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Research Article

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Investigation effects of baking processes on folic acid stability in fortified wheat via high performance liquid chromatography

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ABSTRACT

The main objective of this study was to investigate the floic acid content in various Iranian breads. Analysis of folic acid content eas performed by high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection after extraction and preconcentration of folic acid by using solid phase extraction colume. The effect of various baking process, baking temperature and storage time on the folic acid content of bread in different baking step including wheat, dough before fermentation, dough after fermentation and baked bread was studied. The results showed that fermentation step has important effect in folic acid stability in various flours. Baking temperature and duration do not have *a* significant *impact on folic acid content in various breads. In addition, due to instability of folic acid by exposure to light, air and water, significantly decrease in folic acid contents were observed after storage in stores. Therefore, fresh bread has maximom content of folic acid after backing.*

Keywords: Folic acid; High performance liquid chromatography; solid phase extraction; Fourtified bread.

INTRODUCTION

Folate and folic acid are forms of a water-soluble B vitamin . Folate occurs naturally in food, and folic acid is the synthetic form of this vitamin [1]. The chemical structure of the folic acid is shown in Fig. 1. Folic acid has different important functions in the human body. It plays a major role in the synthesis of red blood cells, in the formation of RNA and DNA [2-5]. Folic acid is used for preventing and treating low blood levels of folate (folate deficiency), as well as its complications, including anemia and the inability of the bowel to absorb nutrients properly [6, 7]. Folic acid is also used for other conditions commonly associated with folate deficiency, including ulcerative colitis, liver disease, alcoholism, and kidney dialysis [8-10]. Women who are pregnant or might become pregnant take folic acid to prevent miscarriage and neural tube defects, birth defects such as spina bifida that occur when the fetus's spine and back do not close during development [8, 11-13]. It is also used to prevent heart disease and stroke, as well as to reduce blood levels of a chemical called homocysteine [14]. Folic acid is used for memory loss, Alzheimer's disease, age-related hearing loss, preventing the eye disease age-related macular degeneration (AMD), reducing signs of aging, weak bones (osteoporosis), jumpy legs (restless leg syndrome), sleep problems, depression, nerve pain and an inherited disease called Fragile-X syndrome [15-17]. Therefore, folate and folic acid have essential role in body healthy, cell division and growth processes. Three ways were used to increase folate intake in the population such as consumption of foods naturally rich in folates, use of folic acid supplements and consumption of foods fortified with synthetic folic acid. Folate is found naturally in some foods include leafy vegetables, okra, asparagus,

fruit, yeast, mushrooms, meat, orange juice and tomato juice. But, most people do not get all the folic acid they need through food alone. Recently, in the many countries, many food products are fortified with folic acid [18-24]. Therefore, folic acid has been added to cold cereals, flour, breads, pasta, bakery items, cookies, and crackers. Folic acid is not stable. It is easily destroyed by exposure to light, air, water, and cooking processes. Like most water-soluble vitamins, folate can frequently be removed from foods during processing. Therefore, it is necessary to determinate loss of folate in the manufacture of foods processing.

Wheat flour in various food products are the mainstays of Iranian diet. Bread was considered the more important food to fortify because it is less expensive than rice and consequently more widely consumed [14, 19, 25-29]. Therefore, Fortification of the wheat flour used for various bread baking with folic acid was recently introduced in Iran. Several methods were used to bake bread. The baking method has essential effect in the folic acid contents in bread.

This study aimed to evaluate the effect of baking process on the level of folic acid in various wheat flour before and after baking in different method. For this purpose, three categories of wheat flour including Taftoun, lavash and barbari were selected as model sample (Fig. 2) and the sample breads were randomly collected from various region of Tehran. For extraction of Folic acid from bread samples SPE Cartridges was used and high performance liquid chromatography with ultraviolet- visible detection was used to detection and determination of folic acid in various bread samples after extraction steps [1, 26, 30-32]. The results obtained from this research can help health professionals and policy-makers to give clear advice about the effects of bread baking method in folic acid level in fortified breads.

EXPERIMENTAL SECTION

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grades. Folic acid standard was obtained from Merck (Darmstadt, Germany). Sodium hydroxide, sodium acetate, acetic acid solution, sodium tetaborate and tricholoro acetic acid were purchased from Merck. HPLC-grade acetonitrile and methanol were obtained from Caledon (Ontario, Canada). Stock solutions of the folic acid about 100 mg L^{-1} were carefully prepared in methanol. They were all stored in the darkness glass container at 2-8 ˚C and working analyte mixtures were daily prepared by dilution with the suitable volume of distilled water.

2.2. Apparatus

Chromatography was carried out by a Knaure model 2500 HPLC (Knaure, Germany) equipped with a Quaternary HPLC pump, a 4-channel mixing valve with a 10 µL sample loop, vacuum degasser and a UV-Vis detector. Separations were performed on an ODS-3 column (150 mm × 4.6 mm, with particle size of 5 µm) from MZ-Analysentechnik (Mainz, Germany). A isocratic elution of 20 mM acetic acid and acetonitrile mixture (50:50) was employed. The flow rate of the mobile phase was set at 0.2 mL min^{-1} and total analysis time was 50 min. The injection volume was 10 µL for all of the samples and detection was performed at a wavelength of 254 nm.

A pH meter Model 827 pH Lab digital pH meter from Metrohm with combined glass electrode was used for monitoring pH adjustment. SPE of folic acid from water samples was carried out using 10 mm height, 1.5 mm i.d., 500 mg of C18 sorbent with 6 mL syringe barrels from Supelco. Ultrapure water was deionized by an Aqua MAX water purification system from Younglin (Seoul, Korea). Sample solution was stirred using a MR Hei-standard magnetic stirrer from Heidolph (Schwabach, Germany).

2.3. Extraction of folic acid from various samples

5.0 grams of each sample (wheat, dough and bread) were transferred in centrifuge tube and 45.0 milliliters of sodium tetraborate solution was added to centrifuge tube. The centrifuge tube was then immersed into the ultrasonic water bath. After that the ultrasonic water bath was switched on for 30 min sonication at 40 kHz of ultrasound frequency and 0.138 kW of power at ambinent temperature. Then, the mixture was centrifuged at 3500 rpm for 10 min to complete phase separation. The upper solution, located at the top of the centrifuge tube, was withdrawn using a syringe and collected in glass test tube to load in SPE column.

As a pretreatment step, the SPE column was conditioned with 5.0 mL methanol and 5.0 mL distilled water. 2.5 milliliters of the sample was loaded into the SPE column at a flow rate of about 0.1 mL min^{-1} with the aid of a

vacuum pump. Then, the column was rinsed by 5.0 mL distilled water to remove the matrix interferences. The extracted drugs in the SPE column were eluted by 5.0 mL phosphate buffer and the eluent solution was collected in test tube and injected to HPLC for analysis.

2.4. Analytical samples

Three caqtegories of wheat flour including Taftoun, lavash and barbari were selected as model sample and the sample breads were randomly collected from various region of Tehran. All sample were collected in four form including fourtified wheat, dough before and after of fermentation and baked bread. The samples were stored at 4˚C.

2.5. Statistical Analysis

Data were expressed as means ± SD (standard deviation) and examined by one-way analysis of variance (ANOVA) to compare different groups. A value of $p < 0.05$ is considered statistically significant. Data analysis was executed in IBM spss statistics 21 (New York, USA).

RESULTS AND DISCUSSION

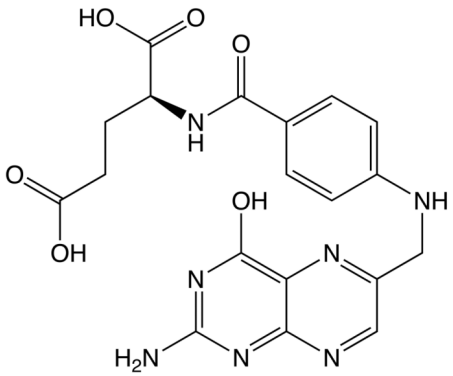
3.1. Investigation of the folic acid content in fourtified wheat

Table 1 shows the folic acid content in fourtifeid wheat. The level of folic acid content in wheat flour were 2.01 ±1.78, 1.47 ± 1.99 and 1.95±1.99 µg L^{-1} for barbari, lavash and taftoon flour, respectively.

Table 1: Folic acid content in bread processing in different breads

Precessing step	(mean ± SD)		
	Barbari flour	Lavash flour	Taftoon fluor
Fourtified wheat	2.01 ± 1.78	1.47 ± 1.99	1.95 ± 1.99
Dough before fermentation	2.32 ± 1.71	1.13 ± 1.87	1.20 ± 1.21
Dough after fermentation	2.27 ± 1.58	1.05 ± 1.17	0.79 ± 0.55
Baked bread	1.98 ± 1.53	1.19 ± 1.39	0.79 ± 0.45

Fig. 1



Log P: -0.86

pKa: 7.61, 4.17, 3.37

Folic acid

Fig. 2

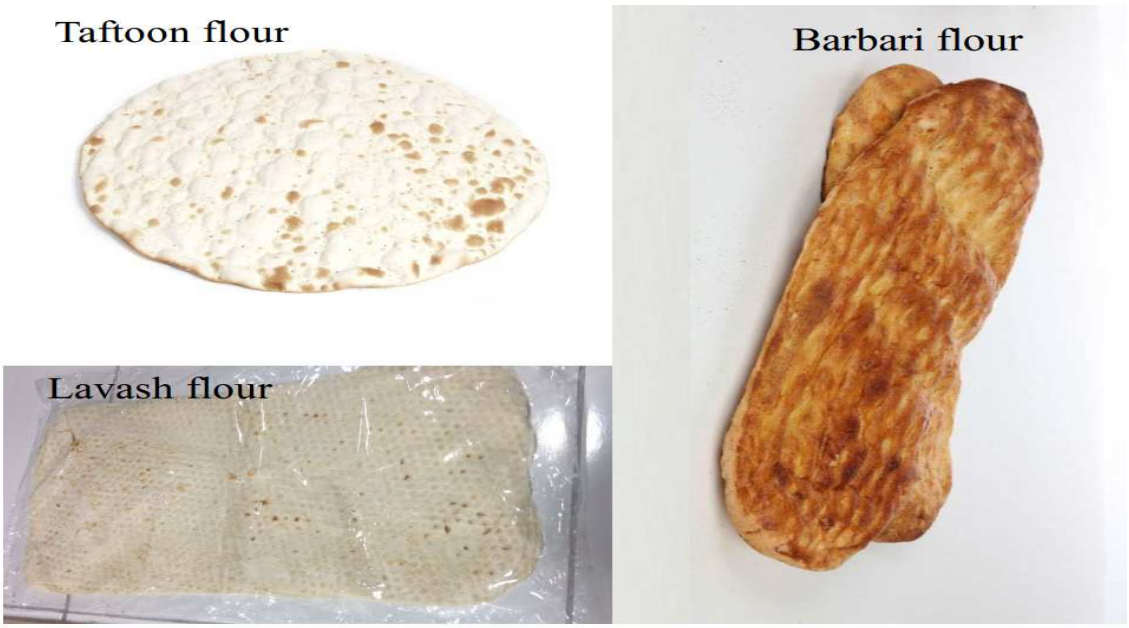

Taftoon flour
Barbari flour
Lavash flour

Fig. 3

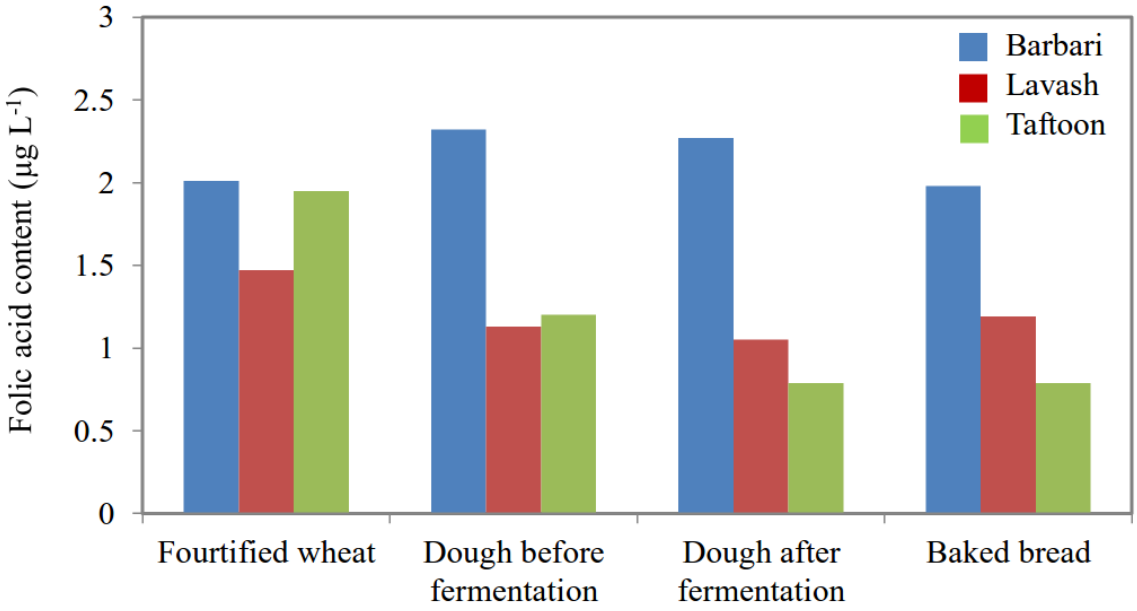
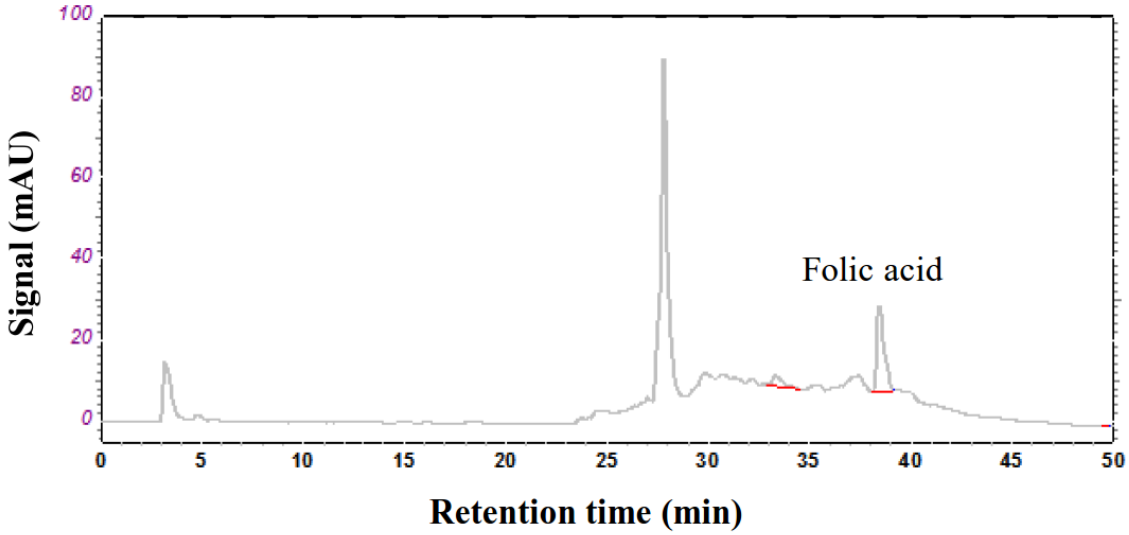

Folic acid content (µg L-1)
3
2.5
2
1.5
1
0.5
0
Barbari
Lavash
Taftoon
Fourtified wheat
Dough before fermentation
Dough after fermentation
Baked bread

Fig. 4



3.2. Investigation of the folic acid content before fermentation of dough

Table 1 also shows the folic acid content in Dough before fermentation. The level of folic acid content in dough before formation were ranged from 2.32 ±1.71, 1.13 ±1.87 and 1.20±1.21 µg L^{-1} for barbari, lavash and taftoon flour, respectively.

3.3. Investigation of the folic acid content after fermentation of dough

As can be seen in Table 1, the mean of the total foloic acid content after fermentation of dough before baking ranged from 2.27 ±1.58, 1.05 ±1.17 and 0.79±0.55 µg L^{-1} for barbari, lavash and taftoon flour, respectively.

3.4. Investigation of the folic acid content after bread baking

Folic acid content for baked bread are reported in Table 1. Mean folic acid values were 1.98±1.53, 1.19 ±1.39 and 0.79±0.45 µg L^{-1} for barbari, lavash and taftoon flour, respectively.

3.5. compartion of the folic acid content in wheat to bread process in various breads

As shown in Fig. 3, folic acid content in all samples were found to decrease significantly after fermentation. Therefore, fermentation step has important effect in folic acid stability in various flours.

By comparion the results that reported in Fig. 3, baking temperature and duration do not have a significant impact on folic acid content in various breads.

In addition, due to unstability of folic acid by exposure to light, air and water, significantly decrease in folic acid contents were observed after storage in stores. Therefore, fresh bread has maximom content of folic acid after backing. Typical Chromatogram obtained for folic acid content in are shown in Fig. 4.

CONCLUSION

In the present study, level of the folic acid content in different baking step including wheat, dough before fermentation, dough after fermentation and baked bread for lavash, barbari and taftoon was determined by high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection. Solid phase extraction was used for extraction and preconcentration of folic acid before analysis. The results showed that fermentation step has important effect in folic acid stability. Baking temperature and duration do not have a significant impact on folic acid content. Additionally, due to instability of folic acid, significantly decrease in folic acid contents were observed after storage in stores. Therefore, fresh bread has maximom content of folic acid after backing.

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