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Measurement of Heavy Metals in Bread with an Emphasis on the Risk Assessment of Aluminum

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ABSTRACT

Introduction: Humans are exposed to aluminum (Al) and other heavy metals through various sources. Scientists have long investigated the effects of Al and heavy metals on human health, reporting a correlation between Al concentrations and health issues such as *Alzheimer's* diseases and cancer. Therefore, a risk analysis study is required to assess the risk of non-cancerous diseases. The present study aimed to measure heavy metals in bread with an emphasis on the risk assessment of Al.

Methods: Various types of flatbread, cakes, and muffins were randomly collected in Tehran, Iran. The samples were prepared based on the modified AOAC official method. Subsequently, test solutions were analyzed for Al, Pb, Hg, Ni, As, Cd, Co, Cr, and Cu via inductively-coupled plasma/optical emission spectrophotometry (ICP-OES). Significant differences between the bread sample groups were determined using one-way analysis of variance (ANOVA) and Duncan's least square difference (LSD) test.

Results: We calculated the concentration, daily intake (DI), national theoretical maximum daily intake (NTMDI), and the hazard quotient (HQ) of Al in Iran. The lowest concentration of Al was observed in Sangak bread, and the highest level was detected in Taftan bread. In addition, the highest Al concentration was observed in cakes (mean: 40.44). The DI of Al was estimated at 0.26 mg/kg, and the NTMDI for adults was 0.005. The HQ of Al in all the bread samples was less than one.

Conclusion: According to the results, the mean acceptable daily intake of Al was 92% of the provisional tolerable daily intake, and the HQ was less than one in the studied bread samples. Therefore, no risk of non-cancer diseases was observed due to the consumption of the bread samples.

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Introduction

Bread contains proteins, vitamins, lipids, and minerals and is considered an important food source in numerous countries, including Iran. The daily average per capita consumption of bread in Iran is 230 grams. Iran has various types of traditional or flatbread (e.g., Sangak, Barbari, Taftan, and Lavash) and baguette (French bread), which are made of wheat flour, water, salt, other ingredients, and food additives. Given the importance of bread as a primary food and its ingredients, it is a notable commodity to be monitored for the presence of excessive additives and/or contaminants [1-3].

Bread ingredients (e.g., wheat and water) could be contaminated with mycotoxins, cyclic hydrocarbons, and heavy metals. Deoxynivalenol (DON) is a mycotoxin most commonly detected in cereal commodities such as wheat [4]. In addition, polycyclic aromatic hydrocarbons are environmental pollutants often found in the air, soil, water, and consequently in bread [3, 5]. Heavy metals and trace elements such as lead, mercury, nickel, arsenic, iron, and cadmium are also important contaminants, which could enter bread through various sources, such as bioaccumulation in bread ingredients (wheat, water, and salt), metal pans used for backing, and food additives [6, 7].

Food additives such as aluminum ammonium sulfate (E523) are often used in baking powder for anticaking properties and are frequently used in various food industries. Using higher levels of aluminum (Al) than the acceptable daily intake (ADI) in daily food materials such as bread could

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lead to the accumulation of Al and adverse effects on human health [8].

In a study conducted in China, Al was detected in 10,514 out of 13,833 samples, and 76.0% of the food samples contained higher Al concentrations than the limit of detection, with levels reported between not detected (ND) and 3,070.0 mg/kg. The highest mean concentrations were also reported in fried dough sticks (a type of bread) and fried cakes (301.2 mg/kg), vermicelli (58.8 mg/kg), and steamed bread and steamed buns (34.2 mg/kg) [9].

In another study performed in 2012, the Al content of samples such as homemade steamed bread, stuffed buns, and fried breadsticks from canteens, small shops, and supermarkets was higher than 100 mg/kg, and the concentration ranges were estimated at 3.6-255.1, 28.0-217.2, and 7.6-185.8 mg/kg, respectively. In addition, the qualified rate was reported to be 46.2%, 61.1%, and 35.3% respectively. In the mentioned study, the quality of other packaged foods (e.g., crackers and potato chips) was considered favorable [10].

In a survey conducted to measure the levels of cadmium (Cd) and lead (Pb) in rice and other foods such as bread in Isfahan (Iran), Cd levels in rice grains and Pb levels were studied. food samples (except Sangak bread) were above the maximum levels based on the Codex Alimentarius and National Standards of Iran. Furthermore, wheat bread samples in Isfahan city and Mobarakeh Steel regions (Iran) were reported to contain a higher estimated weekly intake of Pb compared to other foods [11].

Several studies have investigated the levels of heavy metals in bread. Previous findings in Iran indicate that the concentration of lead, copper, zinc, cadmium, and iron in wheat bread in Golestan Province were below the permissible limits set by the FAO/WHO and the Iranian National Standard Organization, while they posed no threat to the health of the consumers [12]. However, the levels of cadmium in Lavash bread and nickel in Taftan bread have been reported to be high in Tehran city [13].

The present study aimed to measure the levels of aluminum, lead (Pb), mercury (Hg), nickel (Ni), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), and copper (Cu) in flatbread and other flour products, such as cakes and muffins. The national theoretical maximum daily intake (NTMDI) was calculated based on previous studies, and risk assessment parameters were also determined. Due to the high concentrations of Al, we also investigated its possible effects on some diseases.

Materials and Methods

Experimental Materials

Al, Pb, Hg, Ni, As, Cd, Co, Cr, and Cu were purchased from Sigma-Aldrich (Steinem, Germany) with higher purity than 99%. A mixed standard solution was prepared at the concentration of 100 $\mu g/kg^{-1}$, and working solutions were also prepared by diluting the stock solution with double deionized water (Milli-Q Millipore 18.2 MΩ/cm resistivity) for the linear range assay. HNO3, H2SO4, H2O2, HF, HclO 4, and HCl were also obtained from Merck Co. (Darmstadt, Germany). All the plastic and glassware were cleaned by soaking in dilute HNO3 and rinsed with distilled water prior to use.

Sample Collection

As mentioned earlier, Iran has several types of flatbread (e.g., Sangak, Barbari, Taftan, and Lavash), which are baked with wheat flour, while baguette is also available and baked with flour. In this study, flatbreads were randomly purchased from traditional bakeries in the north, west, east, and south of Tehran. In addition, samples of cakes, muffins, and baguettes were randomly obtained from different supermarkets in Tehran. In total, 175 bread samples were analyzed, including five types of Iranian bread and 70 samples of cakes and muffins.

Sample Preparation and Laboratory Tests

The collected samples were digested in accordance with the modified AOAC official method 986.15 and AOAC 999-11. For Al measurement, one gram of the samples was mixed with five milliliters of 70% v/v nitric acid at the temperature of 95◦C for two hours. Following that, the mixture was cooled to room temperature, and two milliliters of 30% H_2O_2 was added to the tube. The mixture was heated at the temperature of 95◦C for one more hour. After digestion, the mixture was cooled to room temperature and brought to 50 milliliters with deionized water.

For the analysis of other elements (except arsenic and mercury), five grams of the samples was weighed, 20 milliliters of 10% HNO_3 was added, and the mixture was shaken. Afterwards, the samples were heated at the temperature of 100°C for two hours and overheated at 350°C by a heater. After cooling, two milliliters of concentrated HNO_3 was added, and the mixture was heated to complete dehydration. At the next stage, the samples were placed in a furnace again for two hours at the temperature of 450°C to obtain white ash, five milliliters of HCl (6 mol/l^{-1}) was added, and the mixture was heated for dehydration. Finally, 10 milliliters of concentrated HNO was added.

For the analysis of arsenic and mercury (hydride generation procedure), 0.3 gram of the samples was added to a 50-milliliter flask, five milliliters of concentrated nitric acid was added to the samples, and the mixture was heated for 60 minutes at the temperature of 150°C. Following that, one milliliter of magnesium nitrate (75 mg/l^{-1}) was added, and the mixture was heated at the temperature of 450°C for dehydration. Two milliliters of HCl (8 mol/l^{-1}) was also added, and the mixture was shaken well. Finally, 200 microliters of potassium iodide (1% w/v) was added, and the sample solution was diluted to 25 milliliters with distilled water. The prepared sample solutions were analyzed for heavy metals via inductively-coupled plasma/optical emission spectrophotometry (ICP-OES) (PerkinElmer, USA).

Risk Assessment

For risk assessment, the estimated overall exposure was compared to the health-based guidance values proposed by the EFSA and JECFA for adults (age of >14 years) [14]. Following that, the ADI, the maximum residue limit (MRL), and the national theoretical maximum daily intake (NTMDI) were calculated. If necessary, default bodyweight values were applied, which have been set at 60 kilograms for adults (EU 10/2011, ISIRI 12968) [14].

✓**Acceptable Daily Intake (ADI)**

The ADI is generally estimated by dividing the no-observed effect level (NOEL) of a test substance by the safety factor and is often expressed in milligrams of additive per kilogram of the bodyweight of humans [15].

✓**Maximum residue limit (MRL**

MRL is derived from the ADI under the assumption of what an average person consumes daily.

✓**National Theoretical Maximum Daily Intake NTMDI**

The NTMDI is the acceptable daily intake, which is calculated based on national data and helps toxicologists to estimate the provisional tolerable daily intake [16].

$$NTMDI = \frac{Maximum\ Limit\ for\ Heavy\ metal(\frac{mg}{kg}) * F(National\ consumption\ of\ a\ product)Kg/day}{Body\ Weight\ (kg)}$$

In this study, the consumption of bread in Iran was considered the mean consumption of different groups in Tehran and other cities [17, 18].

Hazard Quotient (HQ)

A hazard quotient (HQ) refers to the ratio of potential exposure to a substance and the level at which no adverse effects are expected. This index is primarily used by USEPA and is calculated using the following formula:

$$HQ = \frac{ADD}{RfD}$$

Where HQ denotes the hazard quotient (unitless), ADD is the average daily dose (mg/kg/day) or daily intake (DI) [17], and RfD represents the reference dose (mg/kg/day), which is set at one (mg/kg/day) for Al (ATSDR 200815). In this study, DI was calculated using the following formula:

$$DI = \frac{C * CR}{Body\ Weight}$$

Where C represents the total concentration of the elements (mg/kg) in bread, and CR shows the daily consumption rate of bread (0.45 g/day) [14].

Hazard Index (HI) is sum of HQ for each sample.

Statistical Analysis

Data analysis was performed in Excel 2016 and SPSS. Data points were obtained from the mean of at least two independent evaluations and expressed as the mean and standard error of the mean. Significant differences between each sample group were determined using one-way analysis of variance (ANOVA) and Duncan's least square difference (LSD) test.

Results

Aluminum Concentration

According to the obtained results, Al concentrations in the bread samples were within the range of 0.19-131.12 ppm. Figure 1 and Table 1 show the Al concentrations in the bread samples. The highest Al concentration was observed in Taftan bread (mean: 64.05 ppm), while the lowest concentration was detected in Sangak bread (mean: 19.024 ppm). In addition, the range of Al concentration was estimated at 0.48-223.71 ppm in the cake samples and 10.03-76.22 ppm in the muffin samples. The results of ANOVA indicated a significant difference in Al concentration between different types of bread (P<0.05). Furthermore, Duncan's LSD showed a significant difference between Taftan and Lavash bread in this regard (Figure 2).

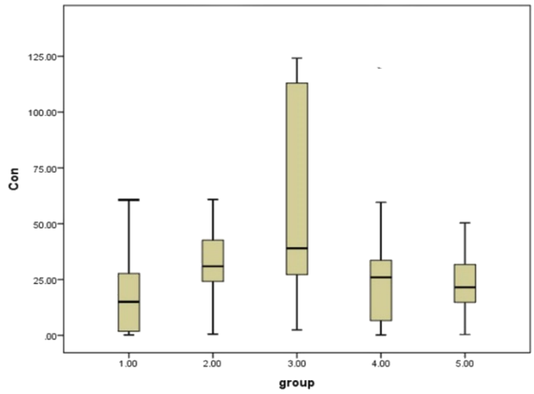

Figure1. Frequencies (Comparison of Average and median) of Concentration of Aluminum in different types of breads: Group 1 is sangak, group 2 is Barbari, group 3 isTaftan, group 4 is Lavash and group 5 is Baguette. Taftan has highest Average of Al concentration and Sangak has the lowest. Median of Sangak is 15.01, Median of Barbari is 30.91, median of Taftan is 38.96, median of Lavash is 25.96 and median of Baguette is 21.50.

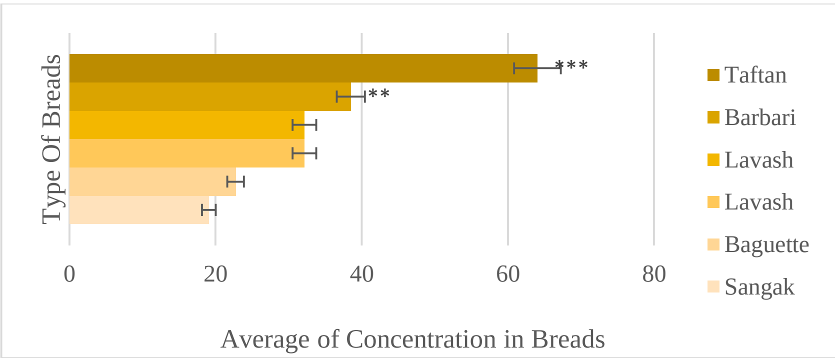

Figure 2. Results of Duncan test. Based on this test Taftan has significance difference with all other types of bread (***) and Barbari has significance difference with all other types of bread except Lavash (**).

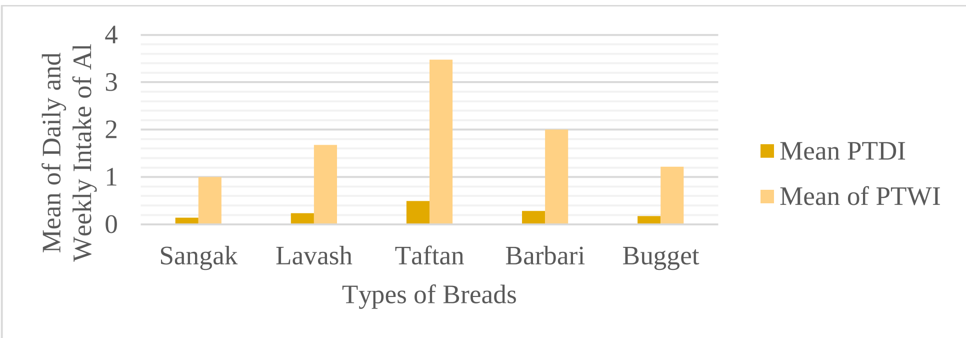

Figure3. Mean of Daily Intake and weekly Intake of Al in all types of bread. It has shown that mean of daily intake and weekly intake of Al in Taftan and Barbari are more than PTDI= 0.3 and PTWI= 2.

Table1. Frequencies (Average, Maximum, Minimum and Range of concentration of aluminum in different types of bread. Taftan has significance difference with all other types of bread (***) and Barbari has significance difference with all other types of bread except Lavash (**).

Type of Bread	Average ±std	Max	Min	Range
Sangak	19.08±17.47	60.69	0.19	60.50
Barbari	38.52±28.34	131.12	0.53	130.59
Taftan	64.05±44.87***	124.11	2.45	121.66
Lavash	32.16±34.21**	129.48	0.19	129.29
Baguette	22.75±12.85	50.37	0.40	49.97

Heavy Metal Concentrations

According to the obtained results, the concentrations of Pb, Hg, Ni, As, Cd, Co, Cr, and Cu were below the detection level (<0.05 ppm) in all the samples. Therefore, risk assessment parameters were not calculated for these elements.

Daily Consumption

Figure 3 shows the mean daily and weekly intake of Al in all the bread samples. Accordingly, the NTMDI of Al in adults was calculated to be 0.005.

Hazard Quotient (HQ)

The obtained results indicated that the target HQ of the Al content in all the bread samples was less than one. In addition, the HQ and ADI were equal based on the reference dose of one.

Discussion

High aluminum levels have been detected in bakery products such as cake (mean: 40.44) and muffins (mean: 33.93). These products used to be consumed by children in schools or at home. Puffed foods are the major source of Al intake in children. In the present study, the mean Al in the bread samples was 32.25, which was lower compared to the cake and muffin samples, and a significant difference was observed between different bread types in this regard. The lowest concentration of Al was detected in Sangak bread, while the highest concentration was observed in Taftan bread. Therefore, it could be concluded that wheat flour and wheat-based products are important sources of dietary Al exposure. Our findings in this regard are consistent with previous studies in other countries. For instance, a study conducted in China indicated the highest mean concentration of Al in different types of cake and bread. Furthermore, a study performed in 2012 showed that the level of Al was high in various types of bread [9, 10]. These findings imply that Al-based food additives are used in preparing different bread types and puffy products (e.g., cakes and muffins).

In the current research, the concentration of heavy metals (Pb, Hg, Ni, As, Cd, Co, Cr, and Cu) was below the detection limit, while some of the previous studies in Iran (Isfahan and Golestan) have shown higher levels than the maximum limit [11-13]. Therefore, our findings indicated a significant reduction in these elements.

As is depicted in Figure 3, the daily and weekly intakes of Taftan and Barbari bread were above the provisional tolerable daily intake (PTDI) and provisional tolerable weekly intake (PTWI). In the present study, the ADI of aluminum in the bread samples was 0.26 mg/kg of bodyweight (BW), which is 92% of the PTDI; this is consistent with previous findings. For instance, a study conducted in China indicated that the mean dietary exposure of the entire Chinese population to Al through Al-containing food additives was 1.795 $mg.kg^{-1}/BW/week^{-1}$, which did not exceed the PTWI despite the dietary exposure [19]. Another study conducted in China in 2010 demonstrated that the mean dietary exposure to aluminum for an adult weighing 60 kilograms was 0.60 $mg/kg^{-1}/BW/week$, which accounted for 60% of the new PTWI as established by the JECFA [20]. Based on the aforementioned findings, it is recommended that the consumption of Al-based food additives be restricted in the processing and cooking of flour-based products.

Risk assessment studies require more data on acute toxicity and the safety factor, which are used to calculate the reference ADI. The acute toxicity of aluminum is relatively low, and its oral lethal dose 50 for rats is 162 mg/kg/BW and 980 mg/kg/BW for mice [22]. The Food Safety Commission of Japan (FSCJ) applied a safety factor of 100 to the no-observed- adverse-effect-level (NOAEL) of 30 mg/kg/BW/day [14]. According to standard 12968 of the National Standards Organization of Iran, the theoretical maximum is the national daily intake, which is calculated in milligrams per person's body weight and expressed as a percentage of the PTDI of the contaminant (heavy metal) and should not exceed the PTDI. The theoretical maximum of the national daily intake is an estimate of more than the actual amount for a pollutant. Furthermore, contaminants may also be reduced during industrial processing, preparation, and cooking. The NTMDI of Al for adults in Iran is set at 0.005, which is less than the PTDI (0.3 mg/kgb/w^{-1}).

When noncancerous HQ reaches the value of one, it represents a high risk of noncancerous diseases in consumers [21]. In the current research, the HQ of each bread sample was less than one, which implied the risk of noncancerous disease. According to a study in Malaysia, aluminum concentration in water was above the standard limit set by the Ministry of Health of Malaysia for drinking water (0.2 mg/l) [23].

Limitations and Recommendations

Due to cost constraints, we were not able to provide more samples. Among the other limitations of our study was the lack of accurate statistical information on the daily consumption of different foods (including bread) by adolescents and children, and the risk assessment of these age groups could not be carried out. Therefore, it is recommended that further investigation be focused on Al levels in other Al-containing food additives, flour, drinking water, and other foods.

Conclusion

In this study, we determined the concentrations of Al, Pb, Hg, Ni, As, Cd, Co, Cr, and Cu in various types of traditional Iranian bread, French bread (baguette), cakes, and muffins. A significant difference was observed between the bread samples in this regard, and Al concentration was highest in Taftan bread (min-max). Fortunately, heavy metals were not detected in the bread, cake, and muffin samples. Therefore, flour-based products are considered an important source of Al.

According to the results, the daily and weekly intakes of Al were below the PTDI and PTWI (except Taftan and Barbari bread) and close to the standard rate. The NTMDI was calculated for Al in Iran based on consumed contents of bread in adults and their body weight. Furthermore, the maximum level (MRL) was calculated based on reliable reports on toxicological properties (NOEL and the safety factor), which also apply to aluminum in food additives. According to our findings, the NTMDI was at the standard rate.

In this study, the risk assessment of Al, as an element associated with immune and brain diseases, was considered significant based on national food safety programs. However, heavy metals were not significantly detected in the samples (<0.05 ppm), and the exact level of these elements could not determine the risk parameters. Although the HQ for each sample was less than one, the risk of Al-related noncancerous diseases was not high. HI was also estimated at 13.22, and its intake through other sources should also be taken into account. In conclusion, high Al concentrations should be properly monitored in Iran, especially in Taftan bread, cakes, and muffins.

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Conflicts of Interest

None declared.

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