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Simultaneous Determination of Nine Elements and Statistical Comparison of Elemental Contamination in Cocoa-Chocolate Based, Milk Based and Fruit Flavored Candies

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Abstract

Candies pronunciation is just enough for arousing the need of tasting it, very especially to the children. As children are the most sensitive group of population, presence of any kind of toxicants in their frequently consumed food products can cause serious health problems. In to the view of this, an approach has been made to estimate the quantity of nine trace toxic elements in three different types of commonly available candies (67 samples) i.e. chocolate based, milk based, and fruit flavored candies. The trace elements were found relatively high in chocolate based candies followed by milk based and fruit flavored candies. The present study enlightens the international food safety authorities to implement strict permissible limits of trace elements in candies. The statistical approach of multiple discriminant analysis model was also used in this study to exhibit the inter-comparison of elemental contamination in similar type of candy samples which in turns proves the non-uniformity and improper manufacturing and processing of candies especially milk based candies.

Keywords: Trace Elements; Toxic Heavy Elements; Candies; Atomic Absorption Spectroscopy; Toxicological Finding and Statistical Comparative Study

Significance

The presence of toxic heavy elements and excessive amount of trace elements in different type of candies become the reason of worldwide concern. Chocolates and candies are usually consumed by the people of all age group, while their consumption by the most sensitive group of population i.e. children and pregnant women, makes this matter even more serious in order to protect public health. Frequent consumption of element contaminated candies could cause chronic toxicity and severe health problems. Keeping this concern in mind, the present study focused on examining the presence of extremely toxic heavy elements i.e. Cadmium (Cd), Lead (Pb) and essential trace elements i.e. Cobalt (Co), Copper (Cu), Chromium (Cr), Iron (Fe), Manganese (Mn), Nickel (Ni), and Zinc (Zn) in different variety of candies which are easily available in the local markets of India. This study provide the pioneer data for inter-comparative analysis and contamination of these elements in three types of candy samples i.e. Cocoa-chocolate based, milk based and fruit flavored candies. Till now, the specific permissible limits for heavy elements and trace elements in frequently consumed candies have not been set by any international food regulatory organization/agencies. Turkish standards have defined the permissible limits for Pb and Cd, specifically in chewing gum and candies. The finding of this study specifically reflects the importance of quality standards in preparations, storage, packaging and transportation of candies. While there have not been limits established for metals in candies yet, there is an ongoing discussion at Codex for establishing limits for both Cadmium and Lead in different type of candies especially cocoa chocolate based candies. It may be worthwhile to highlight this study, being an occurrence data, such as presented in this research paper could be provided help with this process of setting up quality guidelines and permissible limits by International Food Safety and Health Agencies. Hence, such studies as the present study are of great significance to generate data for setting up specific rules and regulations for commonly found elements in different variety of candies and bars.

Introduction

Toxic level contamination of essential trace elements and presence of extremely toxic heavy elements in food products has emerged as a major concern. Essential trace elements are certainly very important for the proper functioning of human body; however, in excess they can produce adverse health conditions. (Duran et al., 2009) Human body needs trace elements only in minute level amounting mg/day, microgram or less than microgram/gram of body weight while mere presence of few heavy elements such as Lead (Pb), Cadmium (Cd) can source severe health issues. Human body can only tolerate an extremely low exposure to these heavy elements.

Generally, humans are exposed to these elements through ingestion (drinking or eating), inhalation (breathing, air) and absorption through the skin. Toxicity of heavy elements is generally classified as Acute Toxicity; sudden high exposure or exposure of about 14 days or less, Intermediate Toxicity; due to the exposure of about 15 to 354 days; and Chronic Toxicity; due to the gradual exposure of more than 365 days. Chronic exposure may result from contaminated food, air, water, dust, by living near a hazardous waste site and maternal transfer in the womb. Symptoms of chronic toxicity are often similar to many common pathological conditions and may not be readily renowned. As such the heavy elements are stable and accumulative in the body tissues means they cannot be metabolized by the body. If the trace elements and heavy elements enter and accumulate in body tissues much faster than their disposal through body detoxification pathways, a gradual build up of these toxins will occur. Their continuous exposure is not necessary to produce a state of toxicity in the body tissue but, in due course, can achieve toxic concentration levels. (Khalid, R. A., Gambrell, R. A., Patrick, W. H. (1978) USDOE Symposium Series) (Hakanson, 1984) (Prusty, 1994)

It does not matter how good health supplements, one takes, excess of elements in the body will damage the natural healing function of the body. Therefore, the quantitative analysis of these elements in various kinds of food products; is very necessary, especially in those food items which are frequently consumed by children and pregnant women. (Duran et al., 2009) Unquestionably, children are the well known consumers of chocolate and candies. Pregnant women are also like to consume specific types of candies to get relief from nauseated feeling. As children and pregnant women, both are the most vulnerable group to any kind of contamination in food products, therefore, the unchecked elemental level in candies can cause serious health problems to them. Thus, there is a high need of determining the content of essential trace elements and toxic heavy elements in different type of candies. Previously, several researchers have reported the study of leaching and contamination of heavy elements in the candies due to element based ink dye of candy packaging. (Duran et al., 2009) (Dahiya et al., 2005) (Kim et al., 2008) These studies have suggested the contamination of candy sample with heavy elements such as Lead (Pb), Cadmium (Cd) due to packaging, processing and transporting. High level of some trace elements in candy samples marketed in Mexico city were also reported by Martinez et al (2010) (Martinez et al., 2010). Despite being the matter of high importance, very fewer reports are published concerning this issue.

Thus, in this study an attempt have been made to analyse the composition of frequently found heavy elements i.e. Lead (Pb), Cadmium (Cd) and common trace elements i.e. Cobalt (Co), Copper (Cu), Chromium (Cr), Nickel (Ni), Iron (Fe), Manganese (Mn) and Zinc (Zn) in different variety of candies i.e. Cocoa-chocolate based, milk based and fruit flavoured candies. Changes in the composition of nine elements have also been studied statistically using multiple discriminant analysis model to reverse classify the type of candy samples based on their inter-related profiling of nine elements. In the present study, sixty seven (67 Nos.) candy samples including cocoa-chocolate based (20 Nos.), milk based (17 Nos.) and fruit flavoured (30 Nos.) candies which were available in local markets of Lucknow city have been quantitatively analysed for the presence of nine elements. This is the first study to report the composition of nine elements and classification of different type of candies based on the profiling of elemental content.

Statistical Analysis

The variations among individual element content in all candy samples have been tested by one way analysis of variance (ANOVA). Data has been summarised in Mean±SD. Individual comparison has been done by Newman Keuls test. Inter-correlation of elements in each type of candy was also performed. Multiple Discriminant Analysis model was used to distinguish the candies based on the quantitative profiling of these nine elements.

Materials and Methods

Sixty seven candies of three different varieties, cocoa chocolate based, milk based and fruit flavoured candies were procured from the local city market of Lucknow, Uttar Pradesh, India. The samples were stored at 4°C until processed. Standard reference material solutions of all nine elements i.e. Cobalt chloride for Cobalt (Co), Copper sulphate for Copper (Cu), Sodium dichromate for Chromium (Cr), Nickel sulphate for Nickel (Ni), Ferrous ammonium sulphate for Iron (Fe), Manganese sulphate for Manganese (Mn), Zinc sulphate for Zinc (Zn), Cadmium nitrate for Cadmium (Cd) and Lead acetate for Lead (Pb) were purchased from Merck India Pvt Ltd. Ultra pure water was manufactured in-house with a water purification system (Milli–Q synthesis Elix-10, Millipore Corp., Mass., U.S.A.). Quantitative determination of all nine elements in each digested candy samples were performed in triplicates by using Atomic Absorption Spectrophotometer (AAS) model GBC (Avanta PM). All the parameters of AAS for the elemental measurement are shown in (Table 1).

Element	Wavelength (nm)	Slit (nm)	Sensitivity (µg/mL)
Iron	248.3	0.2	0.05
Copper	324.7	0.5	0.025
Cobalt	240.7	0.2	0.008
Chromium	357.9	0.2	0.05
Cadmium	228.0	0.5	0.009
Lead	217.0	1.0	0.06
Nickel	232.0	0.2	0.04
Manganese	279.5	0.2	0.02
Zinc	213.9	0.3	0.008

Table 1: AAS parameters for elemental measurements.

Sample preparation

Accurately weighed 1.0 gram of candy sample in a conical flask and subjected to acid digestion with HNO3: H2O2 (3:1) on a hot plate at 60°C. The samples were evaporated slowly to dryness and left undisturbed to allow cooling to room temperature. The digested samples were then dissolved in 1% nitric acid and filtered through whatman filter paper no. 1 and volume was made up to 10 mL in a 10 mL of volumetric flask. Each sample was processed in triplicate. A blank was also processed along with each sample batch [1-10].

Results

In the present study, 07 essential trace elements i.e. Iron (Fe), Copper (Cu), Cobalt (Co), Chromium (Cr), Nickel (Ni), Manganese (Mn), Zinc (Zn) and 02 toxic heavy elements i.e. Cadmium (Cd), Lead (Pb) have been quantitatively analysed in three different varieties of 67 candy samples (20 cocoa chocolate based, 17 milk based and 30 fruit flavoured). The range, standard deviation and average content of all nine elements in each Cocoa-chocolate based, Milk based and Fruit flavored candy samples have been depicted in (Table 2).

Trace	Cocoa-chocolate based candy			Milk based candy			Fruit flavored candy		
Metals	Range	Mean	Std. Dev.	Range	Mean	Std. Dev.	Range	Mean	Std. Dev.
Cr	0.427-3.122	1.129	0.707	0.148-1.799	0.786	0.519	0.213-2.512	0.865	0.519
Pb	0.167-5.124	1.225	1.343	0.241-3.471	1.12	0.911	0.187-2.492	0.744	0.637
Mn	0.046-0.410	0.153	0.082	0.138-0.875	0.337	0.218	0.049-1.489	0.399	0.329
Fe	1.218-10.890	5.768	2.712	0.982-8.691	4.253	1.975	0.925-5.168	2.438	1.074
Ni	0.177-4.256	1.348	1.055	0.065-3.561	1.083	1.005	0.079-1.021	0.306	0.21
Zn	0.277-2.263	0.828	0.623	0.188-1.565	0.802	0.412	0.033-0.401	0.204	0.083
Cd	0.000-1.256	0.305	0.328	0.000-0.095	0.039	0.03	0.000-0.052	0.01	0.015
Co	0.019-0.119	0.062	0.027	0.008-0.158	0.102	0.04	0.021-0.360	0.126	0.083
Cu	0.095-0.550	0.236	0.12	0.148-0.618	0.3	0.133	0.069-0.580	0.263	0.125

Table 2: The range and mean level (µg/g) of all trace metals in each variety of candy samples.

Comparison of average elemental levels among all candy samples were tested by applying one way ANOVA. On comparing, it was estimated that, out of 09 analysed elements, 03 elements i.e. Cr, Pb and Cu were found to be almost equally distributed (P>0.05) among three different variety of candy samples. The remaining 06 elements have showed a significant F ratio which means that the levels of these 06 elements i.e. Mn, Fe, Ni, Zn, Cd and Co are significantly varying among three unlike groups of candy samples as presented in (Table 3).

Trace Metals									
Candy Groups	Mn	Fe	Ni	Zn	Cd	Co	Cr	Cu	Pb
Choc vs Milk	*	*	NS	NS	**	*	NS	NS	NS
Choc vs Fruit	**	**	**	**	**	**	NS	NS	NS
Milk vs Fruit	NS	**	**	**	NS	NS	NS	NS	NS

Table 3: Comparison of mean level of trace metals in candy samples.

Iron (Fe) has been detected at highest level whereas Cobalt (Co) and Manganese (Mn) were found in lowest quantity in each variety of candy samples. It may be mentioned that Fe was found at significantly higher level (P<0.05) in cocoa-chocolate based candies as compared to cocoa-chocolate and milk based candy samples. Manganese (Mn) was detected at considerably lower level in cocoa-chocolate based candies than milk based and fruit flavoured candies. Fruit flavoured candies have shown significantly (P<0.01) low content of Iron (Fe), Zinc (Zn) and Nickel (Ni).

Next to Iron (Fe), cocoa chocolate based candies were found to be contaminated with Nickel (Ni) at second highest level. However, milk based and fruit flavoured candies were found to contain Lead (Pb) and Chromium (Cr) at second highest level respectively. Besides, Cadmium (Cd) was detected in least quantity in milk based and fruit flavoured candies while in cocoa-chocolate based candies, Cadmium (Cd) was measured at significantly high level which raised the concern toward consuming cocoa chocolate based candies. Copper (Cu) was found at relatively high level in milk based candies than fruit flavoured candies and cocoa-chocolate based candies. Iron (Fe), Nickel (Ni), Zinc (Zn), Chromium (Cr), Lead (Pb) and Cadmium (Cd) were measured at highest average content in cocoa-chocolate based followed by milk based and then fruit flavoured candies. Overall it can be assessed that the majority of elements were found at higher levels in cocoa-chocolate based candies than the milk based and fruit flavored candies as also depicted in (Table 2).

Moreover, a relatively high coefficient of variation in between the quantity of each element among different candy groups as depicted in (Table 4), indicates that few candy samples in each variety have high amount of an individual element than others which in turn proving the heterogeneity of the analysed samples. Based on the heterogeneous distribution of these elements, a statistical classification study i.e. multiple discriminant analysis could be helpful in measuring the level of uniformity of elemental distribution in dissimilar candy groups.

COCOA CHOCOLATE BASED

Correlations (Spreadsheet1 26-6-12.sta)

Exclude cases: 21:67

	Cr	Pb	Mn	Fe	Ni	Zn	Cd	Co	Cu
Cr	1	-0.03	0.259	0.089	0.033	0.033	-0.01	0.111	0.454
Pb	-0.03	1	-0.09	-0.03	-0.01	0.105	-0.19	-0	-0.17
Mn	0.259	-0.09	1	0.766	0.438	0.203	0.091	0.488	0.133
Fe	0.089	-0.03	0.766	1	0.024	0.28	0.099	0.269	0.11
Ni	0.033	-0.01	0.438	0.024	1	-0.02	-0.03	0.416	-0.09
Zn	0.033	0.105	0.203	0.28	-0.02	1	-0.21	0.704	0.106
Cd	-0.01	-0.19	0.091	0.099	-0.03	-0.21	1	0.083	-0.21
Co	0.111	-0	0.488	0.269	0.416	0.704	0.083	1	0.232
Cu	0.454	-0.17	0.133	0.11	-0.09	0.106	-0.21	0.232	1

MILK BASED

Correlations (Spreadsheet1 26-6-12.sta)

Exclude cases: 1:20,38:67

	Cr	Pb	Mn	Fe	Ni	Zn	Cd	Co	Cu
Cr	1	-0.17	-0.06	0.494	0.206	-0.16	-0.03	0.47	0.22
Pb	-0.17	1	-0.18	0.038	-0.22	0.03	-0.13	0.08	0.14
Mn	-0.06	-0.18	1	0.21	0.229	0.621	0.379	0.3	0.33
Fe	0.494	0.038	0.21	1	0.117	-0.14	-0.22	0.39	0.22
Ni	0.206	-0.22	0.229	0.117	1	0.632	0.086	0.31	0.61
Zn	-0.16	0.03	0.621	-0.14	0.632	1	0.24	0.24	0.61
Cd	-0.03	-0.13	0.379	-0.22	0.086	0.24	1	0.08	0.26
Co	0.47	0.078	0.304	0.391	0.31	0.243	0.075	1	0.23
Cu	0.22	0.139	0.334	0.217	0.606	0.61	0.263	0.23	1

FRUIT FLAVOURED

Correlations (Spreadsheet1 26-6-12.sta)

Exclude cases: 1:37

	Cr	Pb	Mn	Fe	Ni	Zn	Cd	Co	Cu
Cr	1	0.065	0.423	0.147	0.248	0.01	-0.089	0.111	0.093
Pb	0.065	1	-0.153	0.062	-0.205	0.057	-0.098	-0.173	-0.18
Mn	0.423	-0.153	1	-0.378	-0.059	0.317	0.168	0.112	-0.135
Fe	0.147	0.062	-0.378	1	0.163	-0.311	-0.001	-0.127	0.377
Ni	0.248	-0.205	-0.059	0.163	1	0.118	-0.184	0.129	0.29
Zn	0.01	0.057	0.317	-0.311	0.118	1	0.04	-0.075	-0.098
Cd	-0.089	-0.098	0.168	-0.001	-0.184	0.04	1	-0.006	0.153
Co	0.111	-0.173	0.112	-0.127	0.129	-0.075	-0.006	1	-0.012
Cu	0.093	-0.18	-0.135	0.377	0.29	-0.098	0.153	-0.012	1

Highlighted values indicating high coefficient of variation among individual metals in a single type of candy

Table 4: Variations between individual metal content in similar type of candies.

The literature survey demonstrated that the application of multiple discriminant analysis model have not been explored yet to assess the reverse classification of different type of candies i.e. cocoa-chocolate based, milk based and fruit flavored candy. Applying multiple discriminant analysis on complete candy sample data, fruit flavored candies have shown 100% correct reverse classification which indicates that the nine analysed elements were distributed uniformly among all samples of fruit flavored candies. On the other hand, chocolate based candies have also shown 95% correct classification indicating non-uniform distribution of elements among all samples of chocolate based candies. Conversely, the reverse classification for milk based candies was measured fairly low (64.7%) which proposed that the level of same element could vary significantly in each sample of milk based candies. The significantly varying contamination of candy samples with these 09 elements also indicates the extensive irregularities in candy manufacturing processes, type of packaging, storage and transportation. Thus, a high level and well planned research study is required to confirm the reason of contamination at various steps of candy manufacturing processing, packaging and transporting [11-18].

Discussion

Some elements i.e. trace elements are considered as essential for proper growth and functioning of human body while some are measured as toxic. Fe, Cr, Mn, Co, Cu and Zn are the trace elements, considered as essential nutrients for the normal health functioning and development which acquired less than 0.01% of dry weight of the organism (FAO, 1995) (Taylor, 1996).

On the other hand, heavy elements such as cadmium (Cd) and lead (Pb) are major toxicants where lead is classified as 2B carcinogen by the International Agency for Research on Cancer (IARC). Pb and Cd are also known for their toxicity and other adverse health effects on human body (Goyer, 1991). Gradual accumulation of Pb in body may induce chronic health problems i.e. anaemia, organ damage and neurotoxicity i.e. brain damage, psychological disorders, loss of memory, reduce IQ level (USEPA, 1984). And progressive accumulation of Cd can cause softening of bones, kidney dysfunction, skeletal damage and reproductive deficiencies (USEPA, 1984). Effect of Pb on human body has been extensively studied and Pb is well researched for its antagonistic property towards the central nervous system. Besides occupational, industrial and environmental, the main source of human exposure to these trace elements and heavy elements are consuming contaminated food products. Element composition of various food products poses a major concern towards public health because in excess they can cause numerous severe health issues to humans.

Taking these facts in consideration, international food safety authority and world health organisation have established the provisional tolerable weekly intake (PTWI) for Pb and Cd through food items at 25µg/kg and 7µg/kg body weight (FAO/WHO, 1993) (FAO/WHO, 1989). For a child with body weight 20kg, the PTWI for Pb and Cd would be 500 and 140 µg/week. Besides, the Turkish standards have set the limits for Pb between 1.0 and 2.0 mg/kg in chewing gum and candies respectively (Turkish standards, 1993) (Turkish standards, 1996). As per Anonymous, maximum permitted limit for Pb in cocoa candies is 1.0 mg/kg (Anonymous, 2002). Also by Polish national standard, the maximum permitted levels for Pb and Cd have been decided at 0.30 mg/kg and 0.05 mg/kg in chocolates (FAO/WHO, 2001).

In the present study Pb and Cd were measured at the shocking level of 0.167 – 5.124 µg/g and 0.000 – 1.256 µg/g respectively. Earlier studies have also reported Pb and Cd content in different varieties of candies in the range of 0.049–8.04µg/g, 0.031–2.460 µg/g and 0.001–2.730 µg/g, 0.027–0.825µg/g correspondingly (Dahiya et al., 2005) (Ali et al., 2009). In the year of 2010, Martinez et al have also analysed few candy samples marketed in Mexico and reported the level of Pb ranging from 0.102 to 0.342 µg/g (Martinez et al., 2010). In few analysed candy samples, Pb content was observed more than the permissible level defined for Pb in candies by Turkish standards, 1996. Surprisingly, in many candy samples, Pb and Cd content were more than the maximum limits established for Pb and Cd in chocolates at 0.30 mg/kg and 0.05 mg/kg respectively (FAO/WHO, 2001). The number of candy samples in which Pb and Cd content was found at concentration more than 1.0 or 0.30 mg/kg and 0.05 mg/kg respectively are depicted in Table 2.

Essential trace elements such as, Nickel (Ni), it helps to produce red blood cells and beneficial as an activator in some enzyme systems (National Academy of Sciences, 1975) (Underwood, 1977). However in excess, Ni becomes extremely toxic and leads to decreased body weight, heart and liver damage, severe skin irritation and bronchial haemorrhage (Nielson, 1977). Ni contamination in candies mainly occurs because of its use as catalyst during the manufacturing process i.e. hardening of chocolate by hydrogenation of unsaturated fats and during processing, transportation and storage in nickel containers. Many researchers have reported the daily dietary intake of Ni from 200 to 900µg/day.

But in Indian food, nickel content is reported much higher varied between 240 to 3900 µg/day. As far as Ni is concerned, its maximum permitted level was decided at 0.2 mg/kg, only in some food samples. But in candies, permitted level for Ni has not been defined by any food safety authority.

Previously few researchers have also reported Ni content in the range of 0.041–8.23 µg/g and 0.120–2.588 µg/g in different types of chocolates and candy samples (Duran et al., 2009) (Dahiya et al., 2005). In this study, the level of Ni was measured at 0.177-4.256 µg/g, 0.065-3.561 µg/g and 0.079-1.021 µg/g in cocoa-chocolate based, milk based and fruit flavored candies respectively. In addition to ingestion of Ni through various other food products, the above depicted level of Ni in candy samples from 0.079 to 4.256 µg/g can be considered as harmful to human body.

Traces of chromium (Cr) in the body has been reported as a component of glucose-tolerance factor, acting as a co-factor for insulin action via forming a ternary complex with insulin receptor through peripheral activity, which in turn assist the insulin fixing and control the metabolism of carbohydrate, lipids and proteins. In excessive amount, Cr reduces the effectiveness of insulin at controlling blood sugar and cause stomach irritation, itching, fast and irregular heart rhythms and liver problems. In previous studies Cr content ranged from 0.740 – 6.265 µg/g and 0.088 – 0.39 µg/g have been reported in various candy samples. In present study, Cr content was found varied between 0.427-3.122 µg/g (Cocoa-chocolate based candy), 0.148-1.799 µg/g (Milk based candy) and 0.213-2.512 µg/g (Fruit flavored candy), which shows relatively high level in contrast to previously conducted studies [19-24].

Copper (Cu) is considered as essential trace element for some enzymatic reactions and necessary to control the body pigmentation in addition to Fe and also required for maintaining the body growth, protection from infections and bone abnormalities, glucose and cholesterol metabolism. Excessive accumulation of copper in body tissues can causes Wilson's disease. Fortunately, Turkish standards have took initiative steps to set up permissible limit for Cu in candy samples at 10 µg/g Also, the Spain legislation had set the permissible limit for Cu at 5 µg/g in chocolate and cocoa-sugar (Carbonell-Barrachina et al., 2002). As per Anonymous, it is 15 µg/g and 50 µg/g in chocolate and cocoa-sugar respectively (Anonymous, 2002). Recommended dietary allowance of Cu for 7 to 10 years old child is 1.0 to 2.0 mg/day and 0.5 to 1.0 mg/day for infants as per National Research Council, 1989 (NRC, 1989) Earlier studies have reported Cu content in the range of 0.219–2.455 μg/g and 1.07 – 2.74 μg/g in various candy samples (Duran et al., 2009) (Dahiya et al., 2005). In present study, Cu content was found at comparatively low level between 0.095-0.550 μg/g (Cocoa-chocolate based candy), 0.148-0.618 μg/g (Milk based candy) and 0.069-0.580 μg/g (Fruit flavored candy), which could be considered as less harmful as the detected Cu levels are relatively very low than the permissible limits and the daily dietary intake established by the international standards.

Manganese (Mn), an essential trace element works as a co-factor in vital metabolic processes and fatty acid synthesis. Mn also needed by human body for proper bone and tissue formation and reproductive functioning. In excessive amount, Mn is recognised as a neurotoxic element as it can cause impaired motor skills, neuro-degenerative diseases (Manganism, Alzheimer's disease, Parkinson's disease), cognitive and childhood developmental disorders. As, in this study, content of Mn was also analysed in candy samples and it was measured at levels ranging from 0.046-0.410 μg/g (Cocoa-chocolate based candy), 0.138-0.875 μg/g (Milk based candy) and 0.049-1.489 μg/g (Fruit flavored candy). Unfortunately, neither any food safety authority nor any international health agencies have defined the permissible limits for Mn in food products and thus, the detected levels of Mn in candy samples could not be compared with standards in order to establish the quality of analysed candies for consumption.

Iron (Fe) is the most essential element, present in greater quantity in human body as compared to other trace elements. Fe plays an important role as a catalyst for proper metabolism in human body. Fe level in body must be controlled by regulating uptake because only a small amount of iron loss occurs daily through mucosal and skin epithelial cell sloughing. In some people, regulation of iron uptake is impaired because of genetic defect or genetic susceptibility to iron overload which results in to excessive accumulation of iron in body leads to iron overload disorders, such as hemochromatosis. A previous study had reported the accumulation of iron in the hippocampus region of the brain of those suffering from Alzheimer's disease and Parkinson disease. Excessive iron consumption can typically damage the cells of heart and liver which can cause major adverse health effects such as metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long-term organ damage, DNA damage, coma and even death. As per NRC (1989), the recommended dietary allowances of iron for male and female children of about 10-16 years are 10 mg/day and 15 mg/day correspondingly. Three grams of iron can be toxic for a child of age about 2 years.

Maximum level of iron at 2.0 mg/kg in cocoa fat and a range between 0.2 and 25 mg/kg in some foods products have been stipulated in previous reports (Anonymous 2002). Previous literature have reported iron content in the range of 3.963–9.863 μg/g in different type of candy sample. According to this study, the cocoa and milk based candies have shown much higher level of iron, with highest level at 10.890 μg/g (Cocoa-chocolate based candy), 8.691 μg/g (Milk based candy) and 5.168 μg/g (Fruit flavored candy) which cannot be considered as safe as if, through intake of candies, it could possibly raise to toxic level along with mandatory routine dietary intake [25-29].

Zinc (Zn) is also an essential trace element which works as a catalytic agent in various enzymatic reactions, proper functioning of the brain and central nervous system (Bitanihirwe & Cunningham, 2009). Zinc is also critically required for synaptic plasticity and hence in learning. Recommended dietary intake of Zn for pregnant women and preadolescent children is set at 15 mg/day and 10 mg/day respectively. Even though Zn is essential need for good health, excess of zinc can cause neurotoxicity, depressed growth, reproductive problems, impaired appetite, renal diseases and mal-absorption of other trace elements. Excessive absorption of zinc suppresses copper and iron absorption. Present study have detected Zn in candy samples at the level of 0.277-2.263 μg/g (Cocoa-chocolate based candy), 0.188-1.565 μg/g (Milk based candy) and 0.033-0.401 μg/g (Fruit flavored candy), where level of Zn in fruit flavored candies is relatively very low to believe them as safe to consume. But higher consumption of cocoa and milk based candies can hamper the required absorption of Cu and Fe.

In trace amounts, Cobalt (Co) serves as key element of vitamin B12 which is necessary for the metabolism of every cell in human body, fatty acid synthesis, energy production and for the normal functioning of the brain and nervous system. However, high amount of cobalt in body is considered as a major cause of contact dermatitis. No permissible limits have been determined for Cobalt (Co) in chocolate and candies. Fortunately, in the present study, Co was detected at relatively negligible level in all kinds of candies i.e. 0.019-0.119 μg/g (Cocoa-chocolate based candy), 0.008-0.158 μg/g (Milk based candy) and 0.021-0.360 μg/g (Fruit flavored candy).

Despite of the paramount importance, there are no specific quality assurance guidelines and permissible limits have been set by the international food regulatory agencies and public health safety organizations for commonly found trace elements and heavy elements in frequently consumed candies. Permissible limits for few trace and heavy elements specifically in chewing gum and candies have been commendably set by the Turkish Food Standards [30-34]. The finding of this study specifically reflects the high need of focusing towards defining strict rules and regulations for manufacturing and minimizing metal contamination in different variety of candies.

Conclusion

The present study has reported the level of 09 commonly detected elements in three main varieties of candy. Multiple Discriminant Analysis shows that the pattern of element contamination in similar type of candy samples is non-uniform and thus, suggesting inferior and improper practices of manufacturing and processing candies. The detected level of metals in candies and statistical analysis is indicating that the frequent consumption of candies can silently lead to serious health conditions. Some elements like Pb, Cr, Ni and especially Fe, was found at high concentration in all type of candies which indicates that candies always cannot be considered as safe to eat. Overall outcomes of the present study i.e. presence of toxic heavy elements and high level of essential trace elements in candy samples is pointing towards setting up strict quality guidelines for candy manufacturing, processing, storage and transporting in order to minimize the contamination of candies with toxic elements. In addition, defining proper permissible limits for commonly detected elements in cocoa, milk based and fruit flavored candies and bars is highly important to protect public health especially children, pregnant women. Moreover, it is also imperative to conduct such studies to evaluate metal contamination in various food products in order to aid international food safety standards in defining relevant guidelines and regulations[35-40].

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