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Folate Content in Commercial White and Whole Wheat Sandwich Breads

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After the U.S. mandate of folic acid fortification of enriched grain products, a report indicated higher than expected fortification. Limited information is available on folic acid in enriched products. We measured the folate content in 92 sandwich breads (46 white breads and 46 whole wheat breads) in Birmingham, Alabama, during 2001−2003. The mean folate content in white bread declined significantly from 2001 to 2002 or 2003, whereas the decline in folate content in whole wheat bread containing enriched flour was not significant. White bread contained significantly more folate than whole wheat bread containing enriched flour in 2001 and 2003. In 2002 and 2003, >40% of breads made of enriched flour contained <115 μg of folate/100 g and >70% contained <160 μg/100 g. These percentages were markedly higher than those in 2001. Our data suggest that folic acid in breads containing enriched flour declined after 2001 and monitoring of fortification may be necessary.

KEYWORDS: Folic acid; folate; fortification; bread

INTRODUCTION

In the past decade, strong evidence has emerged indicating that the occurrence or recurrence risk of pregnancy complicated with neural tube defects (NTDs) is reduced by periconceptional folic acid (pteroylglutamic acid) supplementation (*1*−*3*). To reduce the prevalence of NTDs, the U.S. Food and Drug Administration (FDA) mandated that enriched cereal grain products must be fortified with folic acid at the level of 140 μg/100 g of product effective January 1998 (*4*). This fortification resulted in a reduction in the birth prevalence of NTDs as compared to a prior period (*5*, *6*), although the reduction was lower than the estimated 50% (*6*). At the same time, however, postfortification blood folate concentrations have dramatically increased in certain U.S. populations (*7*−*9*). Similarly, in November 1998, Canada initiated the fortification of 150 μg folic acid/100 g of enriched grain products, where an impressive reduction (about 50%) of prevalence of NTDs has been reported (*10*−*12*). Furthermore, starting in January 2000, a slightly higher level of folic acid fortification (220 μg/100 g) to grain products was mandated in Chile, and an increase in plasma and erythrocyte folate concentrations was recently demonstrated in young women (*13*).

Shortly after the initiation of folic acid fortification in the United States, Rader et al. (*14*) took the lead in measuring folate content in enriched products and reported higher than expected values in these products. However, since then, limited information is available documenting the folic acid fortification of enriched grain products. Therefore, we measured the folate content in sandwich breads commercially available in the Birmingham, Alabama, area from 2001 to 2003.

MATERIALS AND METHODS

Reagents used in this study were analytical grade and were purchased from Sigma Chemical (St. Louis, MO). Rat plasma was obtained from Harlan Bioproducts for Science (Indianapolis, IN). The medium used for the *Lactobacillus rhamnosus* (formally known as *Lactobacillus casei*, ATCC 7469) microbiological assay was obtained from Beckton Dickinson (Sparks, MD). A total of 92 white and whole wheat sliced sandwich breads (29, 31, and 32 in 2001, 2002, and 2003, respectively) were purchased from nation or state wide supermarkets in the Birmingham area (**Table 1**). These consisted of 50% white breads, all of which contained enriched flour ($n = 46$), and only 50% of whole wheat breads contained enriched flour ($n = 46$). The first ingredient listed on the package for white bread was enriched wheat flour, and that for enriched whole wheat bread was either "enriched whole wheat flour" or "enriched wheat flour plus whole wheat flour." The list of ingredients of all bread items containing enriched flour included niacin, reduced iron, thiamine, and riboflavin in addition to folic acid. Of 46 whole wheat breads, 17 (37%) were made with whole wheat flour (with or without unbleached wheat flour) without fortification of iron or vitamins including folic acid. We purchased all available sandwich breads around the same time each year. These 92 breads were baked at 12 independent factories, and each factory distributed 2−8 items under different brand names.

All bread samples were processed for folate analysis within 3 days of purchase, which was always before the end of the shelf life specified by the manufacturers. After they were weighed, a randomly selected whole slice of bread was mixed with 5 volumes of 0.1 M potassium phosphate buffer containing 57 mM ascorbic acid with a final pH of 4.2 (*15*). The mixtures were heated at 100 °C for 10 min and treated by the trienzyme extraction method (*15*−*17*). A portion of the heated mixture was treated with α-amylase (prepared from *Aspergillus oryzae*,

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Table 1. Folate Content in Sandwich Breads from 2001 to 2003[a]

	μg/100 g fresh weight			μg/100 g dry weight		
	2001	2002	2003	2001	2002	2003
			white bread			
all with fortified flour	198 ± 55[1] (109–289) *n* = 14	125 ± 40[2] (70–226) *n* = 15	137 ± 36[2] (72–215) *n* = 17	318 ± 112[3] (165–556) *n* = 14	211 ± 68[4] (124–384) *n* = 15	196 ± 55[4] (105–316) *n* = 17
			whole wheat bread			
containing fortified flour	137 ± 75 (44–277) *n* = 10	109 ± 37 (64–159) *n* = 10	103 ± 136 (69–163) *n* = 9	223 ± 75 (44–518) *n* = 10	177 ± 57 (64–159) *n* = 10	141 ± 49 (70–225) *n* = 9
containing unfortified flour	63 ± 19[5] (43–94) *n* = 5	78 ± 42[5] (25–142) *n* = 6	39 ± 6[6] (33–46) *n* = 6	80 ± 17[7] (55–100) *n* = 5	133 ± 67[7] (40–220) *n* = 6	58 ± 8[8] (44–64) *n* = 6

[a] Values are means ± SD (range). Means in a row independently of fresh weight and dry weight bases without a common number are significantly different ($P < 0.005$ for white bread and $P < 0.05$ for whole wheat bread) by ANOVA with Bonferroni's correction. There were no significant differences in whole wheat breads containing fortified flour.

Sigma Chemical) followed by protease (prepared from *Streptomyces griseus*, Sigma Chemical) at pH 6.0 for 3 h each at 37 °C (*15*, *17*). The concentration of α-amylase and protease was 20 mg/mL of 0.3 M potassium phosphate buffer containing 57 mM ascorbic acid (pH 6.0). The solution of α-amylase was treated with charcoal to remove endogenous folate and filtered through a 0.22 μm microfilter, and the protease solution was also filtered immediately before use to prevent bacterial contamination (*16*). After these incubations, the mixture was heated at 100 °C for 5 min to inactivate protease followed by the 2 h incubation at pH 7.5 (37 °C) with folate conjugase from rat plasma, which was treated with charcoal to remove endogenous folate (*16*). Rat plasma folate conjugase used per treatment (100 μL/tube) was able to hydrolyze >180 nmol of synthetic pteroyltriglutamic acid per 2 h at 37 °C. In general, the amount of total folate per the treatment mixture in this study ranged from 3.0 to 16.0 fmol. Thus, the activity of folate conjugase used for each treatment was far in excess, and it was unlikely that the inhibitor(s) of folate conjugase in the bread, if any, led to incomplete hydrolysis of polyglutamyl folate (*16*). A single slice of each brand was used for folate analysis in 2001, and three slices of each brand were used in 2002 and 2003, where the mean values of these three were taken for data analysis. Folate content after the trienzyme extraction method was measured by the *L. rhamnosus* assay (*17*, *18*). [6*RS*]-5-Formyltetrahydrofolate (calcium salt, Sigma Chemical) was used as a standard. The concentration of the stock solution of 5-formyltetrahydrofolate was measured spectrophotometrically before bread folate analysis each year and was stored at −70 °C throughout the assays, which were completed within 6 weeks. The coefficient of variation of day-to-day folate analysis using pooled human plasma samples by the *L. rhamnosus* assay was approximately 10% (*18*).

To obtain the folate content on the dry weight basis, three slices of bread were individually loosely wrapped in aluminum foil and weighed. These were weighed again after placing in a drying oven set at 135 °C for about 12 h. The folate content is expressed as μg of folate (folic acid equivalent)/100 g of fresh or dry weight of bread.

The data are presented as means ± SD where appropriate. The differences between means obtained each year and between means for types of bread were evaluated by analysis of variance (ANOVA) with Bonferroni's correction. A P value < 0.05 was considered significant.

RESULTS

Mean coefficients of variation of the entire bread folate assay procedure (homogenization, heat treatments, enzyme treatments, and microbiological assay combined) were 12.1 and 16.2% in 2002 and 2003, respectively. The mean folate contents of all breads combined were 154 ± 76 (n = 29), 113 ± 43 (n = 31), and 109 ± 49 (n = 32) μg/100 g of fresh weight in 2001, 2002, and 2003, respectively. The overall folate content in breads widely varied from 25 to 289 μg/100 g. The mean moisture contents calculated based on the fresh weight and dry weight were 38, 40, and 29% in 2001, 2002, and 2003, respectively.

Table 2. Number of Breads Made with Fortified Flour by Folate Content (Fresh Weight)

	2001	2002	2003
total	24	25	26
folate content > 329 μg/100 g	0	0	0
folate content < 160 μg/100 g	10 (42%)	19 (76%)	20 (77%)
folate content < 115 μg/100 g	7 (29%)	12 (48%)	11 (42%)

Table 1 shows the folate content in three types of bread based on both fresh weight and dry weight bases. The mean folate contents in white breads containing enriched flour significantly declined from 2001 to 2002 and 2003 ($P < 0.005$ on the basis of fresh weight and dry weight). The decline was smaller and was not significant in whole wheat breads containing enriched flour ($P > 0.05$). White breads containing enriched flour generally had more folate than whole wheat bread containing enriched flour; the differences in 2001 and 2003 were significant ($P < 0.03$ based on fresh weight), although the reason for this difference is unknown. Seventeen of 46 whole wheat sandwich breads sold in our area were prepared with unfortified flour and contained only 60 ± 31 μg/100 g of fresh weight.

The U.S. FDA mandate states "the agency is requiring that these (enriched) products be fortified with folic acid at levels ranging from 95 μg to 309 μg/100 g of product. These values are based on a fortification level of 140 μg/100 g of product" (*4*). These values represent the amounts of added folic acid, not including endogenous (naturally occurring) folate in breads, which generally ranges between 20 and 40 μg/100 g analyzed after trienzyme extraction (*19*, *20*). Using the arbitrary value of 20 μg/100 g of endogenous folate in addition to the amounts of added folic acid, we evaluated the folate content in relation to the target range of 115 (95 + 20) and 329 (309 + 20) μg/100 g. We also examined how many fall below the FDA target value of 160 (140 + 20) μg/100 g. **Table 2** shows the percentages of breads of which total folate content (added folic acid and endogenous folate) are either >329 μg/100 g or <115 or 160 μg/100 g. We found that over 40% of breads, which should have had enriched flour, contained <90 μg of total folate/100 g and >70% of these contained <160 μg/100 g in 2002 and 2003. These percentages were markedly higher than those in 2001. No bread exceeded the upper limit of the FDA suggested range.

DISCUSSION

Our data indicate that the folate content in white breads containing enriched flour in 2002 and 2003 declined significantly as compared to that in 2001. Over 70% of these contained <160 μg/100 g of fresh weight, and over 40% contained <115 μg/100 g in 2002 and 2003. These percentages were markedly higher than those in 2001, indicating that the amount of folic acid added to breads containing enriched flour declined after 2001. The mean folate content in white bread (198 μg/100 g) in 2001 is similar to 177 μg/100 g found by Rader et al. (*14*) before 2000. They suggested that overage beyond the level required by the law should be avoided (*14*).

In the last 6 years since the initiation of the FDA mandate, investigators reported a significant increase in blood folate concentrations and decline in plasma total homocysteine concentrations in certain U.S. populations (*7*−*9*). Subsequently, a higher than predicted folate intake probably due to overfortification was shown by Choumenkovich et al. (*21*) and Quinlivan and Gregory (*22*). On the basis of our data of the decline in folic acid in breads containing enriched flour, however, it remains to be seen whether the greater than expected effect of folic acid fortification in enriched products on blood folate and homocysteine concentrations will be sustained in the future. It may be desirable to monitor folic acid fortification of enriched products as suggested by Rader (*23*).

It is important to realize that not all breads commercially available are fortified with folic acid. We found that about 40% of "whole wheat breads" did not contain fortified folic acid. Life style may affect the type of bread consumed by individuals, and the consumption of "whole wheat bread", which does not contain extra folic acid, may result in the intake of folic acid below the target level of 100 μg per day for women of childbearing age.

We acknowledge the limitations of our study; these include that (i) only sandwich breads were chosen to evaluate folic acid fortification; (ii) the location was limited to the Birmingham area; and (iii) the analysis was performed only during a few months of each year in one laboratory. We do not know whether the results would differ, if the analyses were done more often using a wide variety of cereal grain products from various locations. However, our data cause concern over the level of fortification and, hence, the overall folate intake of the U.S. population.

In summary, we found that the amount of folic acid added to sandwich breads containing enriched flour declined after 2001. It remains to be seen whether changes in fortification will affect blood folate and homocysteine concentrations and the prevalence of NTDs. It may be desirable to establish a monitoring system to document folic acid fortification of enriched cereal grain products.

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