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THE EVALUATION OF SOME HEAVY METALS IN COMMONLY WHEAT BREAD WITH THEIR DAILY INTAKE

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ABSTRACT

Bread is a main staple food and the vital diet that gives approximately 50-90% daily intake of whole calories and proteins. This study was aimed at investigating the content of heavy metals in the different types of wheat bread. A total of 50 various bread categories including Lawasha, Mashini, Samoon, Hawrami and Tiry are provided in bakery stores. The mean concentration of the Cr, Ni, Cd, Pb, As and Co in all the studied bread samples were ranged in ($\mu\text{g kg}^{-1}$) as follows: 0 - 173.7 for Cr, 117.2 - 313.3 for Ni, 0 - 16.1 for Cd, 0.89 - 357.3 for Pb, 5.60 - 33.9 for As and 3.06 - 17.8 for Co. The highest concentration was found for Ni followed by Pb, Cr, As, Cd and Co. The mean daily intake of Cr, Ni, Cd, Pb, As and Co with the consumed bread was 0, 0.5, 4.2, 6.4, 7.4, and 1.6 $\mu\text{g kg}^{-1}$, respectively. The total daily intake of the studied heavy metals in 300 g bread was determined as follows: Lawasha 1.38, Mashini 1.72, Samoon 1.21, Hawrami 0.88 and Tiry 1.21 $\mu\text{g d}^{-1}$ bw. The high contents of daily intake of Cr, Ni, Cd, Pb, As, and Co in bread affect people's health. As a result of this study, it can be concluded that bread types are safe from heavy metals. Total daily intake is lower than the permissible limit which is recommended by FAO/WHO. While heavy metals reduce in bread during wheat cultivation and during the bread-making process is still required.

Key words: Bread analysis, Bread composition, Heavy metals, daily intake.

INTRODUCTION

Bread is a main staple food and the vital diet that gives approximately 50 - 90% daily intake of whole calories and proteins (Feyzi et al., 2017). Globally, the high consumption of bread is thought to be an essential source of minerals (AL Juhaimia et al., 2016), and supplies energy for the body (Lebbos et al., 2019). Beside the bread quality as the main diet source in developing countries, its safety needs to be confirmed and examined in terms of known toxic as trace elements. Bread is also an important and major source of heavy metals (Mestek et al., 2015), this is due to the high consumption of it. But their concentrations depend on many factors. These heavy metals are passed into the food through environmental contaminants (Feyzi et al., 2017; Magomya et al., 2013a). The main source of bread contamination is flour which is produced from contaminated wheat and mode of production and processing (Magomya et al., 2013b; Naghipour et al., 2014). According to (Magomya et al., 2013b) used water heating fuel used for bread production could furthermore be a source of heavy metals.

Jawad and Allafaji (2012) reported that Food processing equipment and vessels have been known as a source of heavy metals such as Pb and Cr in the processed foods.

Still bread products could be unsafe because they can deliver harmful elements to the human body involving heavy metals. Heavy metals have involved public consideration concerning their potential effect on human health problems (Feyzi et al., 2017). Revelation to mineral, Ni and Co are a vital element for human biological function, but become hazardous to human health if they sufficiently available (Al-Kamil, 2011; Lebbos et al., 2019; Ludajic et al., 2016), and As, Pb Cd, and Cr are considered as carcinogen (Edam et al., 2009; Ruttens et al., 2018). They can arise regularly in the body organs and with time might exceed acceptable limits which can cause powerful poisonousness leading to human disorder, defects, illness, malformation and malfunctioning of eventual death (Salazar - Flores et al., 2019).

Trace elements include Cr and Co is thought as vital elements, which are needed by the human body in extremely small amounts. They are vital constituents of biological structures. Micronutrients, such as Cr and Ni may be toxic at excessive levels although they are important for human nutrition and plant growth (Islam et al., 2018; Rahman et al., 2014). On the other hand, toxic elements including As, Cd, and Pb are known to have harmful influences on human health. The US Agency for Toxic Materials and Disease Registry lists all hazardous constituents present in toxic waste sites rendering to their frequency and the severity of their toxicity. The As is greatest harmful on the list of heavy metals (Tokalioglu et al., 2018). These can enter the food chain and form meaningful capacity threats to human health even at low concentrations (Islam et al., 2018; Tokalioglu et al., 2018). In humans, uptake of heavy metals is due to consumption of contaminated foods and the risk increases with increasing the amount consumed. For example, long term exposure to Cr can affect kidney or liver damage. Cd causes renal dysfunction, lung cancer and damages the lung system. Lead leads to serious or chronic damage to the nervous system behavioral diseases. Nickel affects lung cancer, respiratory failure, birth faults, heart illnesses and asthma. Arsenic causes tumors of lung, bladder and skin. Cobalt affects asthma, pneumonia, heart issues, thyroid damage, vomiting and nausea at raised levels (Oyekunle et al., 2014; Ruttens et al., 2018). Thus, the risks related with metal contamination in foodstuffs are of famous concern. Several researchers have approved searches on the concentration of heavy metals in many nutrient supplies in several parts of the world (Salazar-Flores et al., 2019). Bread as a staple food in many homes worldwide, there has been lack of information about its heavy metals concentration.

The aim of current study is to evaluate the safety of bread produced and also determine daily intake of the studied heavy metal. Selected bread samples manufactured will be studied for the presence of selected heavy metals. The heavy metals to be determined include Cr, Cd, Pb, Ni, As, Co. Observing heavy Metals in breadstuff supplies is required for issues of public health concerns.

MATERIALS AND METHODS

Sampling

In the current study, 50 samples of several types of bread which were prepared for selling were collected from different bakeries stores in 2017 in Sulaymaniyah province, Iraqi Kurdistan. This study was achieved by two replicates. These bakeries produce numerous types of bread such as Lawasha, Mashini, Samoon, Hawrami, and Tiry. In selected bakeries two slices of these breads were randomly collected. The samples were stored in a clean paper bag labeled and transported to the laboratory of Halabja Agriculture technical college of Applied Science.

Sample preparation and analysis

At the laboratory, all bread samples were air dried for three days at room temperature in a clean laboratory atmosphere before sub-sample taken from each type of bread according to their preparation process and fermentation time. The dried bread samples were milled using an electric mixer and stored in clean polyethylene bag with 50 mL capacity. About 10 g of samples were transported to the University of Nottingham, UK. About 0.2 g of finely ground samples was digested under microwave heating (Anton Parr, Multiwave 3000) for about 45 min at 2MPa in 4 mL HNO₃ (68%) and 2 mL H₂O₂. The digested samples were diluted to 20 mL with Mili-Q water and stored in universal tube further analysis. All acids were either Analytical reagents (AR) or Trace Analysis Grade (TAG) from Fisher scientific, UK. The concentration of Cr, Cd, Pb, As and Co was measured by ICP-MS (Thermo-Fisher Scientific, ICAP Q, Germany). The digested batch included three practical blanks and three samples of the certified reference material (Tomato leaves) for quality assurance at the laboratory of the University of Nottingham, UK.

Calculation of The Heavy Metals Daily Intake

The daily intake of heavy metals through bread consumption was calculated using an equation.

$$DI_{TM} = \frac{C * DI_{Bread}}{B.W} \dots \dots \dots Eq. 2.1$$

where DI_{TM} is daily intake of trace metal (µg kg d⁻¹), C is trace metal concentration in bread (mg kg⁻¹), DI_{bread} daily intake of bread by adult, and B.W is body weight (kg) in this study the body weight was considered to be 70 kg. An average daily consumption of 300 g of bread was assumed (Udowelle et al., 2017). This method was improved since bread is eaten as a traditional breakfast meal and as in-between meal snacks by the majority of Iraqi people.

Statistical analysis

Data were subjected to one-way ANOVA, including heavy metals concentration and bread types. When significant differences were found in ANOVA, means compared using Least of significant difference (LSD) test at p≤0.05. All analysis was performed using SPSS v.22 for Windows.

RESULTS AND DISCUSSION

Trace elements concentration

In this study, we investigated the concentration of some heavy metals and their daily intake in 50 different bread samples. Figure (1) represents the concentration of the selected heavy metals in the studied bread samples, a significant difference was found between bread types under (p≤0.05). Generally, metals concentration in food depends on some factors such as soil type, pH, organic matter and soil clay contents which have a main role on heavy metals bioavailability (Slepecka et al., 2017). Furthermore, application of chemical fertilizer and pesticide to wheat area and environment pollution (Greger and Löfstedt, 2004). The high concentration of heavy metals can cause many health problems such as skeleton and nervous system, renal and cardiovascular diseases (Udowelle et al., 2017). These heavy metals are also implicated in teratogenesis, mutagenesis and carcinogenesis (Feyzi et al., 2017). Some research in the food contaminants field has been issued by authors (Naghipour et al., 2014). The current study performed on some heavy metals content in selected common bread consumed which evaluates their quality.

Chromium

Of all 5 bread types Cr concentration was below than limited detection of $1000 \mu\text{g kg}^{-1}$. By bread types, the average concentration of Cr in ($\mu\text{g kg}^{-1}$) obtained followed the order: 64.9 for Samoon, 63.3 for Tiry, 36.5 for Hawrami, 24.5 for Lawasha and 15.1 for Mashini (Fig.1A). It is even relatively high which makes bread dietary unfavorable, when not reflecting the addition of heavy metals during the process. Slightly similar Cr concentration in bread from 27.3 to $67.3 \mu\text{g kg}^{-1}$ were reported for 152 white bread samples in a study by (Soares et al., 2010). Bou Khouzam et al. (2012) studied the concentration of toxic and essential elements in Lebanese bread and reported $100 \mu\text{g kg}^{-1}$ for Cr content. Compared to those reported, the concentration of Cr in the studied bread types is in the range reported globally. However, the results are lower than Cr concentration of 700 - 2800 and 860 - 2300 $\mu\text{g kg}^{-1}$ for Barbari and Sangak bread types separately reported by (Naghypour et al., 2014) consumed by Iranian population. The high variation of Cr in studied bread samples may be due to use of metals during the baking process. But, the variation of Cr concentration may be due to type of used flour, environment and soil type of wheat grain grown and used water for preparation of bread yeast (Bawiec et al., 2014).

Nickel

Nickel is one of the essential elements required by the human body to activate body enzymes (Feyzi et al., 2017). But if the concentrations of Ni exceed the allowed limits it may produce toxic results in humans. Its toxicity at higher levels is more outstanding. The very widespread antagonistic health effect of Ni in humans is allergic reaction. The average concentration of Ni obtained were 243.7 for Tiry followed by 194.0 for Hawrami, 177.4 for Samoon, 166.2 for Lawasha and 149.2 for Mashini (Fig.1B), which indicate high concentration of Ni in the studied bread samples. A significant difference was found between bread types under ($p \leq 0.05$). Higher concentration $916 \pm 64 \mu\text{g kg}^{-1}$ in bread reported by (AL Juhaimia et al., 2016). Lebbos et al. (2019) reported $1292 \mu\text{g kg}^{-1}$ of Ni in white bread consumed by the Lebanese population. Compared to those reported, the results of this study indicate that the concentration of Ni in bread types is in the lower range compared to those reported. Variation in Ni concentration between bread types could be an influence of equipment which was used during the bread making process. A similar observation was reported by (Bawiec et al., 2014). Furthermore, it may be due to used water and soil type of grown wheat (Ofori et al., 2016).

Cadmium

The average Cd concentration in ($\mu\text{g kg}^{-1}$) in bread types were 11.5 for Hawrami, followed by 7.36 for Lawasha, 7.05 for Tiry, 4.88 for Mashini and 3.23 for Samoon (Fig.1C). An acceptable limit of Cd in food is $50 \mu\text{g kg}^{-1}$ (Magomya et al., 2013a). From the result achieved all the bread samples analyzed had Cd concentration below the allowable limit. The average Cd concentration in bread samples found in the current study is lower than the average concentrations of this element informed for bread and cereals reported by (Bou Khouzam et al., 2012). And also, higher Cd concentration is informed in the literature with values between $10\text{-}30 \mu\text{g kg}^{-1}$ in white wheat bread reported by (Udowelle et al., 2017). Alomarya and Wedianb (2012) reported average Cd concentration of $16.21 \mu\text{g kg}^{-1}$ for seven bread baked with electricity operated bakeries in Jordan. Subsequently, it can be proposed that the presence of this metal is not due to the baking process but may have derived during wheat growing due to application of phosphate fertilizer which is contain a significant level of Cd (Ludajic et al., 2016). This is caused by the presence of a significant concentration of Cd in bread produced with wheat flour.

Feyzi et al. (2017) reported that the concentration of Cd in bread is powerfully affected by environmental conditions and the conditions of cultivation such as application of high amounts of fertilizers and pesticides. However, low concentration of Cd in studied bread samples may be approved for its lower content in soil. The concentration of Cd in wheat grain is highly associated with its content in grown soil which indicates that wheat adopts Cd from soil (Ludajic et al., 2016).

Lead

The average Pb concentration in ($\mu\text{g kg}^{-1}$) found were 118.4 for Tiry followed by 58.1 for Hawrami, 15.7 for Samoon, 14.6 for Lawasha and 8.36 for Mashini (Fig. 1D), which indicate high variation between bread types. An acceptable limit of Pb in food is ranged from 200 to 2500 $\mu\text{g kg}^{-1}$ (Magomya et al., 2013a; Tortoe et al., 2018). Results of this study discovered that all the bread samples had Pb concentration below the permissible limit. Lead concentration in bread samples of current study was less than those reported by (Feyzi et al., 2017; Magomya et al., 2013a). They reported Pb concentration of 375-2887.2 and 340- 3130 $\mu\text{g kg}^{-1}$ for 40, 15 bread samples in Iran and Nigeria respectively. Magomya et al. (2013a) reported Pb concentration between 340-3030 $\mu\text{g kg}^{-1}$ for 15 white bread samples. Compared to those reported the Pb concentration is less than the range reported globally. Corresponding to Chary et al., (2008) Pb accumulation occurs when high levels of this element are present in the soil. This suggests that the fields in which the wheat was cultivated were not to be polluted by Pb. The bread samples of our study are safe in regard to Pb concentration. However, the variation of Pb concentration in our studied bread samples may be due to equipment used to produce each type of bread. Jawad and Allafaji (2012) studied trace element concentration in Iraqi bread and they reported that Pb increased during bread making-process due to used equipment and efficiencies used in bakeries.

Arsenic

Generally, the average range of As in ($\mu\text{g kg}^{-1}$) were 19.6 for Samoon, followed by 18.0 for Tiry, 10.6 for Mashini, 10.5 for Lawasha and 9.27 for Hawrami (Fig. 1E), with the lowest in Mashini bread and the highest in Samoon bread. Variations in As concentrations in bread samples are recognized to be determined by genetic differences on the one hand, and soil characteristics on the other hand (Ruttens et al., 2018). The As concentration found in our samples overall agrees well with those reported in previous studies. In a survey on market bread conducted by Aliakbar-Malekirad et al. (2016) reported a range of 0 to 12.5 $\mu\text{g kg}^{-1}$ in Iran Barbari bread type, a range from 9 to 48 $\mu\text{g kg}^{-1}$ in bread wheat (average 15 $\mu\text{g kg}^{-1}$) and the highest level $400 \pm 7 \mu\text{g kg}^{-1}$ in white pita bread reported by (Lebbos et al., 2019). Slightly higher ranges were reported by Bou Khouzam et al. (2012) and Tortoe et al. (2018) for Lebanese bread (29–37 $\mu\text{g kg}^{-1}$) and for composite flour bread (10-30 $\mu\text{g kg}^{-1}$). Arsenic is a toxic element. Intake high amounts cause gastrointestinal symptoms, serious disruptions of the cardiovascular and essential nervous systems, and finally death (Feyzi et al., 2017). Furthermore, the results of this study indicated that the concentration range of this toxic element was within the allowable limit in 100 $\mu\text{g kg}^{-1}$ bread recommended by (FAO/WHO, 2016).

Cobalt

Cobalt is vital to life only in small quantities. The average Co concentration in ($\mu\text{g kg}^{-1}$) in the studied bread types were 12.7 for Tiry followed by 12.6 for Samoon, 9.78 for Hawrami, 8.30 for Lawasha and 6.03 for Mashini (Fig. 1F), a significant difference was found between bread types ($p \leq 0.05$). All the studied samples had Co content lower than the permissible limit for Co

30 $\mu\text{g kg}^{-1}$ in foods recommended by (FAO/WHO, 2016). Thus, all the studied samples in this study were safe relating Co content. Average Co concentration was 91 $\mu\text{g kg}^{-1}$ for seven Lebanese bread types (Lebbos et al., 2019). Oyekunle et al. (2014) studied trace metals concentration in bread samples and reported an average of 30-100 $\mu\text{g kg}^{-1}$ of Co for bread loaves in Nigeria. Compared to those reported, the Co concentration in studied bread types in lower range reported globally. But, as shown there is variation between bread types again it may be due to flour type, soil type of growing wheat grain and baking process.

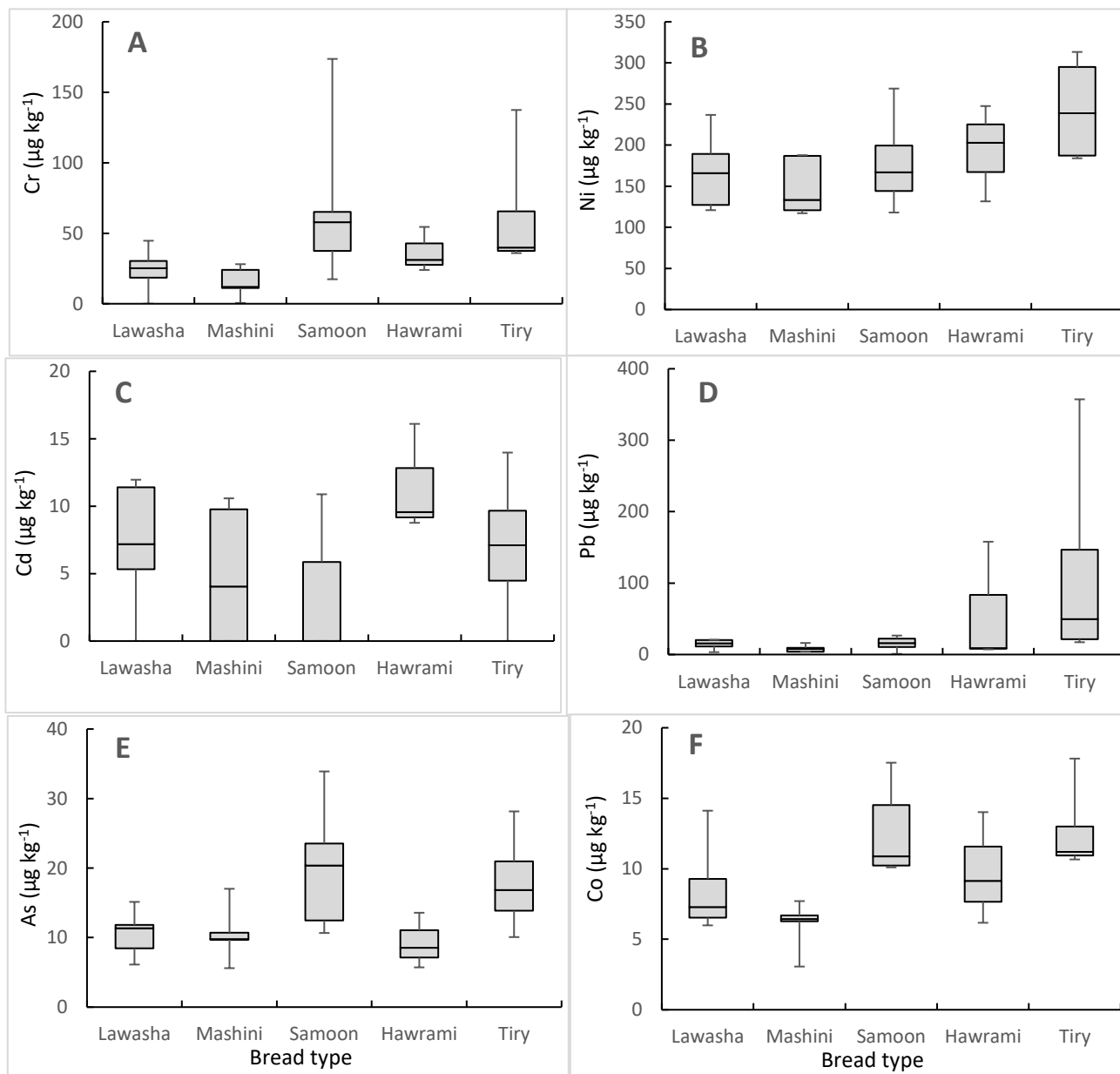


Figure 1. The concentration of the studied heavy metals in different bread types (no = 50).

The variation in selected heavy metals detected in this study may be attributed to variations in composition of flour products from wheat and environmental situations under which this cereal was grown. Wheat variety, growing conditions, and location are all factors that may cause differences in the bioavailability of heavy metals (Gabaza et al., 2018). The wheat growing area in the Kurdistan Region amounts to about 570,000 ha; approximately 500,000 t is harvested every year and wheat production is mostly rain-fed (Mazid, 2015). Wheat grains bread types which are studied derived are grown in different environmental locations and types of soil relating to elemental concentration. These metals basically contaminate plants within the soil or polluted air, which precipitates and falls as rain (Tortoe et al., 2018).

Cluster analysis of selected heavy metals

A dendrogram of heavy metals showed that two logical groups can be recognized in studied bread samples with similarity levels more than 70% (Fig. 2). From left to right group I (Cr and Pb) and group II (Ni and Co) possibly indicating a common source of soil contamination such as phosphate fertilizer and calcareous mineralogy of the wheat growing soils. Greger and Löfstedt (2004) reported that application of phosphate fertilizer to the growing soil causes an increase in the concentration of heavy metals of wheat grain.

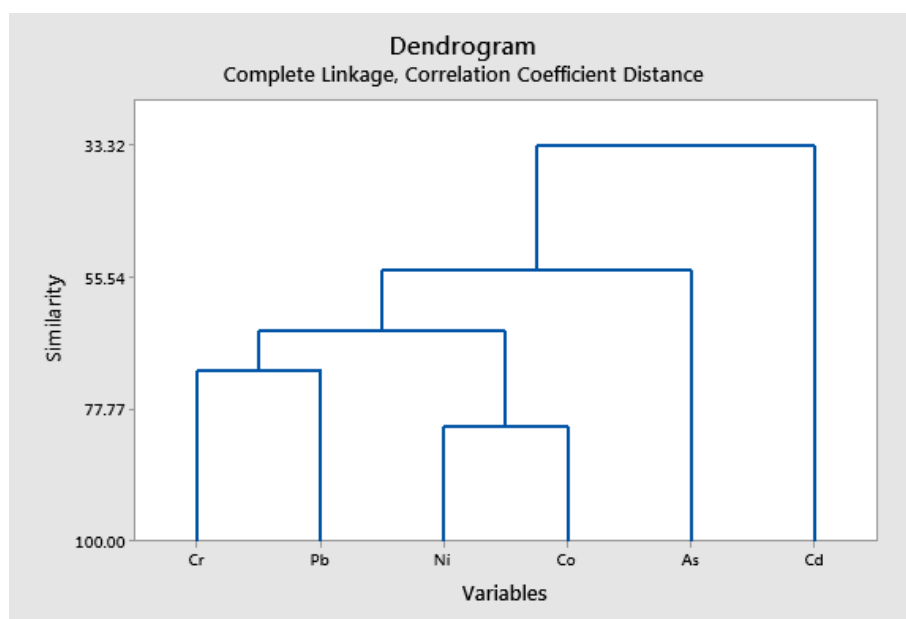


Figure 2. Cluster analysis of selected heavy metals in the studied bread types (no = 50) collected

The daily intake of trace elements

Data in the table (1) presents the daily intake of selected heavy metals in studied bread types. The estimated daily intake (EDI) of the consumption of analyzed bread samples indicated variations. The results showed the order of daily intake of heavy metals concentrations in bread samples was as follows: Ni > Pb > Cr > As > Co > Cd. Expected daily intake of Cr, Ni, Cd, Pb, As and Co rendering to the consumption of different bread types was presented using the market basket. The daily intake of selected heavy metals in studied bread types in ($\mu\text{g kg}^{-1} \text{d}^{-1}\text{bw}$) ranged from 0.10 to 0.22 for Cr, from 0.56 to 0.80 for Ni, from 0.04 to 0.05 for Cd, from 0.05 to 0.57 for Pb, from 0.04 to 0.07 for As, from 0.03 to 0.06 for Co. Results obtained from analyzed bread types

show high variation between metals. The average of EDI of heavy metals for a standard individual in ($\mu\text{g kg}^{-1} \text{d}^{-1} \text{bw}$) were 25 for Cr, 126.27 for Ni, 15.7 for Cd, 28.37 for Pb, 223.6 for As, 11.4 for Co (Koubova et al., 2018; Lebbos et al., 2019; Naghipour et al., 2014). From the results observed the daily consumption of heavy metals in 300 g of bread are below the estimated daily intake.

Table 1. Daily intake of the studied trace metals in the studied bread samples

Bread type	Cr	Ni	Cd	Pb	As	Co	Total
	($\mu\text{g kg}^{-1} \text{d}^{-1}$)						
Lawasha	0.22	0.70	0.04	0.33	0.05	0.04	1.38
Mashini	0.22	0.76	0.04	0.57	0.06	0.06	1.72
Samoon	0.10	0.80	0.05	0.17	0.06	0.04	1.21
Hawrami	0.12	0.58	0.04	0.06	0.04	0.03	0.86
Tiry	0.10	0.56	0.05	0.05	0.07	0.05	0.88
Value (mean)	0.15	0.68	0.04	0.24	0.06	0.04	1.21
DI* ($\mu\text{g kg}^{-1}$)			1	3	2.5		
PIWI**			7	25	15		

*Daily intake of heavy metals and **FAO/WHO provisional tolerable weekly intake

The highest average daily intake of $0.68 \mu\text{g kg}^{-1}$ was achieved for Ni followed by 0.24 for Pb, 0.15 for Cr, 0.06 for As and 0.04 for Cd and Co. Furthermore, in regard to total uptake in bread types the highest estimated daily intake of $1.72 \mu\text{g kg}^{-1} \text{d}^{-1} \text{bw}$ was found for Mashini followed by 1.38 for Lawasha, 1.21 for Samoon, 0.88 for Hawrami and 0.86 for Tiry with an average of $1.21 \mu\text{g kg}^{-1} \text{d}^{-1} \text{bw}$. From the results obtained, daily intake of selected heavy metals is lower than recommended daily intake for standard individuals in all the bread types recommended by the World Health Organization. The estimated daily intake found of heavy metals in this study is below those documented in Misurata markets. Consumers of breadstuff are hopeful to prevent harm to their health. However, estimation of property of such stuffs may increase particular issues, especially their contamination with heavy metals. This is because bread can deliver heavy metals (Slepecka et al., 2017). The proportion of heavy metals in bread indicates the safety of eating this kind of diet. The results indicate that all the bread types consumed are safe due to their low content of the selected heavy metals.

The current study shows an effort to assess the concentration of these heavy metals in different bread types commonly consumed by peoples in Sulaimanyah city and to confirm their unfavorable impact on human health. All the found metals in this study are below the recommended concentration suggested (FAO/WHO, 2016) and other International Agencies. Therefore, it would be useful to investigate the importance of long term exposure to breadstuffs with such contaminants and providing knowledge of their impacts on people's health. Furthermore, the estimated daily intake of these heavy metals was evaluated in sequence to assess the limits of those contaminants in bread types.

CONCLUSION

The results of this study provide appreciated information about the heavy metals contents and their estimated daily intake of studied bread types consumed. All 50 bread samples investigated contained heavy metals in quantities that were in the range of allowed limit. In regards to heavy metals, the highest average concentration in all the bread samples were recorded for Ni

metal while the lowest for Cd metals. The Estimated Daily Intake levels appeared that the intakes of heavy metals from the studied bread samples were below allowable daily intake regulates for the toxic metals and suggested daily intake values for the requirement metals. The highest total daily intake of heavy metals was obtained with Mashini bread while the lowest with Hawrami bread. The results indicate that heavy metals in bread samples are not at risk to consumer's health.

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