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Evaluation of the amount of acrylamide in various edible oils and falafels available in Tehran city fast foods

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ABSTRACT

Healthy and pollution-free foods are very effective in maintaining body health. Among the foods that need to be monitored are falafel and edible oils that can be contaminated with dangerous substances such as acrylamide. In this study, falafel samples were prepared from 3 fast food restaurants in Tehran, and 3 types of common crude oils including sunflower, soybean and canola oils were selected from 5 top-selling brands, and sampling was done randomly. After preparation, these pollutants were evaluated by GC-MS. The average acrylamide in oil samples was 0.08 ± 0.02 mg/kg, which was lower than the European Union standard ($500 \mu\text{g/kg}$ for fried potatoes and $100 \mu\text{g/kg}$ for bread). Among the three crude oils selected, soybean oil had the lowest amount of acrylamide (0.05 ± 0.01 mg/kg) and sunflower oil had the highest amount of acrylamide (0.10 ± 0.03 mg/kg). Also, the average amount of acrylamide in falafel samples was 1.80 ± 0.56 mg/kg, which is higher than the mentioned standards. According to the obtained results and since the amount of acrylamide contamination in the falafels available in fast food restaurants is slightly higher than the standards, it is necessary to carry out more monitoring in this field.

1-Introduction

Edible oils are usually obtained from vegetable or animal fats. Vegetable fats are liquid at room temperature. These types of oils are often prepared from plants, fruit kernels, plant seeds, etc. Of course, most animal fats are solid at room temperature and therefore cannot be considered as oil [1]; But some animals, including fish, whales and some animals that live in cold climates, their body fat is in the form of liquid oil [2]. Edible oils are not completely harmful and their proper consumption can help improve health [3]. Consuming the right edible oils can help in cell growth, improve cardiovascular function, absorb effective nutrients, strengthen the body's immune system, and improve brain and nerve health. In reality, the human body needs oil to absorb fat-based nutrients such as vitamins A, D, E, and K and beta-carotene; Therefore, it is very important for nutritionists to choose the best edible oils to meet the needs of the body. Oil is usually used for frying and cooking food. Sometimes, in order to preserve food, oil is added to them [4, 5].

Falafel is fried balls of cooked chickpeas and spices. A 17 gram ball of falafel is approximately 48% fat, 38% carbohydrates, 14% protein and also contains elements such as sodium, calcium, iron and potassium. Falafel is one of the staple foods of the Palestinian people and is very popular in the Middle East. The consumption of this food is common in the south of Iran and today it is considered as a popular ready-made food in the world. There are many types of falafel such as chickpea and bean falafel, chickpea falafel with meat and chickpea falafel with potatoes in different countries, but the most common one is based on chickpeas and vegetables [6-9].

Healthy food consists of useful components for the health of consumers and is free of harmful and harmful substances [10, 11]. The presence of harmful and harmful substances in food causes disruption in the health of consumers [12, 13]. Detection of these substances is one of the

important aspects in research in the food industry [14, 15]. One of those harmful substances is acrylamide (2-propenamide). Acrylamide is an organic and odorless compound with high solubility in water, molecular weight 71.08, melting point 0.3 ± 84.5 °C and boiling point (136 °C at 3.3 kPa / 25 mm Hg). Acrylamide is mostly produced in carbohydrate-rich foods through a process called the Maillard reaction between reducing carbohydrates such as (glucose) and amino acids (especially asparagine). This chemical compound has wide industrial applications. It can be used in water and sewage treatment, mineral processing and paper and pulp processing. Acrylamide is also used as an additive in cosmetics [8, 16, 17].

After consuming acrylamide-containing materials by mice, acrylamide can be quickly absorbed from the animal's digestive system and distributed in different animal tissues. There are mainly two biochemical pathways for acrylamide metabolism. First, it can be bound to N-acetyl-S-(3-amino-3-oxopropyl)cysteine by glutathione-S-transferase (GST) or it may be converted to glycidamide in another pathway by the cytochrome P450 (CYP450) enzyme complex [7, 18, 19].

Polyacrylamide is a non-toxic polymer that has various uses in industry, but its monomer (acrylamide) is known for its toxic properties that affect various human organs. Acrylamide is a potentially toxic compound with a wide range of side effects. Neurotoxicity is one of the main side effects of exposure to acrylamide. Its neurotoxic effect on humans and rodents has been well studied. Acrylamide inhibits the differentiation of human neuroblastoma and glioblastoma cells. Neurotoxic symptoms of acrylamide in humans include ataxia, skeletal muscle weakness, weight loss, and axonal degeneration. Acrylamide is also described as a genotoxic and carcinogenic agent. Glycidamide as the main metabolite of acrylamide is responsible for genotoxic effects. Exposure of DNA to different doses of acrylamide has been shown to induce high rates of DNA damage. Some studies have also

suggested a link between breast cancer and long-term exposure to acrylamide. There are also various studies on the effect of acrylamide on the immune system. Acrylamide reduces the number of lymphocytes and leads to abnormalities in lymph nodes, thymus and spleen [20-23]. The EU standard level for fried potatoes (ready to eat) is 0.5 mg/kg and for wheat and rye-based products is 0.3 mg/kg [24].

Methods for identifying acrylamide in food include HPLC-MS/MS (high-performance liquid chromatography-mass spectrometry), LC-ESI-MS/MS (liquid chromatography-tandem ionization mass spectrometry), and GC-MS. It has been among the most popular because of lower costs, more convenience and higher accuracy [8, 18, 20, 23, 25-27].

Since the consumption of vegetable proteins, including falafel prepared in edible oils, is increasing in Iran, and also due to the possible risks of acrylamide formation in this food item, it was necessary to conduct the present research. Therefore, the aim of the present study is to investigate the amount of acrylamide in prepared falafels available in different fast food restaurants in Tehran and also to investigate the amount of this pollutant in three types of edible crude oil of different brands using the GC-MS method.

2- Materials and methods

2- 1- Sampling

In this study, falafel samples were prepared from 3 fast food restaurants in Tehran, and 3 types of common crude oils including sunflower, soybean and rapeseed oils in 5 best-selling brands (Iran) were selected from grocery stores, and sampling was done randomly.

2- 2- reagents and instruments

From Merck (Germany), reagents including acrylamide (purity $\geq 99.9\%$), ethanol (laboratory grade), dipotassium hydrogen phosphate, hydrochloric acid (HCl 37%), potassium

hydroxide, tetrachlor (for GC analysis), hexaferrocyanide Potassium (carrez I) and zinc acetate (carrez II) were obtained. Zanthhydrol and acetamide (purity $\geq 99\%$) were purchased from Sigma-Aldrich (USA). Agilent 7890A GC-MS or gas chromatograph with Agilent 5975c mass selective detector (inert MSD). The column was an HP-5ms flexible capillary column (5% phenylsiloxane/95% methylpolyorganosiloxane; length: 30 m \times 0.25 mm; df: 0.25 μ m).

To prepare Carrez I solution, 10.6 g of potassium hexaferrocyanide was dissolved in 100 ml of distilled water. Carrez II solution was prepared by mixing 3 ml of acetic acid with 21.9 g of zinc acetate, then its volume was adjusted to 100 ml with distilled water. The initial standard solution of acrylamide and acetamide (2000 μ g/ml) was prepared in methanol. To obtain the working solution, the upper standard solution was diluted with methanol. The stock and working solutions were kept at 4°C until the experiments [28].

2-3- sample size, method and conditions of investigation

Random sampling was determined from the level of fast foods (for falafel samples) and supermarkets (for edible oil samples) in Tehran. 3 samples of fast food falafel (repeated twice) totaling 6 pieces and 15 oil samples (three types of oil in 5 brands) were selected as replicated twice totaling 30 pieces. The total number of our samples was 36, of which 12 quality control and standard samples were prepared and injected into the GC-MS machine. This study was descriptive-cross-sectional and was conducted at Tehran University of Medical Sciences and Food and Drug Organization partner laboratory in February 1402.

2-4- sample preparation

Each sample was properly homogenized or ground. One gram of falafel and oil samples was weighed and 10 ml of potassium hydroxide (KOH) solution in ethanol was added to the samples and then centrifuged with the help of a centrifuge at a speed of 4000 rpm for 5 minutes. The upper phase was separated. After that, one

milliliter of Carrez I and Carrez II solution was added to the carbohydrate and protein precipitation. The samples were thoroughly mixed and then centrifuged again with the help of a centrifuge at 5000 rpm for 20 minutes. Then 60 microliters of zantidrol and 200 microliters of acetamide were added to the solution. The sample was kept at room temperature (25°C) for 30 minutes, and then 2 ml of hydrochloric acid was added to the samples, and this process was done to complete the derivatization part. Then 1 ml of KOH (2 M) and 2 ml of K_2HPO_4 was added to the sample solution. After stirring the solution, the pH was adjusted in the range of 2.7-6.8. The solution was centrifuged at 5000 rpm for 20 minutes and then the upper phase was separated. In the final stage, 450 microliters of ethanol and 80 microliters of C_2Cl_4 The solution was added to the sample, then the sample was centrifuged at 5000 rpm for 20 minutes. The upper phase was removed and 1 μ L of the sediment phase was injected into the GC-MS [28].

2- 5- CG-MS conditions

Helium was the carrier gas that flowed at a flow rate of 0.8 mL/min. The temperature of the injector was 280°C, the injection mode was splitless and the injection volume was 1 μ L. Next, the injector temperature was maintained at 280°C. 100 °C was the initial temperature of the oven, which was maintained for 1 min, and then the temperature ramp rate was set at 20 °C/min to 300 °C, keeping the temperature for 20 min. The total running time was 21 min. The retention time of internal standard and target compound was 9.9 min and 10.2 min, respectively. Acrylamide was measured in selected samples based on selected ion monitoring (SIM) mode [28].

6-2- method performance

A calibration curve was made from a standard solution of acrylamide in the range of 20 to 400 (ng/g) in methanol. In this study, the limit of quantification (LOQ) and limit of detection (LOD) were calculated using the standard deviation of the response (σ) and the slope of the calibration curve (S) based on the following formula:

Formula 1: $LOQ = 3 LOD$

$LOD = 3.3\sigma / S$ formula 2:

The relative standard deviation (RSD) was determined through the analysis of six replicate acrylamide analytes and was 9.72.

7-2- Statistical analysis

Statistical analysis was performed by SPSS statistical package version 18 (SPSS Inc., Chicago, IL, USA). Statistical significance was evaluated using t-test and independent sample one-way analysis of variance (ANOVA).

3- Results

1-3- Calibration curve and validation of the method

LOQ and LOD levels were 66 μ g/kg and 20 μ g/kg, respectively. The amount of recovery was determined by spotting three real samples of acrylamide in the range of 10 to 50 μ g/L. The recovery rate of the method was 93%. Calibration curve between 20 and 200 μ g/g with linear correlation coefficient (R^2) was determined to be 0.9971 linear.

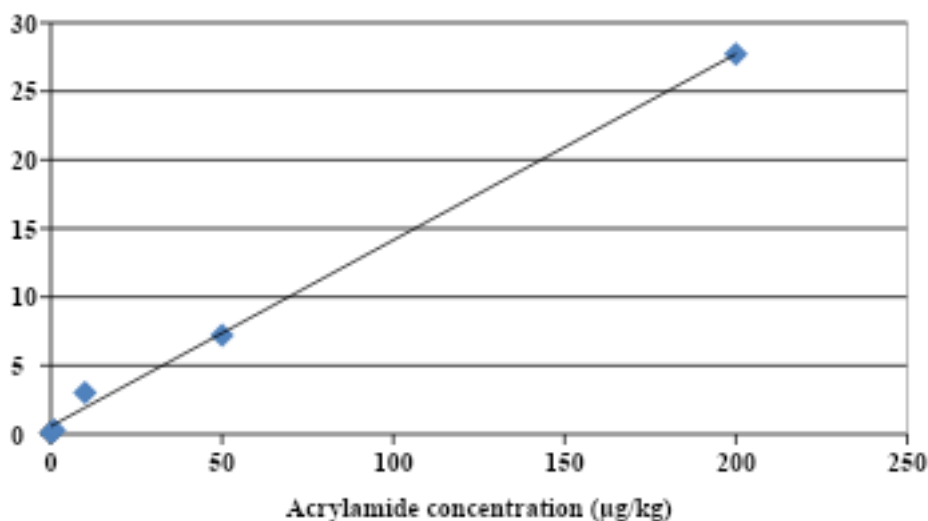


Fig 1- Standard curve for acrylamide concentration

2-3- The amount of acrylamide in all crude oil samples

Table No. 1 shows the amount of acrylamide in common edible crude oils. According to this table, the average acrylamide in oil samples is equal to $0.02 \pm$ It was 0.08 mg/kg , which was

lower than the European Union standard (500 µg/kg for fried potatoes and 100 µg/kg for bread). Among the three selected crude oils, soybean oil has the lowest amount of acrylamide ($0.01 \pm 0.05 \text{ mg/kg}$) and sunflower oil has the highest amount of acrylamide ($0.03 \pm 0.10 \text{ mg/kg}$).

Table 1. The amount of acrylamide in all crude oil samples (mg/kg)

Crude oil		Mean				
Rapeseed oil		0.08 ± 0.02				
Sunflower oil		0.1 ± 0.03				
Soybean oil		0.05 ± 0.01				
Crude oil	Mean	Median	Minimum	Maximum	Std. Deviation	p value
Statistical analysis of all oil samples	0.08	0.08	0.05	0.10	0.02	0.00

3-3- The amount of acrylamide in all fast food falafel samples

Table 2 shows the amount of acrylamide in falafel samples from 3 fast food centers. According to this table, the average amount of

acrylamide is $0.56 \pm$ It was 1.80 mg/kg , which is higher than the mentioned standards. Among the samples of fast food center number 1, the highest amount ($0.24 \pm 3.81 \text{ mg/kg}$) and center No. 2 has the lowest amount ($0.07 \pm 0.32 \text{ mg/kg}$) were contaminated with acrylamide.

Table 2. The amount of acrylamide in all fastfood samples (mg/kg)

Fastfood		Mean				
Fastfood 1		3.81 ± 0.24				
Fastfood 2		0.32 ± 0.07				
Fastfood 3		1.26 ± 0.13				
Fastfood	Mean	Median	Minimum	Maximum	Std. Deviation	p value
Statistical analysis of all fastfood samples	1.80	1.26	3.81	0.32	0.56	0.00

4- Discussion

At the beginning of the discussion, it is necessary to make comparisons with other studies:

Silani et al [29] investigated the amount of acrylamide in different types of nuggets and their results showed that the traditional frying method has a significant effect on the increase of acrylamide compared to the industrial frying method, and it was also found that different cooking temperatures and times increase the formation of acrylamide has a significant effect ($p < 0.05$), but the type of edible oils did not have a significant effect. The highest level of acrylamide was in shrimp nugget (1.5 ± 27 ng/g) fried with rapeseed oil and traditional cooking method (6 minutes at 220°C). While the lowest amount of acrylamide was in chicken nugget (0.1 ± 7.3 ng/g) which was fried with corn oil and industrial method (3 minutes at 180°C).

Pedereschi et al investigated the amount of acrylamide in potato slices and stated that the range of frying time-temperature conditions: 7 minutes at 150°C and 3.5 minutes at 190°C , the acrylamide level of potato slices from 500 to $4500 \mu\text{g/kg}$ increased, which was a 9-fold difference [30].

Matthaus et al evaluated the amount of acrylamide in fried potatoes and stated that the range of frying time and temperature: 10 minutes at 170°C and 190°C , respectively, the level of acrylamide in fried potatoes increased from 800 to $3700 \mu\text{g/kg}$, which A difference was 5-fold [31].

Kenwell et al. measured the amount of acrylamide in potato chips and stated that the range of time-temperature conditions: 1.5, 4 and 12 minutes respectively at 160 degrees Celsius (temperature constant after about 2-1.5 minutes of frying in initial oil temperature of 180°C), the level of acrylamide in potato chips increased from 0.2 to 12 and then decreased to 10 mg/kg (after 12 minutes) [32].

Williams investigated the amount of acrylamide in fried potatoes and stated that the range of frying time and temperature: 3 minutes at 150°C and 5 minutes at 190°C , the level of acrylamide in fried potatoes increased to $7079 \mu\text{g/kg}$ since 1995. found a 3.5-fold difference [33].

Romani et al investigated the amount of acrylamide in fried potatoes and stated that the range of frying time and temperature: 4, 5, 7 and 9 minutes at 120°C respectively, the level of acrylamide in fried potatoes was 50, 68, 208 and $830 \mu\text{g/kg}$ increased [34].

Also, some researchers investigated the amount of acrylamide ($\mu\text{g/kg}$) in consumer breads and stated that its amount is very different, for example Biederman et al. It is 90-40 [36], Pugajua et al. <152-10 [37] and Matheis et al. 36-27 [38]. they reported On the other hand, some researchers reported a higher level of acrylamide contamination in toasted breads, such as Koonings et al., whose level was <1430-30 [39], and Normandin et al., who reported it to be <107-10 [40].

Obesi et al. investigated the amount of acrylamide in consumer cakes with different flavors and their results showed that the maximum and minimum average concentration of acrylamide among the samples respectively correspond to cinnamon cake (212.28 ng/g) and cocoa cake sample (14.1 10 ng/g). The concentration of acrylamide for unflavored samples, cinnamon cake samples, and cocoa cake samples was 61.86 ng/g, 212.28-169.38 ng/g, and 44.64-10.14 ng/g, respectively [28].

The lower amount of acrylamide in crude oils could be due to reasons such as the lack of high heat during the oiling process (acrylamide is usually created through grains and during high heat), the absence of carbohydrates in crude oils, etc.; And the higher amount of acrylamide in fried falafels in fast food was probably due to the use of high heat, the use of leftover or overused oils, and the use of low-quality raw materials (peas, flour, etc.) [28, 29, 31, 33] , 34, 41, 42].

5- Conclusion

The aim of the present study was to investigate the amount of acrylamide in samples of falafels available in fast foods as well as crude oils

produced in Iran. Average acrylamide in oil samples is equal to 0.02 ± 0.08 mg/kg. Among the three selected crude oils, soybean oil has the lowest amount of acrylamide (0.01 ± 0.05 mg/kg) and sunflower oil has the highest amount of acrylamide (0.03 ± 0.10 mg/kg); And the average amount of acrylamide in falafel is 0.56 ± 1.80 mg/kg. According to the results obtained from the present study, the average amount of acrylamide in falafel samples was higher and in crude oil samples, its amount was lower than the European Union standards. According to these results, consumption of falafel can have risks for human health. Among the limitations of the current study is the lack of financial resources, which suggests that in the future, all flour and grain-based products should be evaluated and measured for this pollutant, and a standard should be developed for them in Iran.

6- Thanks and appreciation

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Ethical considerations: The authors have observed all ethical points including informed consent, good behavior, no plagiarism, double publication, data distortion and data fabrication in this article.

7-Resources

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ارزیابی میزان آکریلامید در انواع روغن‌های خوراکی و فلافل‌های موجود در فست‌فودهای شهر تهران

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غذاهای سالم و عاری از آلودگی در حفظ سلامت بدن بسیار مؤثر هستند. ازجمله مواد غذایی که نیاز به نظارت دارد فلافل و روغن‌های خوراکی می‌باشد که می‌تواند آلوده به مواد خطرناکی مانند آکریلامید شوند. در این مطالعه از ۳ فست‌فود موجود در تهران نمونه‌های فلافل تهیه و نیز از فروشگاه‌های مواد غذایی ۳ نوع روغن خام رایج شامل روغن‌های آفتابگردان، سویا و کلزا در ۵ برند پر فروش انتخاب شدند که نمونه‌برداری به صورت رندوم انجام شد. که پس از آماده‌سازی توسط دستگاه GC-MS مورد ارزیابی این آلاینده‌ها قرار گرفتند. میانگین آکریلامید در نمونه‌های روغن برابر 0.02 ± 0.08 میلی‌گرم بر کیلوگرم بود که پایین‌تر از استاندارد اتحادیه اروپا بود (برای سیب‌زمینی سرخ‌کرده ۵۰۰ میکروگرم بر کیلوگرم و برای نان ۱۰۰ میکروگرم بر کیلوگرم بوده است). از میان سه روغن خام انتخاب‌شده روغن سویا کمترین میزان آکریلامید (0.01 ± 0.05 میلی‌گرم بر کیلوگرم) و روغن آفتابگردان بالاترین میزان آکریلامید (0.03 ± 0.10 میلی‌گرم بر کیلوگرم) را دارا بودند؛ و نیز میانگین میزان آکریلامید در فلافل‌ها برابر $0.56 \pm 1/80$ میلی‌گرم بر کیلوگرم بوده است که بالاتر از استانداردهای ذکر شده است. در نهایت با توجه به نتایج به دست آمده و از آنجایی که میزان آلودگی به آکریلامید در فلافل‌های موجود در فست‌فودها کمی از استانداردها بالاتر است، ضروری است که نظارت‌های بیشتری در این زمینه صورت گیرد.

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