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Investigation of Effective Factors on the Formation of Acrylamide and Benzo(a)Pyrene in the Baking Process of Different Bread types Traditional, Semi-industrial and Industrial

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Abstract

Bread as staple of the Iranian may be contaminated by a variety of contaminants such as benzo(a)pyrene and acrylamide. Acrylamide and benzo(a)pyrene are classified as group 2A and group 1 carcinogens, respectively. Therefore, due to the high consumption of bread in our country and the carcinogenic effects of these toxic substances, the effective factors on the formation of these compounds in the process of bread baking were investigated .In this study benzo(a)pyrene and acrylamide were determined in 126 samples of traditional Sangak bread, semi-industrial Sangak bread and industrial bread collected from Tehran and Shiraz cities in IR Iran, then the effect of various parameters on bread contamination with acrylamide and benzo(a)pyrene as well as the relationship between the amount of benzo(a)pyrene and acrylamide in bread were investigated .The results showed that there was a significant difference between the concentration of acrylamide in the different types of bread examined (P<0.001) and between the concentration of benzo(a)pyrene in traditional Sangak breads and industrial breads (P<0.05). On the other hand, there is a significant correlation between temperature and the concentration of benzo(a)pyrene in Shiraz semi-industrial Sangak breads (P<0.05). Also, a significant linear correlation was observed between acrylamide and benzo(a)pyrene concentrations in Tehran traditional Sangak bread samples. This study showed that, reducing the temperature and using the proper baking method could be important factors in reducing acrylamide and benzo(a)pyrene fromation in bread samples.

Keywords: Acrylamide, Benzo(a)Pyrene, Industrial bread, Iran, Traditional bread

Introduction

Bread is an important part of human diet. In some developing countries, bread and cereal products make up 80 percent of the population's staple food, and in almost half of the countries in the world, bread provides food for most of the human (Zahra Hadian, 2011). In

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the diet of Iranians, bread and flour products are the most important food and supplier of most calories, protein and B vitamins and are also minerals (Rajabzadeh, 2017). Mean bread ingestion rate in Iran for urban and rural population has been estimated to be 310 g per person per day (Ebadi Farzaneh, 2015)

At different stages of bread preparation, raw materials and processed bread may be contaminated in different ways. These contaminants include heavy metal such as lead, cadmium and nickel (Khaniki, Yunesian, Mahvi, & Nazmara, 2005), mycotoxins such as deoxynivalenol, zearalenone, nivalenol and ochratoxin (Pacin, Bovier, Cano, Taglieri, & Pezzani, 2010; Patel, 2008; Scudamore, Hazel, Patel, & Scriven, 2009), polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (Ahmed, Hadi, El Samahy, & Youssof, 2000; Eslamizad, Kobarfard, *et al*., 2016; Eslamizad, Yazdanpanah, *et al*., 2016) and acrylamide (Eslamizad, Kobarfard, Tabib, Yazdanpanah, & Salamzadeh, 2020; Eslamizad *et al*., 2019; Keramat, LeBail, Prost, & Jafari, 2011; Mustafa, Kamal-Eldin, Petersson, Andersson, & Åman, 2008).

Acrylamide has been classifed as 'probably carcinogenic for humans'(category 2A) (lnternational Agency for Research on Cancer, 1994). French fries, bread and pastries, coffees and breakfast cereals are foods that play an important role in people's exposure to acrylamide through diet. Other foods make less than 10 percent of the acrylamide intake through the diet (Claeys *et al*., 2010; European Food Safety Authority, 2011; Vinci, Mestdagh, & De Meulenaer, 2012; World Health Organization, 2011).

Another highly carcinogenic compound is benzo(a)pyrene, which is a polycyclic aromatic hydrocarbon (PAHs). This substance is recognized by the IARC as group 1 carcinogen (International Agency for Research on Cancer, 2010). PAHs may be produced in food as a result of thermal decomposition of certain nutrients (such as triglycerides, fatty acids, steroids, cholesterol, and amino acids) (Chen & Chen, 2001). Cereals (bread, cookies, cakes, rice, pasta, etc.) account for 20% and 40% of the daily intake of benzo(a)pyrene and total polycyclic aromatic hydrocarbons relative to total food, respectively (Rey-Salgueiro, García-Falcón, Martínez-Carballo, & Simal-Gándara, 2008).

As mentioned, acrylamide and benzo(a)pyrene are produced during food processing, and bread is one of the most important foods that may be contaminated with these two compounds. Since bread is considered as the staple food in IR Iran, it is important to study the formation of acrylamide and benzo(a)pyrene as well as the factors influencing the formation of these toxins and the relationship between their formation. As far as we know, the present study is the first study to evaluate the effective factors in the formation of acrylamide and benzo(a)pyrene in the process of baking Sangak bread and also to investigate the relationship between the concentration of benzo(a)pyrene and acrylamide in bread.

Materials and methods

Sample collection: 126 samples of traditional Sangak bread, semi-industrial Sangak bread and industrial bread were collected from Tehran and Shiraz. To investigate the affecting factors on formation of acrylamide and benzo(a)pyrene, a questionnaire was completed during sampling.
Determination of acrylamide and benzo(a)pyrene: first, bread samples were blended and homogenized, and then extraction and clean up processes optimized. Acrylamide and benzo(a)pyrene in the samples were quantified using Liquid chromatography mass spectrometry and gas chromatography mass spectrometry, respectively, and the methods were validated. After determining the amount of acrylamide and benzo(a)pyrene in bread, the effect of various factors such as temperature, baking time, degree of baking (raw, normal, toasted and very toasted), dough preparation time and using yeast in the formulation on the bread contamination to acrylamide and benzo(a)pyrene and also the relationship between the amount of benzo (a) pyrene and acrylamide in bread (traditional Sangak bread of Tehran and

Shiraz and Sangak semi-industrial bread of Shiraz) were investigated. Statistical analysis was performed using chi-square test, one-way ANOVA and correlation test in SPSS 17 and GraphPad Prism 6 software.

Results and discussion

The mean concentrations of benzo(a)pyrene in traditional Sangak bread samples of Tehran and Shiraz, semi-industrial Sangak breads of Shiraz and industrial breads of Tehran were 0.83, 0.50 and 0.25 ng/g, respectively. The effect of baking degree and yeast on the amount of benzo(a)pyrene in bread are shown in Tables (1) and (2).

Table 1. The effect of baking degree and using yeast on the amount of benzo(a)pyrene in Shiraz traditional Sangak bread

Parameter		Number of non-contaminated samples (%)	Number of contaminated samples (%)	*P* value
Level of cooking	Raw	0	0	0.91
	Normal	12(85.7)	2(14.3)	
	Toasted	11(84.6)	2(15.4)	
	Very toasted	1(100)	0(0)	
Existence of yeast	Yes	22(88)	3(12)	0.127
	No	2(50)	2(50)	

Table 2. The effect of baking degree and using yeast on the amount of benzo(a)pyrene in Shiraz semi-industrial Sangak bread

Parameter		Number of non-contaminated samples (%)	Number of contaminated samples (%)	*P* value
level of cooking	Raw	0	0	0.47
	Normal	24(82.8)	5(17.2)	
	Toasted	13(92.9)	1(7.1)	
	Very toasted	4(100)	0(0)	
Existence of yeast	Yes	40(77)	6(13)	0.87
	No	1(100)	0(0)	

The results showed that there was a statistically significant relationship between temperature and the amount of benzo(a)pyrene in Shiraz semi-industrial Sangak breads (P<0.05). There was also a significant relationship between the amount of benzo(a)pyrene in traditional Sangak breads and industrial breads (P<0.05). Various studies show that cooking temperature and modification of processing methods could affect benzo(a)pyrone amount in food (Chen, Kao, Chen, Huang, & Chen, 2013; Rey-Salgueiro *et al.*, 2008; Zhang, Chen, & Zhang, 2021).

The mean concentration of acrylamide in Tehran and Shiraz traditional Sangak bread samples, Shiraz semi-industrial Sangak breads and Tehran industrial breads were 26.43, 46.36 and 8.02 ng/g, respectively. The results showed that there was no statistically significant correlation between bread baking time and dough preparation time with the amount of acrylamide in traditional Sangak bread in Shiraz. The results of correlation test showed that there was no statistically significant relationship between bread baking temperature, baking time and dough preparation time with the amount of acrylamide in Shiraz semi-industrial Sangak bread. In addition, the results showed that there was a statistically significant relationship between the amount of acrylamide in different types of breads (P<0.001).

Studies have shown that temperature and baking method have a great effect on the formation of acrylamide (Krishnakumar & Visvanathan, 2014). In the present study,

temperature and baking methods were different and, therefore, there was a significant relationship between the amount of acrylamide and the different types of breads.

The results of analysis of variance (ANOVA) showed that there was a statistically significant difference between the type of bread as an independent variable and the concentration of benzo(a)pyrene as a dependent variable [f(2,134)=62.3, (P<0.05)]. Also, there was a statistically significant difference between the type of bread as an independent variable and the concentration of acrylamide as a dependent variable [f(2,83)=9.36, (P<0.001)] (Fig 1).

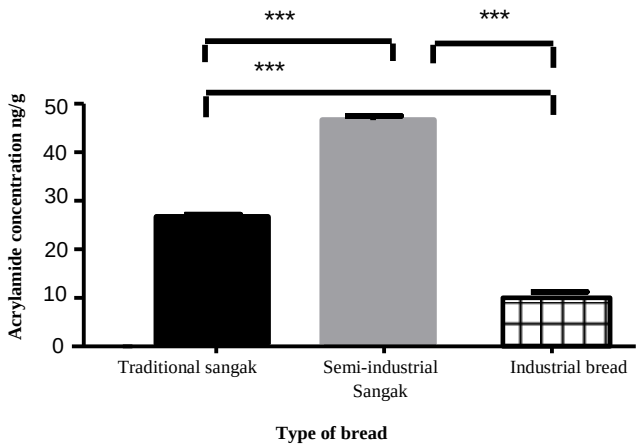

Fig 1. Relationship between acrylamide concentration and type of bread (Tehran and Shiraz traditional Sangak bread, Shiraz semi-industrial Sangak bread and Tehran industrial bread).
The results are shown as mean ± standard deviation. *** P<0.001 Significant difference with the compared group.

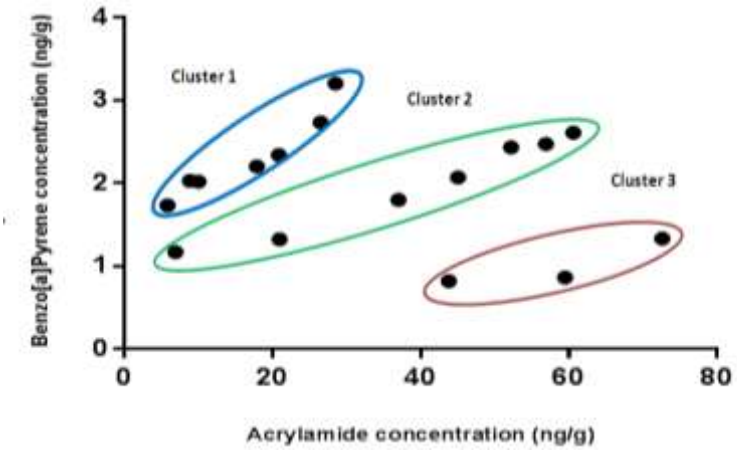

Fig 2. Relationship between the amount of benzo(a)pyrene and acrylamide in bread (Tehran and Shiraz traditional Sangak bread and Shiraz semi-industrial Sangak bread)

The obtained patterns in Fig. (2) show the linear relationship between the concentrations of benzo(a)pyrene and acrylamide in the bread sample. This means that the presence of each of these two contaminants can indicate the presence of the other one.

Conclusions

Considering the significant relationship between temperature and the amount of benzo(a)pyrene in semi-industrial Sangak breads in Shiraz and the lower contamination rate of these two contaminants in industrial breads (that have lower baking temperatures), it can be concluded that reducing the temperature and using the appropriate baking method can be important factors to prevent the formatyion of these toxins.

Also, according to the obtained linear relationship between the amount of acrylamide and benzo(a)pyrene in the bread samples, it can be concluded that any preventive action to reduce one of these two contaminants with a probability of 98% can lead to a reduction of other contaminant.

As far as we know, this study is the first study on the effective factors in the formation of acrylamide and benzo(a)pyrene in the process of baking Sangak bread and also to investigate the relationship between the amount of benzo(a)pyrene and acrylamide in bread. Therefore, it is necessary to conduct additional studies to investigate the affecting factors on the formation of these two compounds and also the relationship between the amount of benzo(a)pyrene and acrylamide in the different types of breads that are used by Iranian people.

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