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## Research article

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## Evaluation of iron content in bakery flour samples of Tehran, Iran

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#### ABSTRACT

Given that iron-deficiency anemia is a major nutritional problem in Iran and bread is one of the main foods in Iranian household basket, a flour fortification program with iron was established in 2001. Thereafter, to quality control of the mentioned program, the iron concentration was measured in flour samples. Accordingly, this study was conducted to investigate the iron content in wheat flour samples in Tehran, Iran. One hundred and twenty-one samples of wheat flour (i.e., Confectionery, Taftoon, Setareh, Barbary, Sangak wheat flour) were randomly collected from bakeries in Tehran by simple random sampling method. The content of iron was determined by flame atomic absorption spectrometry. The mean levels of iron in Confectionery, Taftoon, Setareh, Barbary, Sangak flour were 18.56  $\pm$  5.64 ppm, 28.32  $\pm$  1.74 ppm, 17.21  $\pm$  5.02 ppm, 32.81  $\pm$  3.98 ppm, 14.02  $\pm$  4.99 ppm, respectively. The mean iron concentration of all sample groups was not complied with the minimum recommended level set by the Iranian Ministry of Health and Medical education (40 ppm). The mean iron content of all tested flour was significantly ( $P \le 0.05$ ) lower than the minimum recommended level. The highest iron level was obtained in Barbary (32.81 ppm) and Taftoon (28.32 ppm) flour. While the lowest mean iron level was obtained in Sangak flour (14.02 ppm), followed by Setareh (17.21 ppm), and Confectionery (18.56 ppm) flour. In conclusion, it was identified that the iron fortification program in Tehran, Iran was not well performed as the minimum required level of iron in wheat flours (40 ppm) was not fulfilled. Therefore, the supervision and encouragement of the authorities to provide ironfortified flours is highly recommended.

## 1. Introduction

A large percentage of the world's population suffers from latent hunger and micronutrient deficiencies [1]. According to the World Health Organization (WHO), at least 1 in 5 people in the world suffers from a lack of nutrients, which leads to irreparable damage such as fatigue, hair loss, headache, dizziness, decreased mental function, fetal tube deficits, low birth weight, low intelligent quality (IQ), reduced work abilities, disease, and death in some cases [2,3]. Iron deficiency anemia (IDA) is the most common eating disorder

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worldwide [1]. Iron is a component of red blood cells and is attached to hemoglobin, which is responsible for carrying oxygen and carbon dioxide in the blood [4]. In the early stages of IDA, the body's iron content are depleted and the serum iron level decreases, then the total iron-binding capacity (TIBC) of the transferrin increases, and the serum ferritin, the size of the red blood cells (RBCs), and the hemoglobin content in each RBC decrease [5]. Despite widespread access to iron-rich foods, IDA is still prevalent in the 21st century [6]. According to WHO statistics, anemia affects more than 3 billion people in the world now, and more than 75% of these patients also show symptoms of iron deficiency and depletion of iron in the body [7,8]. The global prevalence of anemia in children of preschool, school age, pregnant women, non-pregnant women, men, and the elderly is equal to 47.4%, 25.4%, 41.8%, 30.2%, and 12.7%, respectively. The highest prevalence of anemia has been reported in Africa and Southeast Asia [9]. According to WHO estimates (2011), the prevalence of IDA among Iranian children, non-pregnant women, pregnant women, and all women of reproductive age was 32%, 28%, 26%, and 28%, respectively [10]. In Yazd (Iran) and Semnan (Iran), the prevalence of anaemia was reported as 9.5% and 4.5% in high school girls, respectively [11].

Given that an iron-deficient diet is associated with many chronic diseases, many countries adopt low-cost, high-efficacy, and lowadverse-effect strategies like food fortification (enrichment) with iron. According to the Codex definition, food fortification refers to the addition of one or more nutrients to the food whether or not it is normally present in the food (such as wheat flour, corn flour, cereals, rice, and spices) to prevent or correct a deficiency of one or more nutrients among people or a particular group [12,13]. As Wheat (*Triticum aestivum* L.) and wheat flour are among the main significant foodstuffs which used to make a variety of confectionery and bakery products in many countries [14], it is generally fortified with compounds such as iron, folic acid, calcium, and vitamins (A, D, and B). These elements are added either directly in the factory or indirectly by fertilizing the crop during the growth phase [15]. Currently, 50 countries have implemented cereal and flour fortification standards [16]. In developed countries, forced wheat flour iron fortification has been implemented for many years and increased the iron intake [17]. Studies have shown that fortification in Europe contributes positively to the nutrition of micronutrients in adults and children [18]. It does not also have the gastrointestinal side effects that are often found in iron supplements and is an effective and safe strategy to combat IDA [19]. The national per capita consumption of wheat in Iran was estimated as 178 kg/year. Moreover, the bread consumption rates were 230–505 g/person/day in 1993 [20]. Taken together, considering the fact that IDA is one of the most common nutritional problems in Iran along with the high consumption rate of bread as a staple food in Iran [12] flour fortification with iron was launched in this country in 2001 to reduce the prevalence of IDA as it can cover a large part of the vulnerable population at low cost as well as wealthy population [20].

In this regard, the Iranian Ministry of Health and Medical Education have set a recommended level of 40–85 ppm as the final content of iron in iron-fortified flour. While the iron-fortified flour premix consists of 30 ppm ferrous sulphate and 1.5 ppm folic acid [20].

By applying the quality assurance protocols like the evaluation of iron content in fortified-flour, the adequacy and quality of these products from production to consumption will be ensured [20]. To the best of our knowledge, there is a limited number of quality assurance studies in terms of the determination of iron content in fortified-flour in Iran hence this study was conducted to quantify the iron content of wheat flour in Tehran, Iran.

#### 2. Materials and methods

#### 2.1. Sample collection

In this study, 121 different samples of Sangak, Barbary, Taftoon, Lavash, Setareh, and confectionery wheat flour were randomly collected from different bakeries in Tehran. Samples were gathered and stored in clean polythene bags according to the protocols of the laboratory. Then, the samples were transferred to the laboratory under suitable conditions (0–4  $^{\circ}$ C) and were kept at –18  $^{\circ}$ C throughout the study period.

#### 2.2. Sample preparation

The preparation steps were performed based on AOAC Official Method 999.11 [21]. Briefly, 5.0 gr of flour samples were weighed using a laboratory scale (with 0.001 gr accuracy). To remove moisture, the samples were placed in an oven at a temperature of  $110 \,^{\circ}$ C until reaching a constant weight. Then, to form ash the samples were burned on an indirect flame inside an electric furnace at a temperature of  $550 \pm 50 \,^{\circ}$ C for 8–10 h. After completely turning the flour sample into white-gray ash, 5 ml of 6 M hydrochloric acid was added to the crucible, so that all the contents of the crucible ash were mixed with the added acid. After placing the crucible on a water bath or heater, the added acid was evaporated. To dissolve the remaining contents inside the crucible, 10–30 ml of 0.1 M nitric acid was added and heated for a few minutes on a boiling water bath or heater, and the resulting solution was carefully transferred to a balloon. It was then reached a final volume of 100 ml using deionized water. At this stage, the sample is ready to be injected into the device.

## 2.3. Instrumental analysis

The iron content of the samples were measured by the flame atomic absorption spectrometry. The iron content was read using standard solutions of Iron (III) nitrate. Adsorption of 0.5, 1, 1.5, and 2 mg/L standard solutions were read at the wavelength of 248.3 nm and then the sample absorbance was read [22,23,24].

#### 2.4. Statistical analysis

Data analysis was performed using SPSS software, version 18 (SPSS Inc., Chicago, IL, USA). The normality of the data was determined by Kolomogorov-Smirnov test. All data were normal. One sample T-test was performed to calculate the significance level of mean difference between groups and the recommended level. The significance of the mean difference in the concentrations of iron between 5 groups of flours were compared at 95% confidence level by one-way analysis of variance (ANOVA) followed by Tukey's test.

### 3. Results

In this study, the iron content in different wheat flour were evaluated. Table 1 displays the results of the iron analysis. The mean levels of iron in Confectionery, Taftoon, Setareh, Barbary, Sangak flour were  $18.56 \pm 5.64$  ppm,  $28.32 \pm 1.74$  ppm,  $17.21 \pm 5.02$  ppm,  $32.81 \pm 3.98$  ppm,  $14.02 \pm 4.99$  ppm, respectively. The mean iron concentration of all sample groups was not complied with the recommended level set by the Iranian Ministry of Health and Medical (40 ppm). The mean iron content of all tested flour groups was significantly (P  $\leq 0.001$ ) lower than the recommended level. The highest iron level was obtained in Barbary (32.81 ppm) and Taftoon (28.32 ppm) flour. While, the lowest iron level was obtained in Sangak flour (14.02 ppm), followed by Setareh (17.21 ppm), and Confectionery (18.56 ppm) flour. ANOVA test showed a statistically non-significant difference between the studied groups in terms of iron concentrations at a 95% confidence interval (P  $\leq 0.05$ ) (Table 2).

## 4. Discussion

Evidences show that more than 40% of the world's population may be under severe threat of micronutrient malnutrition such as iron and zinc deficiency [25]. Among all micronutrients, iron deficiency is a matter of global concern. Due to high-rate consumption of diet that are rich in phytates, the bioavailability of essential nutrients like iron is limited [25,26]. It is a problem of both advanced and developing societies [9]. Fe is one of the trace minerals that are of great nutritional importance. An adult male's body contains 3.6 g of iron, while an adult female's total body contains 2.4 g [27]. Iron is a component of red blood cells and is attached to a protein called hemoglobin, which is responsible for carrying oxygen and carbon dioxide in the blood, Iron also plays an important role in the body's immune system [27]. Anemia and iron deficiency anemia are common diseases in Iran [12]. About 0.8 million deaths occur annually due to iron deficiency disease. It is noteworthy that more than half of the anemia in the world are due to nutritional deficiency of iron [28]. The most vulnerable populations are people of South and Southeast Asia and Sub-Saharan Africa [25]. Anemia is a major public health disorder affecting 1.62 billion people world-wide. The prevalence of anemia is estimated at 43% in low developed countries. Women of reproductive age and children younger than five years are more prone to iron-deficiency anemia. This is due to increased iron requirement during growth and pregnancy. In many developing countries, more than one out of every three preschool children and one out of two pregnant women are estimated to be anemic [25,26]. Due to the worse consequences of iron-deficiency anemia (IDA) including pre-term births, still-births, intrauterine growth and fetal growth retardation, and low birth weight infants, IQ reduction emotional imbalance in children, WHO and UNICEF highlight the urgent of following an integrated, long-term approach to prevent and control of iron deficiency. Commercial iron supplements are quite often ineffective. While, iron fortification of a suitable staple food has become one the main applied strategies implemented to overcome the disease [25,26,29]. Food fortification is an effective, simple, cheap, public health intervention strategy to prevent or correct a nutrient deficiency in the specific population by adding one or more essential nutrients to food. Food fortification based on dietary habits of the targeted population is the most long-term, cost-effective approach in reducing the prevalence of iron deficiency. The efficacy of iron fortification in the improvement of iron status relies on multiple variants like the iron status of the affected population group, the iron compound, the nature of the fortificants, and type of packaging material, transport, storage, processing conditions, and food preparation techniques [30].

The programs of food fortification with iron has been used in developed countries for many years. In general, the most common food carriers used for fortification include cereals (especially wheat and corn) [31]. Cereals, especially wheat, have a high nutritional value for human life in developing countries. Wheat provides almost 70% of the world's food [32,33]. In this respect, wheat flour and bread are nominated as suitable carrier for iron fortification in many Eastern Mediterranean countries, due to the high consumption of bread [30,34] and a flour fortification program with iron in these countries has been proposed as an effective strategy to reduce iron deficiency anemia [20]. Bread provides more than 50–90% of the essential protein and energy for the body [35,36,37]. The main types of bread produced in Iran are Sangak, Barbary, and Taftoon which are made from wheat flour [32,34,38,39]. Apart from being as

## Table 1

les.

Type of flour	Ν	Mean $\pm$ Std. Error	Significance level with minimum recommended level (40 ppm)	Mean Significance level with other groups
Confectionery flour	6	$18.56\pm5.64$	0.001 <sup>a</sup>	0.41
Taftoon flour	47	$28.32 \pm 1.74$	0.001 <sup>a</sup>	0.41
Setareh flour	6	$17.21\pm5.02$	0.001 <sup>a</sup>	0.41
Barbary flour	57	$32.81 \pm 3.98$	0.001 <sup>a</sup>	0.41
Sangak flour	5	$14.02\pm4.99$	0.001 <sup>a</sup>	0.41
Total	121	$\textbf{28.81} \pm \textbf{2.07}$		

<sup>a</sup> The mean difference is significant at the 0.05 level.

#### Table 2

Comparison of mean	levels of iron	in the different	flour samples.
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Flour (I)	Flour (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Confectionery flour	Taftoon flour	-9.75	9.80	0.85	-36.93	17.41
	Setareh flour	1.34	13.05	1.00	-34.84	37.53
	Barbary flour	-14.25	9.70	0.58	-41.15	12.64
	Sangak flour	4.53	13.69	0.99	-33.41	42.49
Taftoon flour	Confectionery flour	9.75	9.80	0.85	-17.41	36.93
	Setareh flour	11.10	9.80	0.78	-16.06	38.27
	Barbary flour	-4.49	4.45	0.85	-16.84	7.85
	Sangak flour	14.29	10.63	0.66	-15.18	43.77
Setareh flour	Confectionery flour	-1.34	13.05	1.00	-37.53	34.84
	Taftoon flour	-11.10	9.80	0.78	-38.27	16.06
	Barbary flour	-15.59	9.70	0.49	-42.49	11.30
	Sangak flour	3.19	13.69	0.99	-34.76	41.14
Barbary flour	Confectionery flour	14.25	9.70	0.58	-12.64	41.15
	Taftoon flour	4.49	4.45	0.85	-7.85	16.84
	Setareh flour	15.59	9.70	0.49	-11.30	42.49
	Sangak flour	18.78	10.54	0.39	-10.44	48.02
Sangak flour	Confectionery flour	-4.53	13.69	0.99	-42.49	33.41
	Taftoon flour	-14.29	10.63	0.66	-43.77	15.18
	Setareh flour	-3.19	13.69	0.99	-41.14	34.76
	Barbary flour	-18.78	10.54	0.39	-48.02	10.44

The mean difference is significant at the 0.05 level.

highly consumed staple foods in Iranian diet, bread is a particularly useful vehicle since the risk of organoleptic deterioration as a result of iron pro-oxidative properties is lower than other foods along with a higher lipid content and longer shelf-life [30]. Flour fortification with iron is being adopted by Egypt, Saudi Arabia, and the Islamic Republic of Iran as a means of combating micronutrient malnutrition. A premix is then added to the flour directly by gravity or by air convection using a pneumatic system [30]. In accordance with our findings, Nejabat et al. demonstrated that the concentrations of Fe in all of the wheat samples were below the permissible limits set by the Food and Agriculture Organization/World Health Organization and Iranian National Standard Organization [40]. Likewise, Tegegne et al. showed that the concentration of Fe in dry weight was  $0.4-36.45 \text{ mg kg}^{-1}$ . The author concluded that the levels of determined Fe in the analyzed cereal samples were found below the permissible limits set by FAO/WHO [41]. Moreover, Alhendi et al. showed that the concentration of all the elements remained below the Iraqi standardization and FAO/WHO limits, which is consistent with our study [42]. Doe et al. indicated that the amount of iron in wheat flour samples was below the detection limit and is consistent with our study [43]. Similar to our results, Sawyerr et al. showed that the concentration of Fe was  $0.1-0.6 \text{ mg kg}^{-1}$  that was within the permissible limit [44]. Consistent with our findings, Pirhadi et al. assessed the concentration of Fe in wheat, flour of Sangak and Lavash bread samples. They concluded that the concentration of Fe in all 270 samples was less than the permitted limit set by the European Commission and JECFA committee [45]. It is demonstrated that factors such as wheat variety, type of fertilizer to wheat fields, type of imported wheat, mixing different types of wheat, and adding premix powder are effective on the level of metal elements in fortified wheat flour [27].

#### 5. Conclusion

According to results of this study, the level of iron was not complied with the minimum recommended level set by the Iranian Ministry of Health and Medical education (40 ppm). The iron content of all flour was significantly lower than the recommended level. So, regular quality control of wheat flour is quite necessary in factories to provide the recommended iron level. The findings of the current study will be served as data to provide useful information regarding the content of iron in fortified wheat flour to inform the regulatory authorities to seek more influential surveillance and encouragement strategies to reach the standard levels.

## Author contribution statement

Sara Mohamadi, Najmeh Yazdanfar, Boshra Ebrahimi-Nejad, Samira Shokri, Mohadeseh Pirhadi, Parisa Sadighara, Tayebeh Zeinali: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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#### Data availability statement

Data will be made available on request.

#### Declaration of interest's statement

The authors declare no competing interests.

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#### S. Mohamadi et al.

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