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ORIGINAL ARTICLE

Effect of Baking Methods Types on Residues of Heavy Metals in the Different Breads Produced with Wheat Flour in Iran: A Case Study of Mashhad

Sajjad Ghasemi[1,2], Mohammad Hashemi[1,2], Mahdi Gholian Aval[3,4], Mohammad Safarian[2], Saeid Khanzadi[5], Arezoo Orooji[6], Seyedeh Belin Tavakoly Sany[*3,4]

[1] *Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

[2] *Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[3] *Social Determinant of Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

[4] *Department of Health Education and Health Promotion, Faculty of Health, Mashhad University of Medical Sciences, Mashhad, Iran*

[5] *Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[6] *Student Research Committee, Department of Biostatistics, Faculty of Health, Mashhad University of Medical Sciences, Mashhad, Iran*

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KEYWORDS

Baking methods;
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ABSTRACT: Food contamination with heavy metals is the main concern for human health that increases globally. Bread wheat is one of the most important grain products for human consumption. The aim of the study is to examine the effect of baking methods on residues of heavy metals in the different breads produced with wheat flour in Mashhad, Iran. A total of 180 samples of different breads produced in 90 bakery shops in Mashhad was analyzed for some heavy metals (Fe, Cr, Cu, Zn, Pb, Hg, Co, As, Al, V, Cd, and Ni) by inductively coupled plasma-optical emission spectrometry (ICP-OES). Results showed that the average concentration of Fe, Al, Zn, Cu, and Cd in some bread samples were significantly ($p<0.05$) different between traditional and industrial baking processes. The average concentrations of the entire test of Fe (46.35±39.78 $mg.kg^{-1}$), Cr (0.28±0.33 $mg.kg^{-1}$), Al (3.49±1.89 $mg.kg^{-1}$), and As (0.479±0.229 $mg.kg^{-1}$) were higher than the current corresponding safety limit in all type of bread. The results obtained indicated that the government requires implementing more remediation or intervention to control and mitigating the contamination burden of heavy metals in traditional bread in the study area to reduce its associated risks for human health.

INTRODUCTION

Bread is one of the most common food sources in human nutrition in the world[1-5], and bread wheat is the main source of the essential mineral compound because of the high rate of consumption of wheat in this product[6]. Bread

*Corresponding author: tavakkolisanib@mums.ac.ir (S. B. Tavakoly Sany)
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plays an important role in providing more than half of the energy as well as fiber, protein, various vitamins and minerals[1, 2, 4, 7, 8]. The daily intake of wheat bread is significantly high in the Iranian population compared with others countries [1, 2][9]. In Iran, the daily intake of bread wheat is 420 g per person. This quantity in Russia, Europe, United States of America, and Turkey is 164, 274, 55, and 400 g per person, respectively[4].

Food pollution with heavy metals is the main threat to human health, and can cause many diseases and complications [10]. High concentrations of toxic heavy metals in the environment, such as Pb, Cd, Cr, Zn, Cu, Co, Fe, Ni, and As, contaminate the water, soil, fauna, and flora[10, 11]. Bread includes several elements and heavy metals such as Zn, Cu, Co, Pb, Ni, Cd, and Fe. These elements in bread were subject of several researches that examined their concentration and effects on human health and environment [12, 13]. Some minerals can result in acute and chronic effects on human health, which is associated with increased inflammation, oxidative stress, and cancer [14, 15].

Several studies reported that rapid urbanization and industrialization are the main sources to develop and spread the toxic level of heavy metals in the environment and food products. Studies have shown that different sources will increase the concentration of heavy metals in bread. It was evidenced that using sewage sludge and chemical fertilizer during the cultivation of wheat significantly increases the levels of heavy metals، such as Zn, Pb, As, Cd, and Cr, in wheat [16, 17]. For example, wheat used to prepare flour may be prepared in a field with a high content of heavy metals in the soil or maybe irrigated with water with a high content of heavy metals. During the flour preparation process, processing equipment and containers can increase concentrations of trace elements (Fe, Cu, Pb, Cr, and Cu) in the final wheat product [18, 19]. Likewise, bread is also contaminated by heavy metals during its preparation and processing phase. During the processing, some additives (such as water, salt, yeast, baking soda) may contaminate the bread with heavy metals. In addition, baking bread in metal trays, traditional ovens and rotating bakeries is another way to contaminate bread due to insufficient supervision and fuel contamination by heavy metals [4]. Further, changes in food processing and cooking methods also have an impact on increasing the possibility of food being contaminated by heavy metals. Consumption of food that contaminated by heavy metals will cause these substances to enter the human body and gradually accumulate, and cause irreparable damage to human health and the environment [6, 20, 21].

Most of the bakeries in Iran use electricity as sources of energy, light oil (diesel), and heavy oil. electricity and light oil (diesel) were commonly used in the automated bakeries or bakeries use the industrial bread process while the heavy oil was used in the traditional bakeries. Data reporting the effect of the types of oven and energy source on the concentration of nutrients in the baked breads are still equivocal and data limited. Likewise, in Iran, the effect of baking methods on heavy metals concentration in bread has not been studied. With regard to the high daily intake of bread wheat in Iran, greater attention needs to provide reliable information on heavy metal contamination in traditional Iranian bread and compare different baking methods. The aim of the study is to examine the effect of baking methods on residues of heavy metals (Fe, Cr, Cu, Zn, Pb, Hg, Co, As, Al, V, Cd and Ni) in the different bread produced with wheat flour in Mashhad, Iran. In this study, we hypnotized that baking methods can influence heavy metals concentration in the different breads that produced with wheat flour.

MATERIALS AND METHODS

Study area and sample collection

In this survey, 180 samples of several types of bread including Sangak, Barbari, and Lavash were collected from traditional and industrial bakeries in different areas of Mashhad, the center of Khorasan Razavi province, Iran from October 2020 to December 2020. The factorial method was used to estimate the sample size based on considering 5 municipal districts in Mashhad, 2 back methods (industrial and traditional), 3 types of bread (Lavash, Sangak, and Barbari), 2 types of samples (dough and bread), and 3 repetitions. Based on cluster sampling

methods, the whole city of Mashhad was divided into 5 clusters (based on municipal zoning). Then, 18 bakeries (9 traditional bakeries and 9 industrial bakeries) were randomly selected from each region, and dough and bread samples were collected from each bakery in 3 repetitions to study the concentration of heavy metals. Mashhad is located in the northeast of Iran, which is a metropolitan city with population of 3.004 million. In this study, bread (n=90) and dough (n=90) samples were randomly collected from 90 bakeries at different areas of Mashhad in 2020.

Preservation and preparation

Polyethylene bottles pre-washed with double distilled water were used to preserve samples. We transported all bread and dough samples in cool and dark conditions, and all samples were stored at 4 °C until laboratory analysis. At the same time, we used the Official Methods of Analysis (AOAC, 2012) in the laboratory for sample preparation [22]. Ten germs (g) of bread and dough samples were precisely weighed, and oven dried at 100 °C to reduce the moisture and constant weight. Then, all oven-dried samples were homogenized, passed through a 2-mm sieve, and stored polyethylene bottles at room temperature for further analysis

Chemical analysis and quality control assessment

In this study, we purchased all reagents, acids, and standard solutions including internal standard solutions, a multi-element solution, and standard stock solutions) from Merck (Darmstadt, Germany). All dried samples were extracted based on the microwave digestion method (EPA procedure 3050B) [23]. About 1 g of dried samples were digested using a mixture solution (HNO_3 and HCl, 3:3) at 90 °C until a transparent solution was obtained (around 2 hours). After cooling, all digested samples were filtered by filter papers (pore size: 0.22 µm, Whatman Filters, UK) and diluted to 20 mL with distilled water. The heavy metals concentrations (Fe, Cr, Cu, Zn, Pb, Hg, Co, As, Al, V, Cd and Ni) in the filtrate were estimated by inductively coupled plasma-optical emission spectrometry (ICP-OES, Spectro Arcoes, 76004555 model, Germany).

During analysis the procedure, we washed all bottles and glass containers in dilute nitric acid, rinsed them in deionized water, and dried at room temperature. The detection limit for each metal was Fe (0.06 $\mu g.L^{-1}$), Cu (0.1), Zn (0.1), Ni (0.12), As (0.2), V (0.06), Co (0.02), Pb (0.5), Hg (0.18), Al (0.38), Zn (0.1) and Cr (0.06), respectively. We used the blank and standards solutions of metal ions to calibrate the ICP-OES. Heavy metals in each sample were estimated in triplicate to check the reproducibility of the analysis. We also used spiked solation (standard reference solutions with known concentration of the metals) and certified reference materials (CRMs) of the plant included rice flour (804 IRMM) to check the measurement accuracy and precision. The percentages of recovery for all heavy metals in this study were acceptable ranges (87% to 112%), which show the precision and accuracy of the laboratory procedure [24]. Since, the reproducibility for all elements were at 95% confidence level, the average concentrations of each metal were used for final interpretation.

Statistical analysis

The statistical software SPSS 16 (SPSS, Chicago, IL) and Excels 2007 (Microsoft Office) were used to perform descriptive statistics and bivariate *analysis (ANOVA and t-test) in* different groups. P-value < 0.05 was considered as significant level.

RESULTS

Table 1 shows the average concentration of trace elements in dough samples (before baking) and bread samples (after baking) from 90 bread bakery shops based on their dry weight. Results showed that the concentration of Pb and Hg in this research was lower than the detection limit in all samples. There is no significant increase ($p > 0.05$) in the average concentration of Cr, Ni, Co, As, and V in the bread samples after baking either in traditional baking methods or in industrial methods. The average concentration of Fe in Lavash ($p<0.05$, sig: 0.032 and 0.041) was significantly different after the traditional and industrial baking process. It was also significant in Barbari ($p<0.05$, sig: 0.042) after

traditional baking, while this significant difference was not seen in Sangak samples (p>0.05, sig: 0.82). A significant increase was observed in the average concentration of Cu in Lavash (p<0.05, sig: 0.049) and Sangak (p<0.05, sig: 0.01) after traditional baking methods, and the concentration of Zn was also significantly high only in Sangak samples after the traditional baking process. Likewise, our finding showed that the average concentrations of Al in all types of bread were significantly increased (p<0.05) after the industrial baking process, while the average concentrations of Cd in all types of bread were significantly increased (p<0.05) after the traditional baking process.

Table 2 shows the effect of baking methods on residues of heavy metals in the different breads produced with wheat flour. Results showed that the average concentration of Fe, Al, Zn, Cu, and Cd in bread samples were significantly different between traditional and industrial baking processes. The average concentrations of the entire test of Fe, Cr, Al, and As were higher than the current corresponding safety limit presented in the FAO/WHO and European Commission standards in all types of bread. However, our finding showed that others elements in the bread samples did not exceed these standards and were in the safe threshold (Table 2).

Table 1. concentration of heavy metals in Iranian bread before and after baking.

Heavy Metals (mg.kg^{-1})		Type of Bread				
		Barbari *(n=60)*		Lavash *(n=60)*		Sangak *(n=60)*
		Traditional oven	Industrial oven	Traditional oven	Industrial oven	Traditional oven
Fe	Before Baking	28.8±8.73	44.16±37.7	46.2±26.10	53.2±30.10	46.4±23.43
	After Baking	31.9±21.10	45.7±32.01	50.5±26.30	56.85±31.40	45.5±20.84
	[a] p-value	0.042	0.42	0.032	0.041	0.82
Cu	Before Baking	2.2±0.84	2.16±0.55	2.35±0.61	2.2±0.51	2.6±0.87
	After Baking	2.3±0.74	2.22±0.75	2.9±0.39	2.4±0.54	2.7±1.19
	p-value	0.89	0.74	0.049	0.11	0.01
Cr	Before Baking	0.32±0.13	0.26±0.29	0.31±0.16	0.27.5±0.26	0.32±0.19
	After Baking	0.33±0.12	0.31±0.32	0.43±0.28	0.28±0.27	0.34±0.20
	p-value	0.33	0.56	0.11	0.24	0.19
Ni	Before Baking	0.14±0.1	0. 12±0.21	0.10±0.12	0.1±0.14	0.11±0.16
	After Baking	0.15±0.1	0.13±0.16	0.15±0.17	0.11±0.16	0.12±0.19
	p-value	0.37	0.43	0.86	0.64	0.91
Zn	Before Baking	9.4±1.84	8.8±2.03	8.9±1.96	9.7±2.42	11.1±2.77
	After Baking	9.85±4.1	8.7±1.91	9.0±1.47	8.7±2.25	12.6±2.70
	p-value	0.89	0.63	0.74	0.37	0.01
Al	Before Baking	4.0±1.35	3.67±0.86	4.02±1.58	3.23±1.04	4.17±1.27
	After Baking	3.98±0.35	3.81±1.12	4.17±1.34	3.65±1.80	3.96±1.08
	p-value	0.082	0.037	0.23	0.041	0.092
As	Before Baking	0.51±0.05	0.45±0.13	0.47±0.06	0.45±0.17	0.53±0.30
	After Baking	0.47±0.07	0.46±0.12	0.47±0.10	0.51±0.14	0.50±0.09
	p-value	0.43	0.82	0.87	0.066	0.082
Cd	Before Baking	0.02±0.005	0.02±0.013	0.02±0.008	0.02±0.01	0.02±0.009
	After Baking	0.03±0.006	0.02±0.014	0.03±0.007	0.02±0.02	0.03±0.01
	p-value	0.034	0.81	0.041	0.92	0.038
Co	Before Baking	0.04±0.06	0.02±0.03	0.01±0.03	0.03±0.04	0.03±0.03
	After Baking	0.05±0.03	0.02±0.06	0.01±0.02	0.03±0.07	0.03±0.04
	p-value	0.45	0.86	0.93	0.91	0.82
V	Before Baking	0.03±0.05	0.05±0.06	0.05±0.06	0.07±0.07	0.10±0.33
	After Baking	0.04±0.05	0.03±0.05	0.05±0.07	0.08±0.09	0.08±0.06
	p-value	0.054	0.16	0.71	0.32	0.083

n, Number of samples: ± Standard deviation; [a] testing significant differences between heavy metal concentration before and after *baking based on ANOVA and t-test*.

Table 2. concentration of heavy metals between different baking methods

Type of Oven	Heavy Metals Concentration (mg. kg^{-1}) *(n=180)*									
	Fe	**Cu**	**Cr**	**Ni**	**Zn**	**As**	**V**	**Co**	**Al**	**Cd**
Traditional	43.9±23.57	2.34±0.7	0.3±0.2	0.11±0.14	9.83±2.57	0.49±0.16	0.11±0.068	0.026±0.03	3.21±1.23	0.027±0.02
Industrial	48.8±32.43	2.14±0.73	0.27±0.27	0.11±0.15	8.74±2.86	0.46±0.13	0.076±0.072	0.025±0.03	3.77±1.32	0.023±0.01
[b] p-value	0.037	0.02	0.28	0.89	0.001	0.98	0.34	0.74	0.002	0.004
[a] Food Safety limit	5	10	0.1	0.5	50	0.1	-	0.1	0.237	0.1

[a] Permissible limit of European Commission and World Health Organization (FAO/WHO); [b] testing significant differences between heavy metal concentration between industrial bread process and traditional process *based on t-test*; *- the food safety limit was not reported.*

DISCUSSION

Heavy metals entering the food chain and reaching critical concentrations will have adverse metabolic and physiological effects on the human body. Therefore, the control of heavy metals to maintain health must be on the agenda. The aim of the study is to examine the effect of baking methods on residues of heavy metals in the different breads produced with wheat flour in Mashhad, Iran.

The study evaluated the values of 12 metals (Fe, Cu, Cr, Ni, Zn, Al, As, Cd, Co, V, Pb and Hg) in bread samples before and after baking. The values of 5 metals (Fe, Cu, Zn, Al and Cd) showed a significant increase in some bread samples baked by both traditional and industrial baking methods and there were no significant increases in Cr, Ni, As, Co and V values. In addition, the Pb and Hg values cannot be detected.

There was a significant increase in concentration of iron in Barbari and Lavash bread baked in traditional ovens and rotary ovens in the present study. The increase in iron in bread may be related to the type of oven and clay that is used in building the traditional ovens [25]. Clay soils is suitable soils for absorption of different heavy metals because of its suitable chemical and mechanical resistance, high cation exchange capacity, layered structure and high specific surface area of clay soils [25, 26]. In addition, due to the presence of silica in their composition, they are a good place to stabilize and adsorb metals (such as iron)[25, 26].

Cast iron is one of the iron alloys commonly used in the production of rotary ovens because of its low cost and ease of production [25]. Using iron in the preparation of ovens is the ways to contaminate baked breads with iron[20]. The difference in iron value between bread samples may be due to the different enrichment of flour used in some samples, which will affect the value obtained [25]. Few studies have been conducted on the evaluation of the heavy metals in bread before and after baking. It was evidenced that reported a significant increase of iron in 4 types of Lavash, Barbari, Sangak and Taftoon bread baked in a rotary oven and 3 types of Lavash, Barbari and Taftoon bread that baked in a traditional oven in Tehran bakeries [25]. A study in Iraq showed that the baking process can significantly increase the iron concentration in bread[20].

In this study, the concentration of copper baked by traditional methods increased significantly, which may be due to the use of traditional ovens when baking bread. As mentioned earlier, traditional ovens are usually made of different soils, the most common of which is clay[25]. Copper naturally exists in the earth's crust, rocks, soil, sediments and water [11, 27]. Hence, there is a possibility of Cu contamination in soils used in the preparation of traditional ovens in different ways. Copper is also exists in the fuel composition used in traditional ovens [28, 29]. Therefore, the soil structure used in traditional ovens and the presence of Cu in the fuel that used in traditional baking methods may be one of the factors that increase this metal in traditional bread. Several studies reported that the concentration of Cu in Lavash and Taftoon samples increased significantly after baking in a traditional oven [20, 25].

In this study, the concentration of Zn significantly increased in traditionally baked Sangak bread. This result could be due to hot pebbles are spread on a bed of the oven[30-32] because Zn is one of the metals found in these pebbles and rubble [33, 34]. In fact, baking Sangak on pebbles may be

one of the reasons for the increase in the Zn concentration in this bread. This result is consistent with study that was reported in Iraq. They indicated that the baking process led to a significant increase in concentration of zinc in bread samples[20].

In this study, the concentration of Al in Barbari and Lavash samples that baked in the rotary oven increased significantly, which may be due to the influence of the oven. Aluminum is the most abundant metal in nature, and it is the most widely used in cooking equipment, including rotary ovens, because of its good electrical conductivity, uniform heat distribution, and rust resistance [35, 36]. In Iran or other countries, there is no research on the effect of baking methods on the Al the concentration in type of bread.

The significant increase in Cd concentration was observed Barbari, Lavash and Sangak that baked in traditional oven. Cadmium enters the soil by different activities like mining, metal and chemical industries, metalworking water, vehicles and consumable fuels, cadmium-containing fertilizers, and pesticides, as well as the production of some metal alloys and battery-making [37, 38]. Thus, contamination of the soils used in building traditional ovens is one of the reasons for the high level of Cd in breads that baked in the traditional way. The type of fuels that used in baking bread are also the main factor to increase Cd in bread[2]. In the traditional baking method, fuel burners or heat source are placed in a corner of the oven to transfer heat to bake bread[39].

Of the 12 metals examined in the present study, the concentration of Fe, Cr, Al and As were higher than the standard, and the concentration of Cu, Al, Ni, Zn, Cd, Co, and V is lower than the food safety limit. Likewise, in this study, concertation of Pb and Hg were below the detection limit. Several ways lead to enter heavy metals in bread, such as agricultural soils, fertilization of growing cereals, fortification of wheat flour with iron, baking equipment and its depreciation[20, 40, 41]. Chromium, Fe, and Al are also released into the environment and food chain through anthropogenic processes, such as combustion of fossil fuels, leather tanning, industrial waste, metal processing, and sewage [42-44].Aluminum and iron naturally exist in the environment., which is are released by natural processes such as soil erosion, rock weathering and volcanic activity, as well as man-made processes such as pesticides, mining and metal industry[45, 46]. Arsenic contamination in environment and foods occurs via both natural and human activities including mines, pesticides and organic fertilizer. Arsenic is used in pharmaceutical, leather, glass and ceramics industries and also used as feed additives for animals[47, 48]. Our findings are consistent with study that was conducted by Tourchi et al. (2017). They claimed that the Fe concentration in the bread samples in Tehran was higher than the allowable limit, while Cu and Pb were within the standard value. However, the chromium was not detected in their study [25, 49].

CONCLUSIONS

Our study findings revealed that bread-baking methods can increase residues of some heavy metals in the different breads produced with wheat flour such as Fe, Al, Zn, Cu, and Cd. Likewise, the concentrations Fe, Cr, Al, and As were higher than the current corresponding safety limit in all type of bread. The results obtained indicated that the government requires implementing more remediation or intervention to control and mitigating the contamination burden of heavy metals in traditional bread in the study area to reduce its associated risks for human health.

Abbreviation:

lead (Pb); cadmium (Cd); chrome (Cr); zinc (Zn); Cu (cupper); Co (Cobalt); Fe (Iron); Ni(nickel); Arsenic (As), plasma-optical emission spectrometry (ICP-OES); Official Methods of Analysis (AOAC); Nitric acid (HNO_3); hydrochloric (HCl).

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Conflict of interests

All authors declared that they have no conflicts of interest in this work.

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ETHICAL CONSIDRATION

This study was conducted after the approval and permission of Mashhad University of Medical Sciences Research Committee IR.MUMS.MEDICAL.REC.1398.623 and was conducted with consideration of Helsinki Declaration in all phases of the study. Confidential data treatment was guaranteed. Availability of data and materials Data from this study will not be openly available until planned publication outputs have been completed.

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