Measurement of Heavy Metals, Saturated, and Trans Fatty Acids in Fast Foods in Tehran

Hossein Abadi, Shayesteh; Azizi, Mohammad Hossein*+●; Moeini, Sohrab; Mooraki, Nargess

Department of Food Technology, Islamic Azad University - North Tehran Branch, Tehran, I.R. IRAN

ABSTRACT: *Heavy metals, saturated and trans-fatty acids are considered one of the most important food contaminants and health threats caused by natural phenomena or human activities. In this regard, fast foods are one of the sources that have to be monitored regularly as the potent points for potentially toxic elements and saturated and trans-fatty acids. The concentrations of lead, cadmium, and arsenic were measured using atomic absorption spectroscopy in fast foods (pizza, falafel, and chicken nuggets). The public health hazard from the consumption of fast food contaminated with heavy metals was determined using estimated daily intake, target hazard quotients, hazard index, and carcinogenic risk. The concentrations of saturated and trans-fatty acids were measured through the Gas chromatography method. A chicken nugget (0.133 mg/kg) had the highest concentration of arsenic, a pizza (0.123 mg/kg) had the highest concentration of lead, and falafel (0.137 mg/kg) had the highest concentration of arsenic. The majority of the estimated daily intake, target hazard quotients, and hazard index were lower than the world standards except for arsenic in chicken nuggets. The mean concentrations of saturated fatty acids in falafel, mixed pizza, and chicken nuggets were 18.02g/100g, 36.35g/100g, and 19.11g/100g respectively. The mean concentrations of trans-fatty acids in falafel, mixed pizza, and the chicken nugget were 1.12 g/100g, 1.32 g/100g, and 0.79 g/100g respectively. The mixed pizza had a higher saturated fatty acids content of 36.35% (heptadecanoic, stearic acid, and short-chain fatty acids). Therefore, heavy metals such as arsenic and saturated fatty acids in fast foods are a risk to the health of consumers and the only solution for this is to minimize the consumption of fast foods.*

KEYWORDS: *Fast food; heavy metals; Saturated fatty acids; Trans-fatty acids.*

INTRODUCTION

Generally, processed foods contain high levels of salt, fat, sugar, and heavy metals. An excessive intake of these nutrients is perceived as the leading reason for an increased risk of the development of several chronic diseases (obesity, diabetes, cancer, and cardiovascular disease). Generally, food processing is associated with negative effects on the quality and safety of foodstuffs. However, food processing is extremely important to extend shelf-life or just to make them edible [1].

Human health is generally harmed by heavy metal-rich foods, and the increased functional foods intake can help to mitigate these deleterious effects. There have been

** To whom correspondence should be addressed.*
+E-mail address: azizit_m@modares.ac.ir
Other address: ● Department of Food Science & Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, I.R. IRAN
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several reports of Heavy Metals (HMs) being transferred from food processing machines and contaminated soils to different foodstuffs such as; tomatoes, chicken, wheat, rice, fish, and seafood, resulting in pollutant levels exceeding those deemed acceptable for human consumption by the World Health Organization and Food and Agriculture Organization [2]. In this sense, lead, arsenic, and cadmium levels in food products are controlled by Regulation 1881/2006 of the 2006 European Union Council, which set maximum levels in processed food of 0.14, 0.2, and 0.02 mg/kg of arsenic, lead, and cadmium, respectively (EC, 2006a) [2,3]. Toxic heavy metals (i.e., Pb, Cd, and As) cause severe health-related issues when they accumulate in living tissues, especially in internal organs (i.e., spleen, liver, pancreas, and stomach) and cause complications such as decreased immunity, fetal growth retardation, psychosocial-social disorders, and malnutrition-related disabilities. [4,5]. The consumption of arsenic, lead, and cadmium-contaminated foods are the main sources of human exposure to these elements [6]. *Kheirabadi et al*. (2016) reported significant amounts of HM, such as Pb, chromium (Cr), nickel (Ni), and Cd in soil and crops, such as wheat, potato, and maize [7]. *Rouniasi et al*. (2016) demonstrated the presence of Cr and As, in soil and leaf, stem, and root of cabbage, lettuce, spinach, and onions [8]. In Bangladesh, *Shaheen et al*. (2016) evaluated meat, egg, fish, milk, vegetables, cereals, and fruit samples and reported the presence of Cr, Pb, As, Cd, and palladium. High concentrations of HMs in vegetables have been reported in China [9].

Other risk factors to human health include saturated fatty acids and trans fatty acids, both of which have considerable adverse effects on human health [10]. SFAs do not contain double bonds, while non-saturated fatty acids are usually found with at least one bound in trans and cis forms. Cis fatty acids are naturally found in foodstuffs, while TFAs are rarely found in foodstuffs and processed foods. In 1976, the average intake of TFAs in Europe varied between 1.2 and 6.7 g/d [11]. TFAs affect lipoproteins in the blood and because of platelets, endothelial vascular, and inflammatory system function, noncontagious chronic diseases like diabetes, asthma, sudden cardiac death, obesity, blood cholesterol increase, and breast, prostate, and colon cancers are developed [12].

Recent studies worldwide indicate very inhomogeneous data as well as very high levels of HMs, SFAs, and TFAs in processed foods such as pizza, burgers, falafels, sausages, and chicken derivatives products such as chicken nuggets, scallions, etc. Therefore, the present study aims to assess the concentration of SFAs, TFAs, and HMs including Pb, Cd, and As the content of high-risk food groups (Fast Food) such as pizza, falafel, and nugget in Tehran, Iran. Also, the estimated daily intake, target hazard quotients, and food hazard index analyses revealed a novel approach to assessing the risk of processed food [13]. Our results can expand our understanding of the human health risks of heavy metals, the saturated and trans fatty acids in processed food in Tehran, Iran. The findings of this study could provide new insights into valuable information for policymakers to manage these contaminants.

EXPERIMENTAL SECTION

Sample collection and preparation

During October and December 2020, three categories of processed foods (n=45) were collected from food chains and restaurants in five different regions of Tehran, Iran, including north, south, west, east, and central. The selected food samples (cooked) were grouped as mixed pizza (n = 15), falafel (n = 15), and chicken nugget (n = 15). All the acquired items were stored in hygienic conditions and by label instructions (when available) and were analyzed before their expiry date. The sample was composed of at least three units of the same item, and it contained approximately 200 g. All the samples were homogenized in a blender (GM200, RETSCH, Germany) (1 to 3 min, 5000 rpm), depending on the food matrix. The samples were then appropriately conditioned based on their perishability (e.g. frozen at -20 °C for highly perishable foods) until analysis [14].

Sample preparation method for HMs

Each food sample (1 g) was put into a beaker (100-mL) and a mixture of concentrated HNO_3 (10 mL) and concentrated H_2O_2 solutions (2 mL) was added, and then the digestion was performed on a hot plate (200 °C). After the digestion, the obtained suspension was filtered through a filter paper with a blue ribbon and completed to 25 mL with distilled deionized water. Analyses for blanks were carried out in the same way without using samples. Then the preconcentration/separation procedure given above was applied to the obtained solutions. After preconcentration, the final measurement volume was increased to 5 mL, and

Table 1: Recoveries, LOD, and LOQ of analyzed heavy metals.

Heavy metals (mg/kg)	Recoveries (%)	LOD	LOQ	Regression Coefficient (r2)
Cd	99.73	0.017	0.058	0.99
Pb	91.65	0.053	0.172	0.97
As	98.11	0.027	0.085	0.91

flame atomic absorption spectroscopy (FAAS) was used for analysis (AAnalyst 800, Norwalk, CT, USA) equipped with a deuterium background correction system and an air-acetylene burner [14,15].

Measuring HMs

A stock solution containing Cd, Pb, and As (100 mg/L) was prepared by dissolving appropriate amounts of $CdCl_2$, $Pb(NO_3)_2$, and Na_3AsO_4 (≥99.0%) (Merck, Darmstadt, Germany) in deionized water. A working standard solution (10 µg/L of each cation) was prepared daily by diluting the stock solution with deionized water. Also, a mixture of standard solution with a concentration of 1 mg/L of each analyte was prepared in deionized water and injected into FAAS each day (three times) for quality control of the detection system [16]. During each batch, quality control, blanks, calibration standard of each analyte, and standard reference materials were tested. Limits of detection and limits of quantification were determined based on the data from the calibration curve according to [17]. The LODs for the heavy metals were 0.018 mg/kg (Cd), 0.052 mg/kg (Pb), and 0.069 mg/kg (As).

The accuracy of the analytical methods (GF-AAS and ICP-AES methods) used was checked using certified reference material (CRM 414, Community Bureau of Reference, Brussels, Belgium). As shown in Table 1, the results obtained were compared with the certified values, and the recoveries were determined according to *Vukasinovic-Pesic et al*. (2017) [17].

Health risk assessment of HMs

To evaluate the potential health risks associated with long-term exposure to heavy metals in fast food, as well as estimations of daily intake and oral reference dose, the target hazard quotient was developed by the United States Environmental Protection Agency [17]. The hazard index approach was employed to assess the human health risks posed by food metals via all processed foods [17].

The hazard quotient or HI, a ratio of the chronic daily intake and chronic RfD was adopted to represent the non-carcinogenic risks to human health:

$$HI = \sum HQi = \sum \frac{CDIi}{RfDi} \quad (1)$$

where RfD is the reference dose (mg/kg-day) (National Bureau of Statistics of People's Republic of China, 2013), and i is the food type. HI < 1 is assumed, to denote safety over a lifetime. In this study, the health risk of heavy metal exposure was assessed for adults.

The human health risks from fast food consumption were estimated based on the THQ, which is a ratio of the EDI of heavy metal to the oral RfD. THQ < 1 indicates that the intake of fast food, by a population, is assumed safe. The EDI and THQ of heavy metals from fast food were calculated using:

$$\mathrm{EDI} = \frac{1}{\mathrm{BW}} \times \sum_{i=1}^{n} \mathrm{CC} \times \mathrm{MC} \quad (2)$$

$$\mathrm{THQ} = \frac{\mathrm{EF} \times \mathrm{EDI} \times \mathrm{ED}}{\mathrm{RFD} \times \mathrm{AT}} \times 10^{-3} \quad (3)$$

where CC is the heavy metal content in a fast-food (mg/kg) and MC is the daily intake of the fast-food (kg/day) [17,18]; EF is the exposure frequency, 365 days/year; ED is the exposure duration, 70 years; AT is the average exposure time for non-carcinogens, 365 × 70 days; RfD is the oral reference dose (mg/kg-day) [18] and i is the metal category (n = 3 in this study). The EDIs and THQs of Cd, Pb, and As, for adults with average and high fast-food consumption, were calculated.

The Carcinogenic risk assesses the potential of humans contracting cancer from oral ingestion of heavy metals over a lifetime. The carcinogenic risk was calculated using the formula [18,19]:

$$\mathrm{CR} = \mathrm{EDI} \times \mathrm{CSF} \quad (4)$$

Where EDI = estimated daily intake. CSF = factor of cancer slope. CR = carcinogenic risk.When the CR ranges from 10^{-6} to 10^{-4}, it represents a no lifetime risk to carcinogens. When the CR value is greater than 10^{-4}, it is considered a substance of carcinogenic worry. The carcinogenic danger of heavy metals from contaminated fast food is described as the possibility of developing cancer within a 70 years' lifetime [19]. The cancer slope factors were taken as 0.0085, 1.5, and 0.38 (mg/kg/day) for lead, arsenic, and cadmium respectively [19].

Fatty acids determination

Fat extraction

For fat extraction, approximately 10 g of sample were weighed and 120 mL of petroleum ether was added [19].

The solution was stirred (60 min), depending on the matrix's complexity, and a separation of phases was obtained. The upper phase (organic phase) was dried with sodium sulfate (Na_2SO_4) and filtered (Whatman® n.° 42, Maidstone, United Kingdom). Petroleum ether was evaporated using a rotary evaporator (Büchi R-210, Labortechnik AG Switzerland).

Preparation of fatty acid methyl esters (FAMEs)

To determine the fatty acids profile of the selected food samples a cold transesterification method was performed according to the [19, 20], with slight modifications. Fat extracted (0.2 g) were weighed and n-heptane (2.5 mL) and of methanolic KOH (0.25 Ml, 2N) were added. After the separation of phases, the upper phase was dried with Na_2SO_4 and filtered (Whatman® n.° 42, Maidstone, United Kingdom). Standard methyl esters were purchased from Merck Company, Darmstadt, Germany.

Gas Chromatography-Flame Ionization Detection (GC-FID)

The fatty acid profiles were determined by first dissolving 50 microliters of the oil sample in 1 milliliter of hexane, then adding 100 microliters of 0.5 methanolic sodium methoxide and shaking the Eppendorf tubes for 15 min. at room temperature. The methylated sample was then placed in contact with anhydrous sodium sulfate to remove any moisture. A gas chromatograph (Hewlett Packard 6890 series GC-Systems, Waldbronn, Germany) equipped with an autosampler, a programmed split/splitless injector, and a Flame Ionization Detector (FID) was used to perform all the gas chromatography analyses. A stabilized non-bonded poly (biscyanopropyl siloxane), SP™ 2560 column (100 m × 0.25 mm i.d. × 0.2 µm film thickness, Supelco™, CA, USA) was employed. Helium was used as carrier gas at 1.0 mL/min flow rate. The split ratio was 50:1 and this was selected after investigating 25:1 and 100:1 split ratios. The oven temperature was programmed as follows: 60 °C (1 min), then to 168 °C at 17 °C/min (held for 28 min), and then to 235 °C at 4 °C/min (held for 15 min). The split valve was opened 2 min after injection. The injector and detector temperatures were 260 °C and 290 °C, respectively. The software used to process peak areas was HP Chemstation (Rev. B.02.01-SR2, Hewlett Packard, California, USA). FAMEs were identified by comparing the retention times of the standards with those of the samples. The relative percentage of each fatty acid was calculated by internal normalization of the chromatographic peak area. The conversion of FAMEs to their fatty acids was done according to AOAC 996.06 [20].

Statistical Analysis

All experiments were carried out on a completely randomized design basis. Statistical analyses were conducted using one-way analysis of variance (ANOVA) at a distinct Duncans analysis level (5%). The Less Significant Differences (LSD) of post hoc tests performed if interactions among Falafel, Chicken Nugget, and Mixed Pizza groups were significant and presented as the mean ± Standard Deviations (SD). The mean and standard deviation were calculated using Microsoft Excel software. SPSS software version 18 was used for data analysis and the graphs were plotted using Excel software.

RESULTS AND DISCUSSION

Heavy metals concentration

Descriptive statistics for the heavy metal content (Cd, Pb, and As), in fast-food food samples (falafel, Mixed Pizza, and Chicken Nugget) the Tehran, are indicated in Table 2. The Pb content in Chicken Nugget (0.068 ± 0.040) and Falafel (0.072 ± 0.049) had significantly ($p \leq 0.05$) higher concentrations related to mixed pizza samples respectively. The As content in Chicken Nugget (0.1 ± 0.047) had the maximum concentrations related to other samples but the Cd concentrations in all samples weren't significantly ($p > 0.05$) different. The As (0.07 ± 0.062) and Cd (0.013 ± 0.012) had the maximum and minimum concentrations in the measured samples respectively.

Health risk assessment of heavy metals

Hazard index

The hazard index (HI) to adults, of heavy metals (Cd, Pb, and As) in fast foods, are shown in Table 3. The average HIs of the heavy metals decreased in the order of As > Cd > Pb, with all values less than 1: 0.5697, 0.0454, and 0.0369, respectively. Therefore, the exposed adults were unlikely to suffer from obvious detrimental health effects. However, some foods in different regions had HI's values of more than 1. Mixed pizza in the west and center, and chicken nuggets in the south, east, and center had 1.7517, 1.0832, 1.1707, 1.1561, and 10.066, respectively. The non-carcinogenic effect decreases with

Table 2: Evaluating the concentration of heavy metals, percentage of fatty acids and trans in different regions in Tehran

Samples	Pb (mg/kg)	As (mg/kg)	Cd (mg/kg)	trans fatty acids (%)	saturated fatty acid (%)	Region
Mixed Pizza	0.032 ± 0.003c	0.043 ± 0.004b	0.004 ±0.006b	0.54± 0.004c	44.16 ± 0.0451a	North
	0.097 ± 0.001b	0.027 ± 0.001b	0.009 ± 0.002b	1.61 ± 0.005b	29.6 ± 0.0312d	South
	0.033 ± 0.002c	0.21 ± 0.006b	0.009 ± 0.001b	1.33± 0.001b	32.35 ± 0.112c	West
	0.02 ± 0.001a	0.047 ± 0.006b	0.012 ± 0.007a	1.76 ± 0.013a	41.2 ± 0.161b	East
	0.023 ± 0.006c	0.123 ± 0.005a	0.027 ± 0.008a	1.62 ± 0.043b	34.43 ± 0.316c	Center
Chicken Nugget	0.030 ± 0.007c	0.03 ± 0.001b	0.009 ± 0.001b	0.24 ± 0.007c	29.11 ± 0.541d	North
	0.087 ± 0.006b	0.133 ± 0.020a	0.013 ± 0.006a	1.11 ± 0.001b	14.58 ± 0.317e	South
	0.057 ± 0.001b	0.09 ± 0.003b	0.027 ± 0.001a	0.42 ± 0.004c	16.34 ± 0.731e	West
	0.107 ± 0.007a	0.13 ± 0.006a	0.013 ± 0.006a	0.93 ± 0.004c	14.59 ± 0.411e	East
	0.06 ± 0.005b	0.117 ± 0.005a	0.008 ± 0.003b	1.21 ± 0.001b	15.48 ± 0.813e	Center
Falafel	0.04 ± 0.007c	0.02 ± 0.000b	0.009 ± 0.009b	0.85 ± 0.004c	17.2 ± 0.503e	North
	0.043 ± 0.006c	0.02 ± 0.000b	0.003 ± 0.000b	1.43 ± 0.007b	11.13 ± 0.411e	South
	0.137 ± 0.001a	0.02 ± 0.001b	0.025 ± 0.008b	1.19 ± 0.0012b	17.05 ± 0.652e	West
	0.053 ± 0.006b	0.02 ± 0.001b	0.016 ± 0.001a	1.31 ± 0.004b	26.73 ± 0.821d	East
	0.087 ± 0.005b	0.02 ± 0.000b	0.006 ± 0.003b	0.82 ± 0.003c	23.43 ± 0.791d	Center
Standard limits	0.20	0.14	0.02	2.00	36.00	

The results represent mean ± SD for three experiments. Different small letters in each column indicate the significant difference between fractions and concentrations ($p< 0.05$).

Table 3: Comparing the hazard index (HI) of fast foods in different regions in Tehran

Samples	Pb	As	Cd	Standard	Region
	0.0195 ± 0.001b	0.3527 ± 0.001b	0.0137 ± 0.001c	0.3859 ± 0.001c	North
	0.0591 ± 0.001a	0.2171 ± 0.001b	0.0217 ± 0.001c	0.2977 ± 0.001c	South
Mixed Pizza	0.0202 ± 0.001b	1.7095 ± 0.002a	0.0221 ± 0.001c	1.7517 ± 0.019a	West
	0.0122 ± 0.001b	0.3797 ± 0.001b	0.0292 ± 0.001c	0.4211 ± 0.007c	East
	0.0142 ± 0.001b	1.0041 ± 0.001a	0.0615 ± 0.001b	1.0832 ± 0.049a	Center
	0.0182 ± 0.001b	0.0391 ± 0.001b	0.0227 ± 0.001c	0.0801 ± 0.001c	North
	0.0531 ± 0.001a	1.0852 ± 0.012a	0.0325 ± 0.001c	1.1707 ± 0.027b	South
Chicken Nugget	0.0345 ± 0.001b	0.7327 ± 0.001b	0.0615 ± 0.001b	0.8322 ± 0.025c	West
	0.0651 ± 0.001a	1.0582 ± 0.011a	0.0325 ± 0.001c	1.1561 ± 0.022b	East
	0.0367 ± 0.001b	0.9497 ± 0.001a	0.0202 ± 0.001c	1.0066 ± 0.001b	Center
	0.0245 ± 0.001b	0.1627 ± 0.002b	0.0542 ± 0.001b	0.2414 ± 0.001c	North
	0.0265 ± 0.001b	0.1627 ± 0.002b	0.0181 ± 0.001c	0.2072 ± 0.001c	South
Falafel	0.0861 ± 0.001a	0.1627 ± 0.002b	0.1501 ± 0.001a	0.3987 ± 0.011c	West
	0.0325 ± 0.001b	0.1627 ± 0.002b	0.0955 ± 0.001b	0.2907 ± 0.001c	East
	0.0531 ± 0.001a	0.1627 ± 0.002b	0.0367 ± 0.001c	0.2524 ± 0.002c	Center
Total mean	0.002b ± 0.037	0.025b ± 0.569	0.003b ± 0.045	0.011b ± 0.651	

The results represent mean ± SD for three experiments. Different small letters in each column indicate the significant difference between fractions and concentrations ($p< 0.05$).

decreasing HI. Thus, the heavy metals in fast food posed no non-carcinogenic risk to the public across Tehran *via* different exposure pathways.

Estimated daily intake

There are toxic elements accumulating in fast food that are harmful to human health. Therefore, a health risk

Table 4: Target Hazard Quotient (THQ) and Carcinogenic Risk (CR) of heavy metals from the consumption of fast food

Samples	Heavy metals	Target Hazard Quotient (THQ)	Carcinogenic risk (CR)
	As	0.552 ± 0.002b	$5.07 \times 10^{-4\,B}$
Mixed Pizza	Pb	0.418 ± 0.001b	$3.66 \times 10^{-4\,A}$
	Cd	0.015 ± 0.001c	$1.15 \times 10^{-5\,B}$
	As	2.120 ± 0.035a	$7.67 \times 10^{-3\,A}$
Chicken Nugget	Pb	0.316 ± 0.007b	$5.12 \times 10^{-4\,B}$
	Cd	0.011 ± 0.001c	$4.43 \times 10^{-5\,B}$
	As	0.011 ± 0.001c	$6.31 \times 10^{-4\,B}$
Falafel	Pb	0.334 ± 0.005b	$2.72 \times 10^{-3\,A}$
	Cd	0.002 ± 0.001d	$3.42 \times 10^{-5\,B}$

The results represent mean ± SD for three experiments. Different small letters in each column indicate the significant difference between fractions and concentrations ($p< 0.05$).

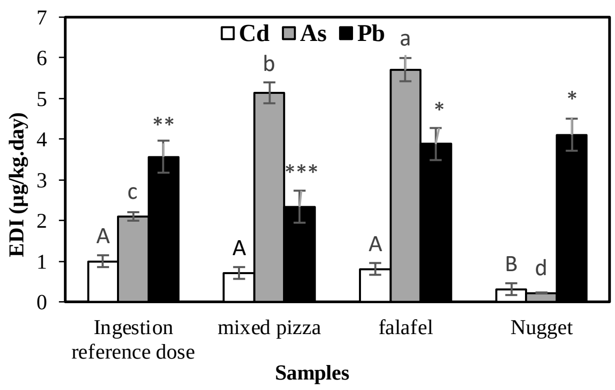

Fig. 1: Estimated daily intake (EDI) of potentially toxic elements in three types of fast food. Different small letters in each column indicate the significant difference between fractions and concentrations (p< 0.05).

assessment is essential for fast food consumption. The EDIs of three toxic elements for adults (70 kg) were calculated based on the concentrations of the toxic elements in the muscle, and their intake by one Persian person (Mainland) [20]. The EDIs of heavy metals, from fast food consumption by adults, are shown in Fig. 1. The EDI in both fast-food samples (mixed pizza and falafel) was significantly ($p \leq 0.05$) greater than the PTDIs (FAO/WHO Expert Committee on Food Additives (Ingestion reference dose (RfD)) in adults [20, 21]. However, it was lower for third elements in chicken nuggets [21]. Therefore, it is suggested that the consumption of mixed pizza and falafel may cause health risks associated with heavy metals.

Target hazard quotient, and carcinogenic risk

The summary of the target hazard quotient and carcinogenic risk due to the intake of three types of fast food from Tehran is shown in Table 4. Arsenic in chicken nuggets had the highest target hazard quotient value (2.12), while cadmium in falafel had the lowest target hazard quotient value (2.22×10^{-3}). The target hazard quotient of Arsenic in chicken nuggets (2.12) was greater than 1. The CR values due to the eating of fast food were shown in Table 4. Arsenic in chicken nuggets (7.67×10^{-3}) and lead in falafel (2.72×10^{-3}) had the highest carcinogenic values, while third elements in mixed pizza had the least carcinogenic risk.

The concentration of SFAs and TFAs

Table 2 shows the average of saturated and trans-fatty acid composition expressed in g/100 g total fatty acids, (mean values and S.D.) and the percentages of saturated, and total trans-fatty acid contents in fast food products (mixed pizza, chicken nuggets, and falafel) in Tehran. Trans fatty acids could be considered low in these kinds of foods varying from 0.79% in chicken nugget to 1.12% in falafel and 1.32 in mixed pizza. Mixed pizza and chicken nugget had the highest and lowest TFAs content respectively. The mean concentration of SFAs in felafel, mixed pizza, and chicken nugget are 18.02, 36.35, and 19.11g/100g respectively (Table 2). In mixed pizza, there is a high proportion of saturated fatty acids (Heptadorcanoic acid (17:0) 22.62─30.42 %, and stearic acid (18:0) 5.53─9.07 (Table 5). Mixed pizza is particularly rich in short-chain fatty acids, the most prevalent are lauric (12:0) and myristic (14:0) acids. Heptadorcanoic acid (17:0) and stearic acid had the highest saturated fatty acids (range 6.81─21.62, 3.11─4.39 %) in falafel and chicken nugget (4.59─23.11, 3.09─5.53 %), respectively.

Discussion

Humans are constantly exposed to the various toxic substances in their environment, which leads to various diseases such as cancer, cardiovascular diseases, and respiratory diseases. Food contamination and the presence of hazardous substances like HMs in foods have become a major concern over the past few years [22]. Today, fast foods are very popular because they are prepared quickly and easily. The HMs contamination in fast foods is undeniable. The HMs concentration compared with the WHO standards that dictate the maximum permissible

Table 5: Percentage of saturated fatty acid components in fast foods in different regions of Tehran

Center	East	West	South	North	Saturated Fat Acid	
0.002a ± 0.63	0.034a ± 0.67	0.004b ± 0.16	0.009b ± 0.2	0.005b ± 0.24	Lauric acid	Mixed Pizza
0.045a ± 2.17	0.007a ± 2.75	0.011b ± 0.67	0.044b 0.54±	0.012b ± 0.8	Myeristic acid	
0.022b ± 22.62	0.135a ± 30.42	1.503b ± 25.22	1.021b ± 25.1	0.771b ± 25.62	Heptadecanoic acid	
0.043b ± 6.5	0.042b ± 6.91	0.102b ± 5.99	0.306b ± 5.53	0.006a ± 9.07	Stearic acid	
0.002a ± 0.25	0.013a ± 0.27	0.006a ± 0.29	0.037a ± 0.31	0.003a ± 0.4	Aracicideic acid	
0.001a ± 0.27	0.015a ± 0.21	0.002a ± 0.18	ND	0.007a ± 0.35	Behynic acid	
ND	ND	0.0012a ± 0.87	ND	0.058a ± 0.82	Lignocric acid	
0.003a ± 0.13	0.043a ± 0.11	ND	ND	ND	Lauric acid	Falafel
0.004a ± 0.35	0.002a ± 0.45	0.003a ± 0.17	ND	0.007a ± 0.12	Myeristic acid	
0.002b ± 18.26	0.045a ± 21.62	0.034c ± 11.65	0.042d ± 6.81	0.054c ± 11.81	Heptadorcanoic acid	
0.001a ± 3.89	0.051a ± 3.69	0.043a ± 4.12	0.047a ± 3.11	0.035a ± 4.39	Stearic acid	
0.012a ± 0.27	0.067a ± 0.32	0.012a ± 0.47	0.012a ± 0.37	0.058a ± 0.57	Aracicideic acid	
0.003a ± 0.32	0.071a ± 0.39	0.047a ± 0.43	0.031a ± 0.56	0.001a ± 0.35	Behynic acid	
0.015a ± 0.14	0.032a ± 0.13	0.078a ± 0.13	0.049a ± 0.17	0.003a ± 0.14	Lignocric acid	
ND	ND	ND	ND	0.047a ± 0.11	Lauric acid	Chicken Nuggets
0.004a ± 0.28	0.008a ± 1.31	0.005a ± 0.26	0.032a ± 0.18	0.047a ± 0.45	Myeristic acid	
0.002b ± 10.77	0.002c ± 4.59	0.002b ± 11.36	0.016b ± 9.97	0.073a ± 23.11	Heptadorcanoic acid	
0.007b ± 3.16	0.004a ± 5.53	0.007b ± 3.34	0.058b ± 3.09	0.009b ± 4.29	Stearic acid	
0.008a ± 0.38	0.002a ± 0.4	0.045a ± 0.47	0.023a ± 0.41	0.003a ± 0.67	Aracicideic acid	
0.002a ± 0.51	0.007a ± 0.31	0.034a ± 0.5	0.041a ± 0.55	0.017a ± 0.26	Behynic acid	
0.012a ± 0.19	0.012a ± 0.14	0.051a ± 0.2	0.058a ± 0.22	0.015a ± 0.14	Lignocric acid	

The results represent mean ± SD for three experiments. Different small letters in each row indicate the significant difference between fractions and concentrations ($p < 0.05$). *ND: Not detected.

concentration of HMs in food. The maximum permissible concentrations of Pb, Cd, and As are 0.2, 0.02, and, 0.14 mg/kg, respectively [23]. The level of Cd in the pizza samples from the central Tehran, in the chicken nuggets from west of Tehran, and in falafels from west of Tehran were higher than others. In other samples, the concentration of HMs was lower than the permissible limits. Those relatively high contaminations of some fast food might be closely related to the pollutants of machinery production and the situation of fast-food cooking [23]. Unfortunately, although Iran has recently opted for the recycling of treated wastewater in irrigation, a great effort is still needed to improve and generalize this reuse. The high levels of lead in all of the studied foodstuffs might be mainly a consequence of road traffic and lead emissions from petrol. Indeed, Iran is, unfortunately, one of the countries where leaded gasoline is still widely used. The variation in heavy metal levels in fast food production could be explained by the distance between sampling points and the oil well, the type and quantity of crude oil used in making fast food, the extent of contamination at each sampling point, the quantity and quality of crude oil that was utilized to cook fast foods, and the extent of contamination of each sampling point [23]. This revealed that the crude oil spillage raised the levels of heavy metals in the oilseeds, and these heavy metals were not removed in the refining process of oilseeds; since crude oil comprises of hydrocarbons and heavy metals. Researchers indicated that the highest concentration of HMs was in chicken nuggets in Bangladesh and reported that the concentration of HMs was higher than the WHO standard [23,24]. *Kheirabadi et al.* (2016) indicated that the concentration of HMs in soil and agricultural products, such as wheat, potatoes, and maize was lower and close to the standard of the Iranian Environmental Protection Organization [24]. *Tajdar-Oranj et al*. (2018) reported a high concentration of Pb and an acceptable concentration of Cd in the noodles available in the Iranian market [25]. The public health hazard from the consumption of fast food contaminated with heavy metals was determined using EDI, THQ, HI, and CR. The average hazard index of metals from the intake of fast foods was lower than 1, which reveals didn't possible health issues for consumers in the future.

The amount of the EDI of the HMs was compared with the ingestion reference dose (RFD) of each HMs. The RFD was calculated from the tolerable weekly intake, determined by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations [25]. The RFD of Pb, As, and Cd is 3.75, 2.2, and 1.0 µg/kg.day respectively [25]. The amount of the EDI of HMs in 22% of fast foods (10 samples of 45 prepared food samples) was higher than the standard RFD. The EDI values calculated in this study, for each metal in each species, were well below the corresponding PTDI values (except As), suggesting that fast-food consumption of the three investigated metals (falafel, mixed pizza, and chicken nugget) will not adversely affect human health in Tehran, Iran. *Nuapia et al*. (2018) conducted a study on cabbage, bean, beef, and fish in South Africa and indicated that EDI of As and Cd was higher than the standard of FDA and EDI of Pb was lower than the standard of FDA [25]. *Haque et al*. (2019) reported that EDI of As and Pb in fast foods was higher than the standard of WHO and EDI of Cd was lower than the standard of WHO [25, 26], which is consistent with the results of the present study. Based on the EDI values obtained in the present study, daily use of fast foods enters a large amount HMs, especially As into the body, which is higher than RFD. The chicken nugget samples from the west of Tehran and As had the highest EDI of HMs, which has negative effects on health.

The THQ of HMs was compared with the standard of the WHO. If the calculated THQ is less than 1, the exposure to that HMs has no adverse effects on the health. If its value exceeds 1, the exposure to the HMs has non-cancerous consequences for the health over time [25, 26]. In the present study, three types of fast foods in five regions in Tehran were collected to assess the accumulation of three toxic elements and the human health risks caused. Interestingly, the results indicated that there is a low non-carcinogenic and cancer risk; the values of THQ and CR were far below the acceptable thresholds (THQ < 1 and 10^{-4} < CR < 10^{-6}). This suggests that there is a low or no human health risk from consuming fast foods. The THQ value is considered to be a reasonable parameter with which to assess the health risk of consuming fast food contaminated with heavy metals [26]. This study showed that As is the most dangerous metal among foodstuffs, since fast foods contaminated by As, will have a relatively higher potential health risks. On the other hand, the potential health risk of Cd is the lowest, which may be ascribed to its higher RfD.

Carcinogenic risk (CR) assesses the potential of humans having cancer due to the oral intake of heavy metals within a 70 years lifetime [26]. The risk of the carcinogenic effect of heavy metals from contaminated fast foods is described as the possibility of having cancer within a 70 years lifetime [26, 27]. Generally, the CR value lower than 10^{-6} is considered to be negligible, above 10^{-4} is considered unacceptable, and lying between 10^{-6} and 10^{-4} is considered an acceptable range [26, 27]. In the present study, the mean CRs of Pb, Cd, and As due to the consumption of fast food were negligible to the acceptable range (Fig. 1). In the present study, the THQ and HI values for the majority of samples were below 1, indicating that the intake of heavy metals from the consumption of these fast food is unlikely to pose a significant risk to humans in the future. *Shaheen et al*. (2016) reported that THQ of Pb, As, and Cd and HI of vegetables and fruits were less than 1. Unlike the present study [27]. *Nuapia et al*. (2018) demonstrated that the HI of foodstuffs such as cabbage, beans, beef, and fish was several times higher than the standard [27]. Additionally, *Fathabad et al*. (2018) indicated that the THQ of HMs in fruit juices and compotes in Tehran were As> Pb> Cd, which is similar to the pattern in the present study. The HI of mixed pizza, chicken nugget, and falafel were 0.3153, 0.3561, and 0.0941 respectively; and THQ of Pb, As, and Cd were 0.0147, 0.2279, and 0.0125 respectively. It is worth noting that As allocated a very high ratio of HI values (90% ≈) in the fast foods [27, 28]. *Abtahi et al*. (2017), in a review study, assessed the HMs concentration of rice in 26 provinces in Iran and indicated that As had the highest concentration in rice from the provinces of the north of Iran such as Tehran, Mazandaran, Babol, etc., [27, 28] which is in line with the study by *Bamuwamye et al*. (2015) [28]. Given that HMs arise from natural activities and human activities and the variables such as geographical location, industrial development rate, water quality, water purification quality, air contamination, soil contamination, and waste management, there were many differences in the HMs concentrations, EDI, THQ, and HI of foods in the present study and other similar studies.

SFAs and TFAs determination

As the results showed, mixed pizza had the highest SFAs and TFAs content. Mixed pizza, chicken nugget, and falafel show a mean saturated fatty acids content of 36.35% (range 29.6-44.16 %), 19.11 %(range 14.58-29.11%), and 18.02 % (range 11.13-26.73%) due to the presence of hydrogenated oils (heptadecanoic, stearic acid, and short-chain fatty acid) in the fat fraction which came from the cheese and components which have been previously fried. In mixed pizza, in descending order, the samples with the highest SFAs content were from the north, east, central, west, and south regions. Fatty acids in natural vegetable oils are mostly unsaturated and are all of the cis conjugation types; trans isomers appear when vegetable oils are partially hydrogenated to make kinds of margarine, shortenings, and a large variety of food products [29]. Compared with cis unsaturated fatty acids, the structure, physical properties, chemical stability, and physiology of trans fatty acids resemble those of saturated fatty acids. The proportion of trans fatty acids could be considered low (<1.76%) in all samples because hydrogenated oils are not present. falafels have been manufactured (majority of them) with oil immersion technique cooking in lauric oils (coconut and palm kernel oils) and palm oil. The present data indicate that mixed pizza and falafel contain trans fatty acids derived from hydrogenation of oils whereas the chicken nuggets are from natural origin (reactions of biohydrogenation in ruminants) or resulting from heat treatments of oils, most probably the deodorization. Pizzas show a mean saturated fatty acids content of 36.35% (percentage of total fatty acids) due to the presence of cheese and short-chain fatty acids. *Asgary et al*. (2009) studied SFAs content in the most popular brands of vegetable oils and showed that more than one-third of SFAs in the products were trans in hydrogenated vegetable oil (1-3%) [29]. *Nazari et al*. (2009) examined the content of SFAs in dairy and corn products and found that stearic acid (C18:0) and palmitic acid (C16:0) had the highest concentrations. In snacks and bread products, the most common TFAs were alaidic acid with concentrations ranging from 2.4% to 18.5%. In addition, in dairy products, vaccenic acid (C18:1, 11T) was the main TFAs with a concentration range of 2.1% to 11.5% [31]. Pasdar (2014) showed that the highest content of SFAs was in Kebab dishes served in restaurants and among different dishes, Kebab-e-Barg had the highest level of SFAs (46.76%) [30]. *Fernández et al*. (2000) conducted a study in Spain and reported that saturated fatty acids content in the chicken burger, hamburgers, French fries, and pizza was 28.4%, 41.7%, 43.4%, and 45.5% respectively; these figures for TFAs were 2.4%, 3.7%, 20.9%, and 3.1% respectively [32].

CONCLUSIONS

Based on the results, the concentration of trans and saturated fatty acids, EDI, THQ, HI, consumption, and absorption of the contaminants in fast foods in Tehran are worrying. Most of the hazard factors of HMs were lower than the standards of WHO and FAO. The EDI values from this study indicated that there was no risk to human health from the consumption of these fast foods. All THQ and HI values suggested that there was no non-carcinogenic risk to human health from exposure to an individual or combined metals through the consumption of these fast foods. In addition, exposure to As through the consumption of the chicken nuggets considered in this study resulted in a carcinogenic risk. The carcinogenic risks from exposure to As, Pb, and Cd through ingestion of mixed pizza and falafel were acceptable and negligible. It is important to note that excessive consumption of fast foods should be avoided, as they may have harmful effects on the health of those who consume them. It is necessary to regularly test the levels of heavy metals in fast foods and to conduct regular questionnaire surveys to obtain updated information on the average daily consumption of fast foods and heavy metals and to understand the possible chronic effects of fast food consumption on consumers. The results of this study will provide a database for the development of a rational fast-food consumption plan for adults. Therefore, it is important to track and prevent As from entering foods through air or water and prevent the complications and consequences of exposure to these HMs such as gastrointestinal diseases and damage to the liver and intestine. On the other hand, the fast-food samples examined in this study were in an acceptable condition in terms of fatty acids; while SFAs in the pizza and falafel samples were worryingly high. Therefore, heavy metals such as As and SFAs in fast foods are a risk to the health of consumers and the only solution for this is to minimize the consumption of fast foods. This suggests that more attention should be given particularly to the lead levels to assure food safety and to protect the citizens from fast food that might injure their health. This study provided some recommendations to minimize the exposure of aliments to lead fuel and improper irrigation water and oilseed. This is the role of government institutions to strengthen national regulations and set effective and efficient procedures for better quality control of irrigation water and oilseed.

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