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The Effect of Baking Process on Stability of Fortified Folic Acid in the Breads

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ABSTRACT

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Aim: determine the effect of different procedures of bread preparation including fermentation and baking on folic acid content of most popular types of breads.
Background: Folic acid deficiency can be harmful specifically for the vulnerable population worldwide. The most reliable way to provide enough folic acid to population is adding it to the staple foods. Bread is the most important staple food for Iranian population. However, food preparation may affect folate stability.
Methods: The aim of this study was to determine the effect of food processing, including fermentation and baking on folic acid content of folic acid fortified Iranian breads such as Lavash, Taftoon and Barbari. The flour was fortified with folic acid (0.15 mg/100 g). Breads were prepared by fortified flour, sourdough, salt and water.
Results: Folic acid was extracted first and then was determined using the HPLC after the preparation procedure. The folic acid content in the all of the three types of flour (Lavash, Taftoon and Barbari) was changed significantly during the fortification. There was no significant difference in folic acid content between dough before and after the fermentation process. The folic acid content was decreased after the baking of all types of breads. This reduction was lowest in Barbari breads. This might be due to the lower baking temperature and higher thickness of Barbari.
Conclusion: Approximately 24-40 percent of the folic acid content of the three types of breads was decreased due to the baking process.
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Introduction: Folate is a water-soluble vitamin. The synthetic form of folate is folic acid. Folic acid is one of the essential nutrients that body needs to survive. Getting enough supply of folic acid from natural sources of food is difficult, because, natural

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folate sources have only 60% of synthetic vitamin activity. Besides the supplementation of folic acid, the most reliable way to provide enough folic acid to human body is folic acid fortification of the staple foods such as wheat flour or corn bulgur (1). As a coenzyme, folate is a single-carbon transporter which is essential for the synthesis of nucleic acids DNA and RNA (2). Another important role of folate is its involvement in the conversion of harmful homocysteine to the safe methionine and also the production of S-adenosyl-methionine as an important methyl donor. Studies have indicated that folate deficiency can lead to a critical health problems in human such as cardiovascular disease, dementia, depression, Alzheimer's disease, neural tube defects (NTD), megaloblastic anemia, cancers, premature delivery, low birth weight, fetal growth retardation, preeclampsia, and congenital heart defects (3-6). Scientists estimated that consumption of folic acid before and during the pregnancy could decrease the risk of NTD by 50-60 percent. However, as women usually are not aware of their pregnancy at the beginning of the pregnancy, they generally miss the folate supplementations. Therefore, food fortification is an effective way to increase the folate intake for the vulnerable population (7). The intake of folate is 45% less than the recommended amounts in the countries without the national fortification programs (3). Bread is produced and consumed widely by the majority of people every day. Therefore, the fortification of flours could be a cost-effective national program to decrease the risk of folate deficiency (8). Results of a study performed in Iran showed that the prevalence of low folate and vitamin B_{12} levels and hyper homocysteinemia is noticeably high (9). In other studies in Iran and Canada, the rate of NTDs and the prevalence rate of spinal bifida decreased significantly after a flour fortification program with folic acid (10, 11). The study done by Abdollahi *et al* in Golestan province showed that the average daily intake of folic acid was 198 micrograms, and 14.3% of healthy women had folic acid deficiency in their fertility age. Despite the absence of a national program to monitor congenital defects, the incidence of these defects in several studies in Iran has been reported to be high compared to many other countries (10, 12). Therefore, daily intake of folic acid is recommended to prevent these diseases. Mandatory folic acid fortification program was implemented in Iran since 2007 (13, 14). According to the codex definition, enrichment involves the addition of one or more essential nutrients to the food with the aim of preventing or correcting the complications caused by deficiency of that nutrient in general or a specific group of populations (15). World Health Organization (WHO) has suggested 1.5 mg/kg of folic acid for enrichment of flour (16, 17). Folic acid loses its activity in acidic and alkaline environments, presence of light, oxidizing or reducing agents. However, folate is relatively stable while exposed to heat and humidity (8). Some studies revealed that food processing such as fortification can even increase the folate content (18). Barbari, Taftoon and Lavash breads are the main types of bread made in Iranian bread industry. Therefore, the aim of this study was to determine the effect of different procedures of bread preparation including fermentation and baking on folic acid content of most popular types of Iranian breads. such as Lavash, Taftoon and Barbari.

2. Materials and Methods

2.1. Preparation of fortified breads

Wheat flour is provided by Derakhshan Co. Iran. The specification of flours is mentioned in Table 1. Wheat flour is fortified by adding

the Premix of folic acid and ferrous sulfate (200 ± 10 grams per 1000 kg of wheat flour) and mixing it with a rotary blender to ensure a thorough diffusion of folic acid into the flour (this premix of folic acid and ferrous sulfate is produced inside Arad Charta Company, Poonan, Iran). The premix contains: 42% dry ferrous sulfate (88% purity), 0.75% folic acid (95% purity) and 57.25 % maize starch. The final folic acid concentration of flour is 1.5 mg/kg of dry weight.
The initial sourdough is a mixture (mixing time was 10 minutes) of 200 ml of vinegar, 500 g of yogurt, 20 kg of flour (Taftoon or Lavash or Barbari, it depends on type of bread) and 8 liters of water (28^c-30^c c). Then the prepared dough is put in a stainless steel dish to rest for 6-8 hours at the temperature 25-30^c c and pH = 4-4.5. Then the initial sourdough is prepared (soft & tightness when tiny bubbles are seen in the sourdough). This initial sourdough used for all of dough (Taftoon, Lavash, Barbari).
To make final sourdough for Lavash and Taftoon breads, the initial sourdough is added to 65 liters of water. Then this combination is added to 120 kg of flour and 15 liters of water. For Barbari bread, the initial sourdough is dissolved in 60 liters of water and then 80 kg of flour is added to it. Then this compound is mixed using a mixer. The mixing process continued until absorption of water was completed and a uniform sourdough is obtained. Finally, the sourdough was placed at a temperature of 28-30 °C for 150 minutes, until the sourdough is become ripe. The dough of bread (Lavash, Taftoon, Barbari) is prepared by mixing 80-100 kg of wheat flour (it depend to type of bread), water, sourdough and 0.8 to 1.2 g of sodium chloride. The ingredients are mixed using a mixer for 10 min at speed of 35 rpm to make it uniform and it is allowed to be fermented at a temperature of 28-30 °C for 2 hours. The appearance of bubbles in the dough, rising up to about 15 cm, creating a loafing-like texture inside the dough and its pleasant smell were considered as the signs of enough fermentation and the dough being ready.
For preparation of Barbari bread, 600 gram of dough was separated and placed to rest for 10 minutes. Then, that shaped into oval sheets with a thickness of 2cm, a length of 50 cm and a width of 20 cm. For preparation of Taftoon bread, 300 gram of dough was separated and placed to rest for 10 minutes. Then that shaped into round sheets with a thickness of 1cm, and diameter of 50 cm. For preparation of Lavash bread, 200 gram of dough was separated and placed to rest for 10 minutes. Then that shaped into oval sheets with a thickness of 3 mm, a length of 50 cm and a width of 30 cm.
Finally, thin sheets of dough are transferred to a fire oven for cooking with a paddle. Breads are baked at the temperature 332°C for 1-2 min, 315°C for 2-3 min, and 250 °C for 15-20 min for Lavash, Taftoon, and Barbari breads, respectively. The baked breads are allowed to cool at room temperature (Table 2).

2.2. Sampling the fortified breads

Forty-five breads are chosen randomly to analyze their folic acid content. These included: 3 samples from each types of unfortified flour (Barbari, Taftoon, and Lavash breads), 3 samples from each types of standard fortified flour (Barbari, Taftoon, and Lavash breads), 3 samples from each types of fortified dough (Barbari, Taftoon, and Lavash breads) before standard fermentation, 3 samples from each types of fortified dough (Barbari, Taftoon, and Lavash breads) after fermentation and 3 samples from each types of bread (Barbari, Taftoon, and Lavash breads) after baking. Flour samples were stored at ambient temperature and were used directly for analysis. Dough and bread samples were frozen to prevent deterioration and stored at -20˚C until the analysis.

2.3. Folic acid extraction and determination

In order to extract folic acid, 17.4 g of Potassium dehydrate phosphate (K_2HPO_4 mw: 174.18, Merck, Germany) was dissolved in distilled water and reached a volume of one liter to obtain a solution of 0.188. Then % 0.1w/v of ascorbic acid (Merck, Germany) and % 0.1 w/v of 2 mercaptoethanol (Merck, Germany) were added to the buffer. The slurry was then adjusted to pH = 8-8.5 and the stock of folic acid standard using concentration of 1 mg/ml was prepared. It should be noted that the maximum storage time for folic acid was one week at -20 ° C and other standards were provided daily.

The standard solutions using concentration of 20000, 10000, 5000, 2500, 2000, 1000, 750 ng/ml were prepared for plotting the calibration curve. To prepare calibration curve, 100 µl of the standard solution in the specified concentration range was injected into the HPLC column (C18) and measured by a diode array detector (DAD, 360 nm). The retention time for folic acid was captured for 25 minutes. Standard calibration curves were plotted by the concentrations of folic acid standards against absorbance.

Diethylene Triamine Pentaacetic Acid (DTPA, MW = 393.35, Sigma-Aldrich, USA) is used as sequestering agents to remove trace metals. This compound bonds to iron which is added as premix to wheat flour and prevents its participation in chemical reactions. To prepare DTPA solution, using 1 mmol/1 L of concentration, 0.4 DTPA was mixed with distilled water to reach the volume of 1 liter and then 1N NaOH (Merck, Germany) was added to solution until dissolving DTPA.

Flour samples were used directly while frozen dough and bread samples were milled at cold temperature. At first, extracted solution containing 500 ml DTPA and 5mL dibasic-phosphate (containing 2-Mercaptoethanol and ascorbic acid, pH= 8-9) was added to 2 g of the samples. Then the solution was shaken for 30 min and centrifuged for 10 min at 9000 rpm at 4 °C. The supernatant was removed using a syringe and passed through the PTFE nozzle filter (pore size = 1 micron). The final purified solution was collected in a 1-ml amber vial and 100µl of extracted samples were injected into a high performance liquid chromatography (HPLC) column (19). Analyses were performed using HPLC (Agilent, USA) equipped with C18 column (25 cm × 4.6 mm × 5 µm), UV detectors (DAD model 1260 G1315D, USA) set at 290 nm. The isocratic mobile phase containing formic acid 0.2% in deionized water: methanol (88:12) was used with a flow rate of 1ml/min. The amount of injection was 100µl and column temperature was 50°C. All data were re-arranged based on recovery results and reported as mean ± SD on a moisture-free basis. HPLC was validated for accuracy, precision, linearity and stability (20). Validation was carried out based on the ICH Q2R1 guideline (21). The limit of quantitation (LOQ) and limit of detection (LOD) were determined using the signal-to-noise ratio. Analytical Method Validation for Linearity, Sensitivity and Accuracy was done according to (22) that LOD and LOQ were calculated using the formulae LOD = 3× s/b and LOQ = 10 × s/b, respectively, where s (standard deviation on the intercept point of calibration curve) and b (slope of the calibration curve).

Variables were reported as mean ± standard deviation (SD). The normality of data was tested using Kolmogorov Smirnov test. One-way analysis of variance was conducted to compare folic acid content between different products. The Tukey's test was used to determine which of the means differed. The data was analyzed in SPSS version 24. Differences were considered significant when p≤0.05.

3. Results

The characteristics and processing condition is presented in Tables 1 and 2. The folate content of the unfortified flour and fortified flour presented in Table 3. The folate content of unfermented dough, fermented dough, and bread (after baking) are presented in Table 4. The folic acid content in the all of the three types of flour (Lavash, Taftoon and Barbari) had a significant difference between before and after the fortification with folic acid. There was no significant difference in folate content between dough (Lavash, Taftoon and Barbari) before and after the fermentation process. However, the folate content was decreased significantly after baking in all types of bread and this reduction was lowest in Barbari bread (24.28%) compared to Taftoon (39.82%) and Lavash (35.3%), which was due to the higher thickness and lower baking temperature in Barbary compared to two other types of breads (Fig. 1).

Table 1. Characteristic of flours

BREAD	Ash* (%)	Humidity (%)	Protein* (%)	Gluten (%)	Acidity (%)	Extraction (%)
Barbari	0.70-0.85	14.20	11.50	27	2.40	78-82
Lavash/ Taftoon	0.85-1.13	14.20	11.50	26	3.50	82-87

***Based on the dry weight**

Table 2. Preparation conditions for different types of bread

Conditions	Barbari	Lavash	Taftoon
Baking temperature (˚C)	250	332	315
Baking time (min)	15-20	1-2	2-3
Fermentation temperature (˚C)	28-30	28-30	28-30
Fermentation time (min)	120	120	120

Table 3. Compare folates content (µg/100 g dry basis) between unfortified flour and fortified flour

Bread	Unfortified flour	Fortified flour
Lavash	135±11.48[a]	1473.5±53.18[b]
Taftoon	267±97.92[a]	1514.25±32.44[b]
Barbari	174±36.23[a]	1535.75±52.7[b]

*Values are means and standard deviations of triple measurements.
** The different letters indicate significant differences (P < 0.05).

Table 4. Comparison of folates content (µg/100 g dry basis) between flour, unfermented dough, fermented dough, and bread in different types of bread

Bread	Flour	Unfermented dough	Fermented dough	Baked bread
Lavash	1473.5±53.18[b]	1213.75±126.18[b]	1226.25±86.6[b]	793.5±23.38[d]
Taftoon	1514.25±32.44[b]	1168.75±65[b]	1221.75±48.93[b]	735.25±14.63[d]
Barbari	1535.75±52.7[b]	1418.75±155.16[b]	1288.5±216.39[b]	975.75±51.13[c]

*Values are means and standard deviations of triple measurements
** The different letters indicate (referring to the row) significant differences. (P < 0.05)

4. Discussion

The results of this study showed that the content of folic acid did not change significantly in the dough before fermentation compared to fortified flour.

Also the amount of folate did not change significantly after fermentation compared to amount before the fermentation. Our results are different compared to some published studies such as Kariluoto *et al* in which the total content of folate increased significantly after the fermentation (23). Furthermore, Gujska *et al* and Osseyi *et al* reported that the level of all folate content was increased in the dough (11, 24). We also found that folate content was decreased significantly after baking of all types of breads and reduction was lowest in Barbari bread. The folate content was decreased significantly after baking in all types of breads and this reduction was lowest in Barbari bread, which was due to its higher thickness and lower baking temperature compared to two other types of breads. The thickness of Lavash bread is very low. Taftoon is a bit thicker than Lavash, and Barbari is the thickest bread used in this study. Thickness of bread probably decreases the temperature of the bread in the baking process. This is probably responsible to a higher decrease of the folate content in Lavash compared to Barbari bread. Our result was in same line with the results of Kariluoto *et al* (23), which showed that the content of folic acid in rye and wheat breads was reduced by 25 % and also with the study of Anderson *et al* in which it was observed the heat degradation of folic acid in bread between 21.9 and 32.1% during baking (11, 25). Moreover, in the study of Omar *et al*, the total content of folic acid in Baladi bread was reduced after baking (24). However, our results are in contrast with some other findings including Osseyi *et al* who revealed that folate content in breads is not reduced after baking and Vahteristo *et al* who showed folate content is increased after the baking process in fish and chicken fillets (25). It is difficult to elaborate the reason of this discrepancy because the amount of folate after fermentation and during baking process is being affected by various factors, such as the type of fermentation and composition of sourdough and their micro flora. Sourdough is a combination of water and flour in which lactic acid bacteria (LAB) initiate lactic acid fermentation. The mechanisms of reduction or increase in the amount of bioactive compounds in sourdough under process are mainly unidentified, however, some studies have indicated that a number of LAB are able to produce folate (16). Moreover, Kariluoto *et al* believed that synthesis of folate was suppressed due to the reduced pH during the fermentation process (23). Other factors that may affect the folate content including the type and duration of fermentation process, the bread type, the natural amylolytic and biological activity of flour which depends on flour, preparation of flour in food processing of bread and baking temperature (23).

According to Iranian national statistics, Iranian population consume around 320 grams of bread daily (unpublished data: Iranian Ministry of Health), which equals 60 percent flour (192 grams daily). After considering the amounts of folic acid loss during the baking process (which is around 25-40%, according to the type of the breads), Iranian bread consumers receive 40-60% of their daily folate requirements from folic acid-fortified breads, which is a considerable part of their folate needs.

5. Conclusions

In our study, the folate content is decreased significantly after baking process in all types of fortified flat breads (Lavash, Taftoon and Barbari) due to the high temperature. However, Barbari bread showed the lowest reduction compared to the others. It may be due to the lower inside-temperature in baking process and also the higher thickness of bread. Furthermore, no change of folate after fermentation may be due to the composition of sourdough. The analysis of the bioavailability of folic acid inside the human body is recommended.

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