.3-6.26, 2.39-18.62, 8.89-25.62 and 21.92-71.85 $mgkg^{-1}$ respectively. The total hazard quotient ranged between 0.06 – 0.25 for Ni while it ranged between 0.03-0.13, 0.03-0.09, 0.06-0.47, 0.0015-0.0042 and 0.029-0.10 for Pb, Zn, Cu, Cr and Fe respectively. The correlation analysis and metal distribution pattern showed that the proportion of cocoa solid in chocolate determined the level of copper and zinc in the products. The low values of calculated target hazard quotient suggests the safety of the investigated chocolates with respect to heavy metal contamination.

Keywords: *Heavy metal Contamination, Chocolates, Health implication, Southwest*

INTRODUCTION

Chocolate is a typically sweet, brown food preparation of *Theobroma cacao* seeds. The seeds are usually roasted and ground in form of a liquid paste or in block. Much of the chocolate consumed today is in form of sweet chocolate, a combination of cocoa seeds, cocoa butter or other fat and sugar. Milk chocolate is a sweet chocolate that additionally contains milk powder or condensed milk, while the white chocolate contains cocoa butter, sugar and milk but no cocoa solids (Miller et al. 2006).

Another type of chocolate which is not so popular among children is the dark chocolate. This contain 70-80% cocoa solid. It is loaded with nutrients that can positively affect human health. Dark chocolate contains more antioxidant activity polyphenols and flavanols than many fruits (Stephen et al. 2000). The flavanols in dark chocolate can stimulate the endothelium, the lining of arteries to produce nitric oxide which lowers resistance to blood flow and therefore reduces blood pressure and improves blood flow (Fisher et al. 2003); increases high density lipid (HDL) and lowers total low density lipid in men with elevated cholesterol (Baba et al. 2007); protects lipoproteins against oxidative damage (Rein et al. 2000) reduces insulin resistance- a common risk factor for many heart disease and diabetes (Grassi et al. 2005); reduces risk of cardiovascular death (Buijsse et al 2006); lowers the risk of calcified plaque in the arteries (Djousse et al. 2011); improves blood flow to the skin, increases skin density and hydration (Heinrich et al. 2006); increases blood flow to the brain (Francis et al. 2006); improves cognitive function in elderly people with mental impairment (Desideri et al. 2012). Though some industries produce chocolate in Nigeria, but greater proportion of chocolates in Nigerian markets are imported from different parts of the world.

In spite of the various health benefits and pleasure associated with the consumption of chocolates of cocoa origin, the issue of heavy metal contamination in the product has become a global concern. Before chocolate is ready for consumption, the product passes through diverse industrial processes which involve machinery and packaging (provides means of protection, marketing and safe handling). Some of the colored printing done on the wrappers for the purpose of enticement, beautification have metals origin. Heavy metals such as Pb, Cr, Ti, Zn and Cu can migrate from the printed surface to the product through blocking, rubbing, peeling and diffusion (Bradley et al. 2005). The consumption of chocolate is common among Nigerian children especially those in the cities. Contamination of imported food products with heavy metals may cause a serious risk for human health because of the physiological effects of heavy metals. Consumption of even small quantity of metals can lead to considerable bio-toxic effects. Though individual metal exhibit specific signs of toxicity, many illness like gastrointestinal disorders, diarrhea, stomatitis, depression, pneumonia and many other have been reported as general signs associated with Cd, Pb, As, Hg, Zn, Cu and Al consumption. In addition, young children are considered to be at greatest risk due to their ability to effectively absorb metals and thereby suffer physiological development retardation (Kocak et al. 2005).

Apart from natural source and production chain, heavy metal contamination in chocolate can arise from environments where these products are kept (Ashraf, 2006). With the exception of supermarkets, many of these products are not stored under safe hygienic conditions. They are often displayed in open trays or containers in the markets or hawked along the streets (Iwegbue, 2011). Contamination may arise from such unsafe storage conditions.

Data on metals concentration in foods at the point of consumption is necessary in order to estimate health risk associated with heavy metal contamination in chocolate. In Nigeria, data on heavy metal contamination in chocolate and potential health risk associated with long term consumption among children is limited. Hence, the study evaluates the level of selected heavy metals and potential health risk indices associated with consumption of common brands of chocolate in Ibadan, southwestern Nigeria.

MATERIALS AND METHODS

The sampling was carried out in June 2014. Thirty (30) chocolate samples containing 26 milk chocolate and 4 dark chocolates were purchased from selected supermarkets within Ibadan metropolis. Ibadan is the largest indigenous city in West Africa which is located in the south western part of Oyo state, Nigeria. Each of the samples was homogenized by blending in a stainless steel blender. After homogenization, 2g of each sample was weighed into a 50ml beaker followed by the addition of 20ml Nitric /Perchloric acids) (3:1 v/v) and digested for 2 hours at the temperature of 150^{o}C. After digestion, the digests were analyzed for Fe, Cu, Zn, Pb, Cr and Ni using Buck 210 VGP Atomic Absorption Spectrophotometer.

Quality control

For every metal analysis, samples were prepared in triplicates. All glass wares used in the course of the study were soaked in 10% HNO_3 for 18 hours and later rinsed with deionized water to prevent contamination from glass ware. The Atomic absorption spectrometer was re-calibrated after analyzing ten samples. Accuracy of the analytical procedure was determined by introducing known amount of heavy metal and re-analyzed. Spiked recovery were 98% for Pb, 97% for Fe, 99% for Cu, 98% for Zn, 97.5% for Cr and 98% for Ni. Blank solutions were prepared in order to make room for contamination from analytical reagents used. The detection and quantification limits (LOD and LOQ) were obtained on the basis of the concentration of the analyte that produced signal-to noise ratio of 3x standard deviation of low concentration/slope of the calibration line and 10x standard deviation of low concentration/slope of the calibration line respectively. The detection limits in mg kg^{-1} of the heavy metals were Ni (0.003), Pb (0.05), Zn (0.003), Cu (0.003), Cr (0.002) and Fe (0.001).

Estimated Daily Intake

In order to appraise the health risk associated with heavy metal contamination in the studied chocolates, estimated daily intake of metals was calculated using the formula:

$$EDI = \frac{C \times CR \times EF}{Bw} \quad \ldots\ldots (1)$$

Where

EDI = estimated daily intake: It is generally the number of milligrams of the contaminant that enters the body for each kilogram of body weight (mg/kg/day).

C = concentration of the contaminant in the exposure pathway being considered (mg/g) of food.

CR= contact rate; Amount of food taken per day (g/day) (chocolate of 20g per day was considered for each child in the study)

EF = Exposure Frequency: This number indicates how often the individual is exposed during a year and the number of years (365 days for 6 years was considered in the study)

Bw = Body weight (kg) (Since the study was focused on children, an average weight of 20kg was used)

Target Hazard Quotient

The health risk associated with heavy metals exposure through the consumption of chocolate was evaluated using the target hazard quotient (THQ) (Liu et al., 2006).

$$THQ = \frac{EF \times FD \times DIM}{RfD \times W \times T} \quad \ldots\ldots (2)$$

Where EF is the exposure frequency (365d/year), FD is the exposure duration (6 years), DIM is the daily metal ingestion (mg/person/day), RfD is the oral reference dose (mg/kg/day), W is the average body weight (20kg), T is the average exposure time for non carcinogen (365 days/year x number of exposure years). THQ is a highly conservative and relative index (Wang et al, 2005). If THQ is less than 1, there is no obvious risk from the substance over a lifetime exposure, while if THQ is higher than 1, the toxicant may produce an adverse effect. The higher the THQ value, the higher the probability of experiencing long term carcinogenic effects. (Song et al. 2009)

RESULTS

Results of metals in imported chocolate

Observation from the study shows that, milk chocolate predominates the population of chocolate within the city where the study was carried out. Among the chocolates obtained at random, dark chocolate was 13.33% of the whole population while milk chocolate was 86.70% of the population.

The concentration of lead (Pb) in the chocolate ranged between 0.11 and 0.47 $mgkg^{-1}$ with a mean value 0.23 $mgkg^{-1}$. There was however no detectable Pb in 10% of the samples analyzed. Results show that Pb concentration was higher in most of milk chocolate than dark chocolates.

Concentration of nickel in the chocolate ranged between 1.26 and 3.79 with an average value of 2.60 $mgkg^{-1}$. The concentration of zinc in the chocolate ranged between 8.80 and 25.62 with a mean value of 13.95 $mgkg^{-1}$. The concentration of Fe in the studied samples ranged from 21.92 to 71.85 with a mean value of 41.88 $mgkg^{-1}$ while Cu level in the analyzed chocolates ranged from 2.39 to 18.62 with an average value of 5.79 $mgkg^{-1}$. Results show that all the dark chocolate among the studied samples had much higher copper residue compared with milk chocolate. On the other hand, the level of chromium in the chocolates ranged between 2.30 and 6.26 with a mean value of 4.13 $mgkg^{-1}$.

Estimated daily intake

The calculated estimated daily intake of Pb in the studied samples ranged between 0.10 and 0.43 with a mean value of 0.22 µg kg bwd^{-1} (Figure 1). The calculated daily intake of Cr obtained from the consumption of 20g of studied chocolates ranged from 2.3 to 6.25 µg kg bwd^{-1} while the estimated daily intake of Cu in the studied chocolates ranged from 0.24 to 1.86 µg kg bwd^{-1}. The calculated daily intake of Fe from the daily consumption of 20g of the chocolates ranged between 20.35 and 71.85 with an average value of 39.21 µg kg bwd^{-1}. Estimated daily intake of Cu in the studied chocolates ranged from 0.24 to 1.86 with a mean value of 0.58 µg kg bwd^{-1}. Daily intake of Zn from a daily consumption of 20g of the selected chocolates ranged between 8.90 and 131.5 with a mean value of 19.41 µg kg bwd^{-1}. On the other hand, the calculated estimated daily intake of Ni in the samples ranged from 1.25 to 5.55 with an average value of 3.0 µg kg bwd^{-1}per 20g chocolate.

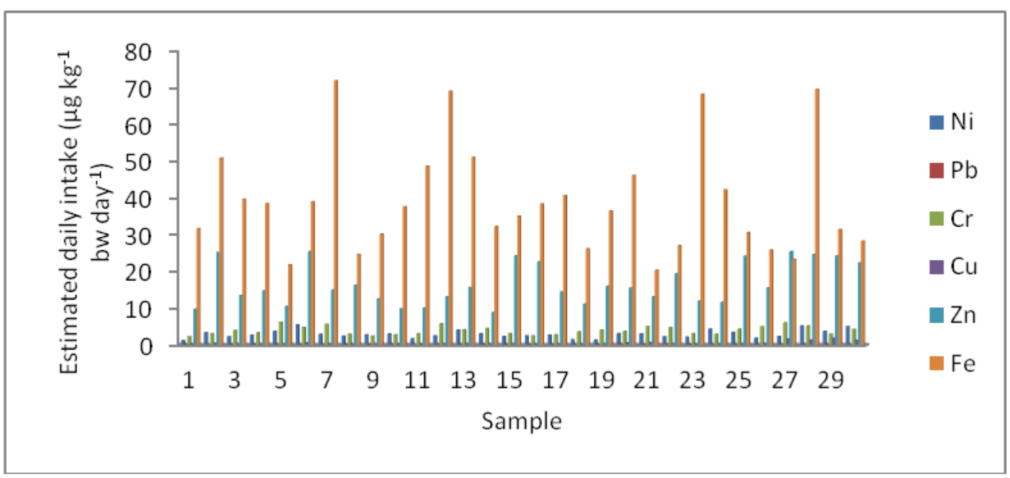

Figure 1: Estimated daily Intake of metals in chocolate brands

Table 1: Levels of heavy metals in various chocolate brands.

	Concentration of heavy metals in chocolates ($mgkg^{-1}$)					
	Ni	Pb	Cr	Cu	Zn	Fe
1(milk)	1.26 ±0.05	0.25±0.03	2.30±0.07	3.33±0.10	9.79±0.13	31.66±1.02
2(milk)	3.43±0.06	0.00±0.00	3.18±0.12	5.16±0.21	14.20±0.10	50.79±2.13
3(milk)	2.29±0.02	0.32±0.02	4.03±0.08	4.96±0.20	13.15±0.15	39.65±1.41
4(milk)	2.69±0.10	0.19±0.02	3.40±0.10	4.10±0.15	14.75±0.20	38.52±1.26
5(milk)	3.79±0.13	0.12±0.01	6.26±0.21	2.39±0.12	10.56±0.23	21.92±0.93
6(milk)	2.56±0.09	0.43±0.01	4.78±0.13	6.58±0.11	13.40±0.21	39.00±1.02
7(milk)	3.50±0.08	0.47±0.03	3.68±0.10	2.93±0.10	10.00±0.11	71.85±2.07
8(milk)	2.52±0.04	0.23±0.01	3.01±0.11	3.16±0.13	12.30±0.13	24.61±1.14
9(milk)	2.78±0.10	0.30±0.02	2.52±0.07	5.07±0.20	12.61±0.09	30.20±1.03
10(milk)	2.37±0.09	0.31±0.03	2.84±0.14	3.56±0.09	9.92±0.12	37.62±1.30
11(milk)	3.48±0.07	0.00±0.00	3.20±0.10	2.76±0.05	10.11±0.24	48.58±1.20
12(milk)	2.62±0.06	0.36±0.05	5.86±0.16	4.21±0.12	13.21±0.21	69.02±2.16
13(milk)	3.10±0.09	0.11±0.02	6.15±0.20	3.65±0.06	11.71±0.15	51.11±1.83
14(milk)	2.24±0.04	0.28±0.05	4.60±0.15	4.50±0.10	8.89±0.17	32.26±1.24
15(milk)	2.42±0.07	0.17±0.03	5.18±0.10	5.10±0.12	14.31±0.13	35.12±1.13
16(milk)	2.61±0.05	0.42±0.06	2.62±0.11	3.29±0.16	12.62±0.10	68.41±1.41
17(milk)	2.76±0.08	0.20±0.01	2.81±0.09	4.23±0.10	14.48±0.24	40.62±1.30
18(milk)	1.72±0.07	0.10±0.02	6.21±0.15	3.17±0.07	11.02±0.21	26.20±1.20
19(milk)	1.46±0.04	0.26±0.01	4.13±0.96	3.84±0.09	13.02±0.14	36.46±1.03
20(milk)	1.80±0.05	0.32±0.04	3.79±0.12	6.20±0.13	14.56±0.30	46.13±0.92
21(milk)	3.04±0.06	0.45±0.03	5.12±0.14	6.98±0.16	13.14±0.16	70.36±1.60
22(milk)	2.32±0.08	0.33±0.04	4.86±0.05	5.06±0.06	10.28±0.04	27.05±0.83
23(milk)	2.18±0.03	0.18±0.03	5.72±0.17	3.80±0.05	12.00±0.08	68.17±1.48
24(milk)	2.36±0.06	0.21±0.04	2.98±0.10	3.65±0.13	11.63±0.10	42.21±1.36
25(milk)	2.21±0.06	0.29±0.02	4.42±0.21	4.31±0.15	14.18±0.14	30.62±2.53
26(milk)	1.96±0.03	0.00±0.00	5.10±0.08	4.62±0.18	13.62±0.16	25.84±1.16
27(dark)	3.12±0.11	0.14±0.02	4.12±0.13	16.56±0.14	25.40±0.21	23.32±1.81
28(dark)	3.28±0.12	0.12±0.03	3.61±0.10	14.28±0.12	24.86±0.26	69.52±1.10
29(dark)	3.43±0.25	0.11±0.01	3.02±0.11	18.62±0.09	25.62±0.24	31.41±1.82
30(dark)	3.52±0.36	0.12±0.03	4.30±0.21	13.76±0.07	23.29±0.18	28.29±1.06
Mean	2.60	0.23	4.13	5.79	13.95	41.88
Min	1.26	0.00	2.3	2.39	8.89	21.92
max	3.79	0.47	6.26	18.62	25.62	71.85

Key: milk= milk chocolate; dark= dark chocolate

Target hazard quotient (THQ)

THQ though, does not provide quantitative estimate on the probability of an exposed population experiencing a reverse health effect, it offers indication of the risk level due to contaminant exposure. The THQ index can be defined as the ratio of determined dose of a pollutant to the reference dose (RfD) (µg/kg bw/d).The following were the values used for oral reference dose: Fe (0.700), Zn (0.300), Cu (0.040), Ni (0.02), Pb (0.0035) and Cr (1.5) (USEPA, 2010)

The calculated target hazard quotient of Ni for a child that consumes 20g chocolate every day of the week for six years (THQ_{365}) ranged between 0.06 and 0.26

The calculated Target hazard quotient of Pb ranged from 0.03 to 0.13 for a child that consumes 20g chocolate everyday of the week while the calculated total hazard quotient of Cr, Cu, Zn and Fe had range of 0.0015 – 0.0042, 0.06-0.47, 0.03 – 0.08 and 0.029-0.10 respectively for a child who consumes 20g of the chocolates on a daily basis for a period of six years.

Table 2: Calculated Total hazard Quotients of heavy metals in chocolates.

	Target Hazard Quotient 365 $_{days}$					
	Ni	Pb	Zn	Cu	Cr	Fe
1(milk)	0.06	0.07	0.03	0.08	0.0015	0.044
2(milk)	0.17	0.00	0.04	0.13	0.0021	0.071
3(milk)	0.11	0.09	0.04	0.12	0.0027	0.056
4(milk)	0.13	0.06	0.05	0.10	0.0023	0.054
5(milk)	0.26	0.03	0.04	0.06	0.0042	0.031
6(milk)	0.18	0.13	0.06	0.16	0.0032	0.055
7(milk)	0.25	0.05	0.05	0.07	0.0038	0.100
8(milk)	0.13	0.07	0.05	0.08	0.0020	0.034
9(milk)	0.14	0.09	0.04	0.13	0.0017	0.042
10(milk)	0.16	0.09	0.03	0.09	0.0019	0.053
11(milk)	0.09	0.00	0.03	0.07	0.0021	0.068
12(milk)	0.13	0.10	0.04	0.11	0.0039	0.097
13(milk)	0.21	0.03	0.05	0.09	0.0028	0.072
14(milk)	0.16	0.08	0.03	0.11	0.0031	0.045
15(milk)	0.12	0.05	0.06	0.13	0.0021	0.049
16(milk)	0.13	0.12	0.06	0.08	0.0018	0.054
17(milk)	0.14	0.06	0.05	0.11	0.0019	0.057
18(milk)	0.08	0.03	0.04	0.08	0.0025	0.037
19(milk)	0.07	0.08	0.05	0.10	0.0028	0.051
20(milk)	0.16	0.09	0.05	0.16	0.0025	0.065
21(milk)	0.15	0.12	0.04	0.17	0.0034	0.029
22(milk)	0.12	0.10	0.06	0.13	0.0033	0.038
23(milk)	0.11	0.05	0.04	0.10	0.0021	0.095
24(milk)	0.22	0.06	0.04	0.10	0.0020	0.059
25(milk)	0.18	0.08	0.05	0.11	0.0030	0.043
26(milk)	0.10	0.00	0.05	0.12	0.0034	0.036
27(dark)	0.12	0.04	0.08	0.41	0.0041	0.033
28(dark)	0.26	0.10	0.07	0.36	0.0035	0.097
29(dark)	0.19	0.13	0.09	0.47	0.0020	0.044
30(dark)	0.26	0.04	0.08	0.34	0.0029	0.040
Mean	0.15	0.07	0.05	0.15	0.0027	0.055
Min	0.06	0.03	0.03	0.06	0.0015	0.029
Max	0.26	0.13	0.09	0.47	0.0042	0.100

Key: milk= milk chocolate; dark= dark chocolate

Table 3: Correlation among heavy metals in chocolates

	Fe	Cu	Zn	Pb	Cr
Cu	0.259				
Zn	0.413	0.961**			
Pb	-0.073	-0.093	-0.179		
Cr	-0.374	0.175	0.163	0.196	
Ni	0.059	0.353	0.470	-0.458	0.703*

DISCUSSION

Nickel

The range of Ni concentration in the study was lower than the range reported by Dahiya et al.(2005) during the assessment of 69 different brands of chocolate and candies in Mumbai, India. Observations showed that, out of the 30 brands of chocolates, the dark chocolates were higher in nickel than several of the milk chocolates except a few milk chocolates (4) that had higher concentration of Ni than the dark chocolate. Similar observation of high nickel in dark chocolate was reported by Dahiya et al. (2005). Prakash et al. (2014) reported nickel concentration ranging between 0.77 and 5.29 $mgkg^{-1}$ in five brands of chocolate from Tinuchirappalli, India. However, the highest value recorded for nickel in their study was higher than the highest value obtained in this study. A good proportion of nickel in the chocolate was supplied by cocoa beans which is a major component of dark chocolate. Despite the possibility of cocoa solid being a source of nickel in chocolate, the major source of nickel contamination in chocolate results from the manufacturing process when hardening is done by hydrogenation of unsaturated fats using nickel as catalyst (Dahiya et al. 2005). Nickel is regarded to be a natural constituent of diet and its compounds are generally recognized as safe when used as ingredient in human food (IRIS, 2003). The range obtained in the study is similar to that reported by Ochu et al. (2012) from the assessment of the level of nickel in imported chocolate sold in Nigeria. This suggests that chocolates sold in various parts of Nigeria may have common production origin but different points of entry into Nigerian markets. Nickel is found in small quantities in many foodstuffs (0.001-0.01$mgkg^{-1}$) and in higher concentrations in grains, nuts, cocoa products and seeds (up to 0.8$mgkg^{-1}$) (National Food Agency of Denmark, 1995). Nickel is found as complex bound Ni^{2+} ions in diets (Codex, 1995). According to Codex (1995) nickel intake via foodstuff does not cause hazards for the majority of consumers. Food intake, gastric emptying and peristalsis movement of the intestine are the substantial significance for the bioavailability of nickel. The absorption of trace nickel ions released in the gastrointestinal tract may be 40 times higher than that of complex-bound nickel from foodstuff (Sunderman et al. 1989).

The estimated daily intake of nickel in the study falls within the World health organization tolerance daily intake for nickel (5$\mu gkg^{-1}bwday^{-1}$). Acceptable or tolerable daily intake is a measure of the amount of a specific substance originally applied in food or drinking water that can be ingested orally on a daily basis over a life time without an appreciable health risk (WHO, 1987). It is expressed usually in milligrams of the substance per kilogram of body weight per day. Despite the fact that the range of estimated daily intake obtained for nickel falls within the acceptable values, over-indulgence of children in relation to excessive chocolate consumption may lead to exceedance of the limit. The study focused on situation where a daily consumption of 20g of chocolate is made by a child who weighs 20kg. The range of estimated daily intake of nickel reported by Ochu et al. (2012) is similar to what was obtained in the study. On the other hand, Iwegbue (2011) reported a higher range of estimated daily intake of nickel (0.9-58.6 µg kg^{-1}bw day^{-1}) in some ready-to-eat foods consumed in Southern, Nigeria. In addition, much higher dietary Ni contributions ranging from 200 to 900 µg kg^{-1}bw day^{-1} have been reported (Clemente et al., 1980; Larsen et al. 2002), 240 – 3900 µg kg^{-1}bw day^{-1} (Krishnamurti and Puspha, 1991). According to Duran et al (2009), the dietary intake of Ni does not lead to any health risk in the general population but could be troublesome to some sensitized individuals. Nickel at trace amount may be beneficial as an activator of some enzyme systems (Under Wood, 1977). At higher levels, it accumulates in the lungs and may cause bronchial hemorrhage. Other symptoms of nickel toxicity include nausea, weakness, dizziness (Nielson, 1977).

The calculated target hazard quotients for nickel (Table 2) indicates that, a child who weighs 20kg and consumes 20g of chocolate everyday of the week for a period of six years is not likely to have any health concern as a result of low THQ calculated for nickel in all the 30 samples which was found to be less than 1. A hazard quotient is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected. If the target hazard quotient is calculated to be less than 1, then no adverse health effects are expected as a result of the exposure. However, if the calculated target hazard quotient (THQ) is greater than 1 then, there is a reason for health concern (Sharma and Agrawal, 2005). It must be noted that THQ is not a measure of risk (Chien et al. 2002; Wang et al. 2005 and Hague et al. 2008) but indicates a level of concern. In a related account, Iwagbue et al. (2013) also reported THQ for 15 ready –to –eat food in southern Nigeria where 11 samples had THQ values less than 1 while the rest 4 samples had THQ values higher than 1. It is however advisable for children who are regular consumers of chocolate to be moderate in eating the commodity.

Lead

Result shows that several of the milk chocolates had higher concentration of Pb than the dark chocolates. The range of Pb (0.23-0.47 $mgkg^{-1}$) found in the study was lower than the range (0.009 – 0.92 $mgkg^{-1}$) reported by Al-Mayaly (2014) for Pb in some kinds of candy imported from Turkey and China. Iwagbue et al. (2013) reported a range of 0.009 to 3.8$mgkg^{-1}$ Pb in some ready-to-eat foods in Nigeria. The reason given for high Pb content of the foods was as a result of the way and manner the foods are being handled by vendors who usually display them in trays without cover or hawked along the major roads where contamination with Pb and other metals from automobiles emission cannot be ruled out. The concentrations of Pb in the various brands of chocolates were below 1$mgkg^{-1}$ guideline value for Pb in foods. The Codex Alimentarius, Global foods Standards developed by FAO and WHO limits the Pb contents of cocoa powder or beans to 1mg of Pb per kilogram product. Based on the distribution of Pb metal in the examined chocolates, it was clearly evident that, the proportion of cocoa solid in chocolate does not solely determine the quantity of Pb that is present in the commodity. This suggests possible contributions from manufacturing and other ingredients that make up milk chocolate. Other authors had earlier suggested possible means of Pb contamination in chocolates and candies. Fuortes and Bauer, (2000) suggested the possibility of Pb leaching into the product from tainted wrappers. Pb can be introduced into the product during processing such as drying, storing and grinding. Processing or storage of products in Pb contaminated containers may also contribute to Pb transfer in chocolate. Due to cases of Pb contamination in foods, the US Food and Drug Administration (FDA) and State Health Department sometimes issued warnings about contamination of Pb in candy imported from Mexico (FDA, 2001). According to the report of Fuortes and Bauer, (2000), the Centre For Disease Control reported the case of six Californian children who suffered from Pb toxicity after eating candy imported from Mexico. The detection was made during routine blood Pb screening. It is generally assumed that Pb contamination cannot be avoided in cocoa products due to the fact that majority of cocoa beans used for the production of cocoa based products are grown in locations that still use leaded gasoline. This may not be totally correct as there are other means through which Pb transfer could occur. In a study conducted by Aikpokpodion et al. (2013), an attempt was made to evaluate the impact of post harvest handling and processing on Pb contamination in cocoa beans. Standard procedures were used in processing the beans as well as drying them in environment free of vehicular movement. In spite of all the necessary precautions adopted to prevent cross contamination, 73% of the total number of cocoa samples analyzed exceeded 1mg Pb kg^{-1} cocoa beans set as the maximum residue limit. Analysis of varieties of chocolate products from various countries completed by Swiss group in year 2002 showed that the Pb content of the items ranged from 0.0011 to 0.769mg Pb kg^{-1} chocolate which was below International standard limit (1mg kg^{-1}) (Mounicou et al., 2003). It has been reported that, 5 to 7 % of Pb contained in cocoa is bio-available to the body (Mounicou et al. 2002). However, this may not be the same for children who react more to Pb toxicity compared with adults. The study by Yanus et al. (2014) reported that more heavy metals entered a child's blood streams than an adult's due to lower body weight and higher digestive tract uptake. Results from certain studies have shown that Pb exposure can delay kids' intellectual development (Huang et al 2012).

Estimated daily intake of Pb (0.10 to 0.43 µg kg^{-1} day^{-1}) in the study (figure 1) was lower than the range reported by Iwegbue (2013) (0.1-4.3 µg kg^{-1} day^{-1}) from the study of heavy metals in selected food in southern Nigeria. The values of estimated daily intake of Pb obtained in the study were lower than the values (3.6 µg kg^{-1} day^{-1}) set by Joint Experts Committee on Food Additives as the provisional Tolerance Daily Intake (World Health Organisation, 2000). Observation from the study showed that milk chocolate presented higher values of estimated daily intake of Pb compared with dark chocolate.

The level of target hazard quotient for Pb obtained for the 30 brands of chocolate investigated in the study (0.03-0.13) shows there may not be any health concern for a child (20kg) who consumes 20g of the chocolate everyday of the week. However, children who are lovers of chocolate should not be encouraged to go into excessive consumption of the product considering the physiological implication of abnormal concentration of Pb in human system, with emphasis on young children. According to the report of Yanus et al (2014), young children risk exceeding recommended limits for Pb when consuming chocolate in excess. Yanus et al. (2014) assessing the human risk of trace metals in chocolate stated that although the Pb concentration found in a variety of global brands of chocolates, were below the USA standard of 1,000ngg^{-1} limit, the Pb concentration should still be considered a health concern.

Children who are big consumers of chocolate may be at risk of exceeding the daily limit of Pb due to their low body weight and higher digestive tract uptake. Children may be vulnerable to Pb exposure via chocolate consumption. One cube of dark chocolate can contain up to 20% of oral limit of Pb in the body. In addition, chocolate may not be the only source of heavy metals in their nutrition as other diets taken may also contain trace metals thereby increasing the risk of exceeding the daily limit. The risk does not apply to adults in the same degree because their digestive absorption of metals is poor (Oliver, 2014). Given the various observable considerations, it is advisable for children to eat milk or white chocolate in large quantity rather than eating dark chocolate which contain higher proportion of cocoa solid.

Copper

Copper at trace level is an essential metal and serves as anti-oxidant which helps the body to remove free radicals, prevent cell structure damage (Salama and Radwan, 2005). The level of copper in the 30 brands of chocolate examined ranged from 2.39 to 18.62 $mgkg^{-1}$. The higher values were recorded for dark chocolates while the lower values were recorded for milk chocolates. Result (Table 1) shows that the range of Cu concentration was similar to the range reported by Joo and Bett (1996). The level of copper obtained in dark chocolate in our study (13.76-18.62$mgkg^{-1}$) was higher than the level (4.12-6.34$mgkg^{-1}$) reported by (Falandysz and Kotecta, 1994) in dark chocolate in Malaysia.

Generally, cocoa solid is a major source of copper intake. Little wonder why its concentration in milk chocolate was significantly lower than Cu levels in dark chocolates with higher proportion of cocoa solid. Naturally, cacao as a crop has the ability to bio-accumulate copper in its tissue which makes the seed (cocoa beans) naturally high in copper content. (Olaofe, 1987) reported a mean concentration of copper residue (8.2 $mgkg^{-1}$) in 11 cocoa samples originating from Ondo State, Nigeria. In the same country, Aikpokpodion et al, (2013) reported mean values of 25.00, 26.00 and 18.00 mg Cu kg^{-1} for cocoa beans obtained from Ogun, Ondo and Cross River States, respectively. In a study carried out by Sager, (2012), copper concentration in cocoa and its products ranged from 3.47 – 31.60 $mgkg^{-1}$. The presence of considerable amount of copper in cocoa-based products is not peculiar to chocolate. In USA, chocolate drinks and chocolate cakes were found to range among the 20 top foods for copper (Sager, 2012). The total dietary copper intake by males and females was positively associated with the consumption of chocolate foods, which is the main source of Cu intake (Sager, 2012). Among chocolate foods, the dark chocolate made the highest contribution to the mean daily copper intake within a 3-day dietary record study, followed by chocolate pie and chocolate milk (Joo and Belts, 1996). Joo et al. (1995) reported a mean copper concentration of 8.8$mgkg^{-1}$ in dark chocolate while Dos Santos et al. (2005) reported copper concentrations ranging between 26.6 and 31.5mg kg^{-1}. These values were higher than copper level recorded in the present study.

Africa produces 73% of the world cocoa (World Cocoa Foundation, 2012). Hence, the issue of copper residue in chocolate cannot be discussed without a mention of the level of metals in cocoa beans. A comparison of copper content of cocoa beans obtained from south western Nigeria at different times as reported by Olaofe, (1987) (8.2$mgkg^{-1}$) and Aikpokpodion et al. (2013) (26.0$mgkg^{-1}$) suggests that copper concentration in cocoa beans increases with time. Between 1987 and 2013, there was a mean increase of 17.8mg Cu kg^{-1}cocoa beans obtained from Ondo State, Nigeria. The increase in copper concentration of the beans with time is mainly due to continuous use of copper-based fungicides by cocoa farmers. Most cocoa farmers in Nigeria and other cocoa producing countries in West Africa use copper-based fungicides to manage black pod disease infestation in the plantations. After application, greater portion of the fungicide goes to the soil while the rest is absorbed in cocoa tissue. As a result of the non biodegradability of copper in soil, it accumulates over time thereby increasing the concentration of copper in soil which ultimately leads to high copper content of cocoa beans via translocation and bioaccumulation. The report Lee and Low (1985) also support the contribution of cocoa solid to copper content of dark chocolate. In their study, they observed that, the level of copper in chocolate increased with the proportion of cocoa solids in the final product. A range of 13.78 - 18.28mgkg^{-1} Cu was recorded for dark chocolate while a range of 2.54-2.87mgkg^{-1} was recorded for milk chocolate. The conclusion was that, dilution by the addition of other ingredients in place of cocoa mass was responsible for the decrease in copper content of milk chocolate.

Among the 30 brands of chocolates examined in the study, only the dark chocolates exceeded 10mgkg^{-1} limit set for Cu in foods (European Commission, 2006). This suggests that excessive consumption of dark chocolate by children may expose them to copper toxicity. The milk chocolates on the other hand had copper concentration below the European limit.

The estimated daily intake of copper in the study ranged between 0.24 and 1.86 µgkg^{-1} bw day^{-1} with a mean value of 0.58 µgkg^{-1} bw day^{-1}. The Joint Expert Committee on Food Additives (JECFA) set Provisional Maximal tolerable daily intake (PMTDI) of Cu at 500 µgkg^{-1} bw day^{-1} while the safe upper level recommended by EVM (2003) is 160 µgkg^{-1} bw day^{-1}. The mean estimated daily intake of Cu obtained in the study was 0.17% and 0.36% of PMTDI and safe upper limit respectively.

Target hazard quotient (Table 2) calculated for copper in the study ranged from 0.06 to 0.47. This suggests that consumption of 20g chocolate on a daily basis may not pose health threat to chocolate consumers since the obtained values are much lower than 1.

Chromium

Chromium is a mineral that humans require in trace amount although its mechanisms of action in the body and the amounts needed for optimal health are not well defined. It is found primarily in two forms : first, trivalent Cr^{3+} which is biologically active and found in food. The second form is hexavalent (Cr^{6+}) - a toxic form that results from industrial pollution. Chromium is known to enhance the action of insulin (Mertz, 1993) a hormone critical to the metabolism and storage of carbohydrate, fat and protein in the body (Porte et al. 2003). It has been estimated that humans require mainly 1 µg day^{-1}. The concentration range of chromium in the study (2.3 – 6.26 mgkg^{-1}) was lower than the range (0.8 – 21.4mg kg^{-1}) reported by Iwagbue et al.(2013) in selected ready-to-eat foods from Southern Nigeria. Chromium was found higher in milk chocolate than dark chocolate which suggests that various ingredients added to milk chocolate introduced a measure of chromium metal into the product. Milk chocolate is usually made from 27-30% cocoa solid by weight, 12% milk solids, cocoa butter, sugar, lecithin, vanilla, fruits and nuts while dark chocolate is made of 47-85% cocoa solid, cocoa butter, sugar, lecithin and any other flavor. Iwagbue (2011) reported Cr content of some chocolates and candies in Nigeria ranging from 0.04 to 3.0 mgkg^{-1}. The main form of Cr found in food is the trivalent form (Anderson, 1994). In order to prevent deficiency of Cr in human, the committee on medical aspect of food policy (COMA) recommended that Cr should be above 0.1 and 1 µg kg^{-1} bw d^{-1} for children and adolescent respectively (MAFF, 1999).

The EVM guidance value for trivalent Cr is 150µg kg bw d^{-1} (EVM, 2003). The range of estimated daily intake of Cr in the study (2.3 – 6.25 µg kg^{-1}bwday^{-1}) was lower than the range (1.1 – 28.4 µg kg $^{-1}$bw day^{-1}) reported by Iwagbue (2013) in some foods in Nigeria. The calculated daily intake of Cr in all the samples considered in the study was found to be between 1.53 and 4.17% of the EVM Guidance level. This is an indication that chocolate sold in Ibadan city, Nigeria, are safe with regard to chromium contamination in chocolates.

The low values of target hazard quotient obtained for Cr in the investigated chocolates (Table 2) is an indication that a child who has a daily consumption of 20g of any of the imported chocolate examined in the present study is not likely to have any health concern as a result of Cr toxicity. This is due to the fact that the THQ values obtained in the study ranged between 0.15 and 0.42%

Zinc

Zinc is an essential mineral that stimulates the activity of about 100 enzymes in the body. It also supports human healthy immune system. It is necessary for the synthesis of DNA, essential for wound healing, supports healthy growth and development of the body during adolescence, childhood and pregnancy. Though the actual amount of zinc necessary to support human body is quite small, its effects on the body are astronomical (www.fitday.com/fitness-articles/nutrition/vitamins). The mean (13.95mg kg^{-1}) concentration of Zn in the studied chocolates was lower than the mean value of Zn reported by Sager (2012) in dark chocolates but higher than value (12mg kg^{-1}) reported for milk chocolate. The level of Zn in the study (8.89 – 25.62 mgkg^{-1}) was similar to Zn level (8.90 – 20.00 mgkg^{-1}) observed by Iwagbue et al. (2013) in some ready-to-eat foods in Nigeria. The pattern of zinc distribution within the 30 chocolate samples analyzed followed that of copper. All the dark chocolate were higher in zinc than the milk chocolate which shows that, cocoa solids are rich in zinc and its proportion in chocolate could determine the level of zinc in the product. According to Edward group (2015), the occasional indulgence in a square of dark chocolate may offer boost to human zinc level. Soylak et al. (2005) reported mean concentration of Zn ranging from 6.8 to 20.4 mgkg^{-1} in appetizers and snacks. Salaman and Radwan (2005) reported Zn in the range of 2.35 – 13.70 mgkg^{-1} in cereals, Saracoglu et al. (2004) reported Zn levels in the range of 3.1 and 16.1mgkg^{-1} in biscuits in Turkey while Gopalani et al. (2007) reported Zn level in Indian biscuit ranging from nd-13.4 mgkg^{-1}. The values of Zn obtained from all the studied chocolates were below the guidance value of 150 mg kg^{-1} set for Zn in foods. The Joint Expert Committee on Food Additive (JECFA) Provisional Maximal tolerable daily Intake (PMTDI) for Zn is 1000µhkg^{-1} bw day^{-1} (WHO. 1982). The expert group on vitamins and minerals (EVM) safe upper level for Zn per day is equivalent to 700µgkg^{-1} bwday^{-1} in 60kg adult and 233.33µgkg^{-1}bwday^{-1} in 20kg child for a total dietary intake (EVM, 2003), the range of estimated daily intake in the study (8.90-131.50 µgkg^{-1}bwday^{-1}) was found to be between 3.8 and 56.4% of the recommended upper safe level. The findings show that the daily recommended intake of Zn cannot be attained by the consumption of 20g of chocolate for a child that weighs 20kg. This is in consonance with the report of Sager (2012) whose study on trace element in chocolate concluded that the recommended daily intake of 150 mg kg^{-1} Zn in diet can be hardly reached. In table 2, the estimated target hazard quotient (0.03 -0.08) ranged between 3 and 8% of the safe THQ value. This indicates that the daily consumption of 20g chocolate by a child of average weight of 20kg is not likely to pose health threat on children who eat chocolate on daily basis.

Iron (Fe)

Iron is essential for the proper growth and development of the human body. It helps metabolize proteins and plays a role in the production of hemoglobin and blood cells. Iron deficiency can lead to conditions like anemia, chronic anemia, cough and pre-dialysis anemia. The health benefits of Fe include the eradication of different causes of fatigue. Fe also plays a key role in strengthening the immune system by making it strong enough to fight off infections. Fe treats insomnia and regulates body temperature (www.newsmax.com/tlnewsmax/article/371069).
Among the various metals considered in the study, Fe is the most abundant. It ranged between 21.92 and 71.85mgkg^{-1} with an average value of 41.88 mgkg^{-1}. The range of Fe obtained in the study was higher than concentration range (1.7 – 12.3mgkg^{-1}) reported by Iwagbue (2011) but lower than concentration range of Fe (nd – 102mgkg^{-1}) in Chocolate and candies reported by Ochu et al. (2012). The range was also lower than the level (20.1 – 167mgkg^{-1}) obtained in selected chocolate in Austria as reported by Sager, (2012). Result show that the concentration of Fe in many of the milk chocolate was higher than Fe content of some dark chocolate. On the other hand, some dark chocolates had higher Fe content than some milk chocolates. The high Fe content of the milk chocolate may be due to Fe content of the ingredients used. In a related but different report, Pennington and Young (1990) reported that US milk chocolate contained 8 times less Fe than dark chocolate. Among nutritional food intakes in Poland, chocolate contained significant higher Fe (25mgkg^{-1}) than milk, honey and eggs (Falandysz and Kotecta, 1994). This implies that, chocolate is a good source of mineral iron that the body requires.

Iron is mainly a deficiency problem and not a toxicological problem and it is generally acknowledged to be the most common single nutritional deficiency in both developing and developed countries (Nordic Council of ministers, 1995). Under normal conditions, about 5-15% of Fe in food is absorbed (Elinder, 1986). Iron salts like ferrous sulphate and ferrous succinate are commonly used for the treatment and prevention of iron deficiency in humans (Beliles, 1994).

The estimated daily intake of Fe from the consumption of 20g of chocolate per day in the study (20.35-71.85 µgkg^{-1}bwday^{-1}) is similar to the range (10.8 – 71.80 µgkg^{-1}bw day^{-1}) reported by Iwegbue et al.(2013). In table 2, the calculated estimated daily intake was found to be in the range of 4.07 and 7.8% of the lower limit (10mg day^{-1}) of the recommended dietary allowance of iron (Demirezen and Uruc, 2006). This implies that, the consumption of chocolate on a daily basis is a good source of nutritional iron and not likely to cause health concern.

Correlation analysis (Table 3) showed that copper and zinc had significant (P = 0.1) positive relationship. This indicates that, the proportion of cocoa solids in the investigated chocolates was mainly the determining factor for copper and zinc levels in the products. Chromium and nickel also had significant (P = 0.05) positive correlation which indicates similar sources of the metals in chocolates.

CONCLUSION

The study revealed that the concentration of the metals considered in the study were within the acceptable limits except for copper in dark chocolates. The study showed that, daily consumption of 20g of any of the investigated chocolates cannot supply the required daily amount of Cr, Zn and Fe needed by the body. Hence, other food sources will be required to supply the nutritional deficit. Quantity of cocoa solids in chocolate determined the level of copper and zinc in the commodity.

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