

# Folate in Beer

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## ABSTRACT

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Folate levels in a range of beers and other beverages have been measured using high-performance liquid chromatography with fluorescence detection (HPLC-FLD) and by liquid chromatography tandem mass spectrometry (LC-MS/MS). The data from the LC-MS/MS study was revealed to be unreliable, through the masquerading of polyphenol degradation products as folates. Using the HPLC-FLD procedure most beers ranged between 2.2 and 24.2 µg per bottle, or up to 6.1% of the recommended daily allowance. Some imported beers contained no detectable folate and we believe that this is due to the folate decreasing in level during storage and transportation. Wine, vodka and whiskey contained no detectable folate, while orange juice contained folate at a comparable level to that found in the beer with the highest folate.

**Key words:** Beer, folate, high-performance liquid chromatography with fluorescence detection, liquid chromatography–mass spectrometry.

## INTRODUCTION

There have been several reports of beer as a source of B-vitamins. It is clear that thiamine is relatively deficient in beer, but that others in the B complex may be present in significant quantities. In reality, the number of original scientific publications offering substantive values for these vitamins in beer is relatively low. Previous discussions of folate in beer have been those by Walker et al.<sup>7,8</sup> and Pietercelie et al.<sup>5</sup> In this paper, we report recent work to quantify folates in a range of beers. We have used two methods for quantification, namely high-performance liquid chromatography with fluorescence detection (HPLC-FLD) and liquid chromatography tandem mass spectrometry (LC-MS/MS).

## MATERIALS AND METHODS

### Beers

Beers were purchased in local stores and stored at 4°C. The beers either were ones purchased fresh specifically for this project or were beers that had been obtained earlier for previous unrelated investigations. Two 14 mL ali-

quots were taken from each brand of beer and one 50 mL aliquot was reserved for future analyses. All beer samples and method validation samples were analyzed on the same day. Values shown are for individual brands of beer, but the commercial names have not been used.

### Chemicals

The natural folates 5-methyltetrahydrofolate (5MTHF), 5-formyltetrahydrofolate (5FTHF), tetrahydrofolate (THF), 5,10-methylenetetrahydrofolate (5,10-CH<sub>2</sub>-THF), 5,10-methenyltetrahydrofolate (5,10-CH=THF) and pteroyl-triglutamic acid (PteGlu<sub>3</sub>) were ordered from Schircks Laboratories (Jona, Switzerland). Folic acid (FA), lyophilized rat plasma, α-amylase from *Aspergillus oryzae*, and protease from *Streptomyces griseus* were from Sigma-Aldrich (St. Louis, MO). The internal standard of [<sup>13</sup>C<sub>6</sub>]pABA was from Beta Chem (Leawood, KS). All other chemicals, including HPLC-grade acetonitrile, were obtained from Fisher Scientific (Fairlawn, NJ) unless otherwise specified.

### Preparation of buffers

The extraction buffer of 0.1 M potassium phosphate, pH 6.0, with 10 mM ascorbic acid was prepared daily and as previously described<sup>1</sup> except that neither 2-mercaptoethanol nor sodium azide were added. The standard preparation buffer contained 0.02 M potassium phosphate buffer, pH 7.2. The SPE elution buffer contained 0.1 M potassium phosphate, pH 6.0, 1 M sodium chloride, and 250 mL/L acetonitrile. The HPLC buffer contained 0.03 M potassium phosphate buffer at pH 2.2. The enzyme solution of α-amylase was prepared by dissolving 60 mg into 3 mL of extraction buffer. The protease solution was prepared by weighing out 25 mg and dissolving in 25 mL of extraction buffer<sup>1</sup>. The lyophilized rat plasma was prepared by dissolving in 1 mL of extraction buffer. The citric acid buffer was prepared from citric acid monohydrate dissolved in water before pH adjustment with 50% sodium hydroxide (w/w).

### Preparation of standards

Standards of FA, 5MTHF, 5FTHF, THF, 5,10-CH<sub>2</sub>-THF, and 5,10-CH=THF were prepared in 50 mL of 0.02 M potassium phosphate buffer, pH 7.2, whereas PteGlu<sub>3</sub> was prepared in 25 mL of the same buffer for analysis by HPLC-MWD/FLD. Fifty µL aliquots of the dissolved standards were mixed with 950 µL of phosphate buffer and analyzed by UV-Vis to confirm gravimetric concentrations. After analysis by UV-Vis, the samples were mixed with 100 µL of 1% ascorbic acid in water (w/w) and analyzed by HPLC (described below) to determine purity and retention time. Calibration standards of these

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folates were prepared in 10 mL volumes ranging in concentration from 26,000 ng/mL to 0.1 ng/mL in the potassium phosphate buffer and 1 mL of 1% ascorbic acid.

Calibration standards of methyl-*p*A BA for LC-MS/MS analysis were prepared by treating one mL working stock solution (365  $\mu\text{mol/L}$  *p*A BA in methanol) with 100  $\mu\text{L}$  trimethylsilyldiazomethane and 200  $\mu\text{L}$  methanol. After reacting at room temperature for 30 min, the working stock solution was evaporated to dryness at 50°C under argon. The standard was re-suspended in one mL acetonitrile and from this first working stock solution, a second working stock solution (14.6  $\mu\text{mol/L}$  methyl-*p*A BA) was prepared. This second working stock solution was used to prepare the methyl-*p*A BA standards in acetonitrile, which ranged from 0.15 to 7.3  $\mu\text{mol/L}$ . A constant volume of the derivatized IS (prepared like the working standards described above) was added to each calibration standard to provide ratios of analyte to IS ranging from 0.05 to 2.5 after derivatization.

### Preparation of samples

Beer, wine, liquor, and juice samples were analyzed by pipetting 14 mL of sample into 15 mL centrifuge tubes. The pH was randomly checked to ensure that the pH was between 5.5 and 6.0. Fifty  $\mu\text{L}$  of  $\alpha$ -amylase was added and the samples were vortexed for 30 sec. The samples were incubated at 37°C for 1 h and then all were treated with 50  $\mu\text{L}$  of protease, vortexed, and incubated at 37°C for 3 h. Following treatment with the protease, the samples were heated at 95°C in a water bath for 15 min to deactivate the protease. The samples were cooled on ice before addition of 20  $\mu\text{L}$  of the rat plasma reconstituted with 1 mL extraction buffer. The samples were again vortexed for 30 sec and incubated at 37°C for 16 h. Finally, the samples were heated at 95°C for 15 min, cooled on ice, and centrifuged at 6000  $\times g$  for 5 min using an International Clinical centrifuge (Model CL, International Equipment Company, Needham Heights, MA). The samples were cleaned up and concentrated using SAX SPE cartridges (Alltech, Deerfield, IL) and then analyzed by HPLC-MWD/FLD.

The samples were also analyzed using a previously described LC-MS/MS method employed for red blood cell folate analysis<sup>3</sup> with some slight modifications. In this method, two aliquots of the beer samples that had been cleaned up with the SAX SPE cartridges were used for LC-MS/MS analysis. One of these aliquots was prepared to measure the endogenous *p*A BA concentrations and the second aliquot was treated with HCl to determine the folate fraction. The folate concentration in these samples was then the difference of  $C_{p\text{A BA as folate}} - C_{\text{endogenous } p\text{A BA}}$ .

Briefly, a 500  $\mu\text{L}$  aliquot of the beer sample (cleaned up through the SAX SPE cartridges) was mixed with 10  $\mu\text{L}$  of [<sup>13</sup>C<sub>6</sub>]-*p*A BA IS. The pH of one of these aliquots was adjusted to pH 4.5 with treatment of 2 drops of 1 N HCl. The sample was cleaned up using a C18 HLB OASIS SPE cartridge as described below. After clean-up and elution from the SPE column with 1 mL of ethyl acetate/hexane (95/5, v/v), the sample was derivatized with 200  $\mu\text{L}$  methanol and 40  $\mu\text{L}$  trimethylsilyldiazomethane at room temperature for 30 min with shaking. After derivatization, the sample was evaporated just to dryness with

argon and mild heat (50°C). The sample was resuspended in 100  $\mu\text{L}$  of acetonitrile, transferred to an HPLC PolySpring™ insert, and stored at -20°C until analysis.

A second 500  $\mu\text{L}$  aliquot of the beer sample was mixed with 10  $\mu\text{L}$  [<sup>13</sup>C<sub>6</sub>]-*p*A BA IS and treated with 500  $\mu\text{L}$  12.1 N HCl. The headspace of the tube was filled with argon and capped before heating at 110°C for 4 h. After 4 h, the tubes were allowed to cool to 25°C. The pH of the hydrolysate was adjusted to 4.5 with 2 mL of a solution containing an 85/15 (v/v) ratio of 1.25 M citric acid buffer (pH 5.0) and 50% sodium hydroxide in water (w/w). The samples were cleaned up using the C18 HLB OASIS cartridges described below and then derivatized and re-suspended in acetonitrile as just described. The samples were stored at -20°C until analysis.

### SPE treatment

The Extract-Clean™ SAX SPE column with 500 mg sorbent bed and 9 mL volume was prepared by first swelling the sorbent bed with 15 mL of extraction buffer. This volume was allowed to flow through by gravity. The sample supernatant (approximately 14 mL) was applied to the column and allowed to flow through by gravity. The column was washed with 15 mL of extraction buffer and the folates eluted with the addition of 6 mL of elution buffer as previously described<sup>1</sup>. The samples were heated at 50°C under a stream of argon for approximately 30 min or until the sample volume was 5 mL or less. The sample volume was brought up to 5 mL, if necessary, with extraction buffer. Fifty  $\mu\text{L}$  of 10% ascorbic acid solution in water (w/w) was added and the sample was vortexed. Five hundred  $\mu\text{L}$  of the sample was transferred to an HPLC vial for analysis by HPLC-MWD/FLD.

Samples were also cleaned up using the C18 HLB OASIS SPE cartridges (60 mg sorbent, 3 mL) from Waters Corporation (Millford, MA) prior to analysis by LC-MS/MS. The cartridges were conditioned with 2 mL methanol followed by 2 mL DI water that flowed through by gravity. The sample was then loaded onto the column, allowed to flow through by gravity, and then 2 mL of water/methanol (95/5, v/v) was applied to wash the column. The *p*A BA analyte and IS were eluted with 1 mL of ethyl acetate/hexane (95/5, v/v) and this fraction was collected. The top layer that contained the ethyl acetate was removed and transferred to a 2 mL HPLC vial and derivatized as described above.

### HPLC analysis

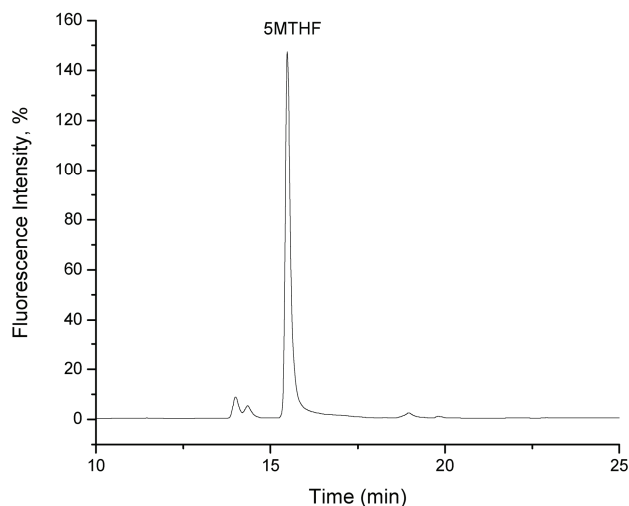
All standards and samples were quantified using the Agilent 1100 HPLC system as previously described<sup>2</sup> with a few modifications. The HPLC system consisted of an inline mobile phase degasser, a quaternary pump, an autosampler, a thermostated column compartment (which was held at 25°C during analysis) a multi-wavelength detector (MWD) and a fluorescence detector (FLD). The system was controlled using Agilent ChemStation software. Separation of folates was completed on an Alltech Adsorbosphere C18 column, 150  $\times$  4.6 mm i.d., 3  $\mu\text{m}$  packing with a C18 Brownlee guard column (Alltech and Associates, Deerfield, IL). The flow rate was 0.4 mL/min and the injection volume was 40  $\mu\text{L}$ . The mobile phase consisted of mobile phase A: HPLC buffer (0.03 M phosphate buffer,

pH 2.2) and mobile phase B: acetonitrile. The gradient program was as follows: 0 to 5 min, 94% A and 6% B; during 20 min, the gradient was raised linearly to 24% B and kept constant for 2 min. The conditions were returned to 94% A and 6% B within 3 min and held for 15 min to re-equilibrate the column. The detection of folates was completed using a MWD (at 290 nm) and an FLD (excitation was set at 290 nm with emission at 360 nm). Comparison of retention times with those of folate standards and the ratio of MWD/FLD signals were used for peak identification.

The instrumentation and chromatographic conditions for the LC-MS/MS analysis have been described previously<sup>4</sup>. Briefly, A Perkin-Elmer Series 200 LC system (Perkin-Elmer, Shelton, CT) was combined with a Sciex API 2000 triple-quadrupole MS system (Perkin-Elmer) controlled by Analyst 1.3.1 software (Applied Biosystems, Foster City, CA) for quantitation of methyl-*p*ABA. The samples were separated using a chromatographic system and mixed with a post-column reagent before ionization as previously described<sup>4</sup>. Briefly, a Synergi 4  $\mu$  Hydro-RP column (150  $\times$  4.6 mm i.d.; Phenomenex, Torrance, CA) with an isocratic mobile phase of aceto-nitrile/water (70/30, v/v) at 0.5 mL/min was utilized for analyte separation for a six min chromatographic run. A post-column reagent of 0.025% formic acid in acetonitrile was added at 0.2 mL/min and then split 1:1 (source: waste) prior to entry into the source. Optimized settings for the sample ionization by positive ion atmospheric pressure chemical ionization were described and the multiple-reaction monitoring mode was utilized for data acquisition.

### Quantification

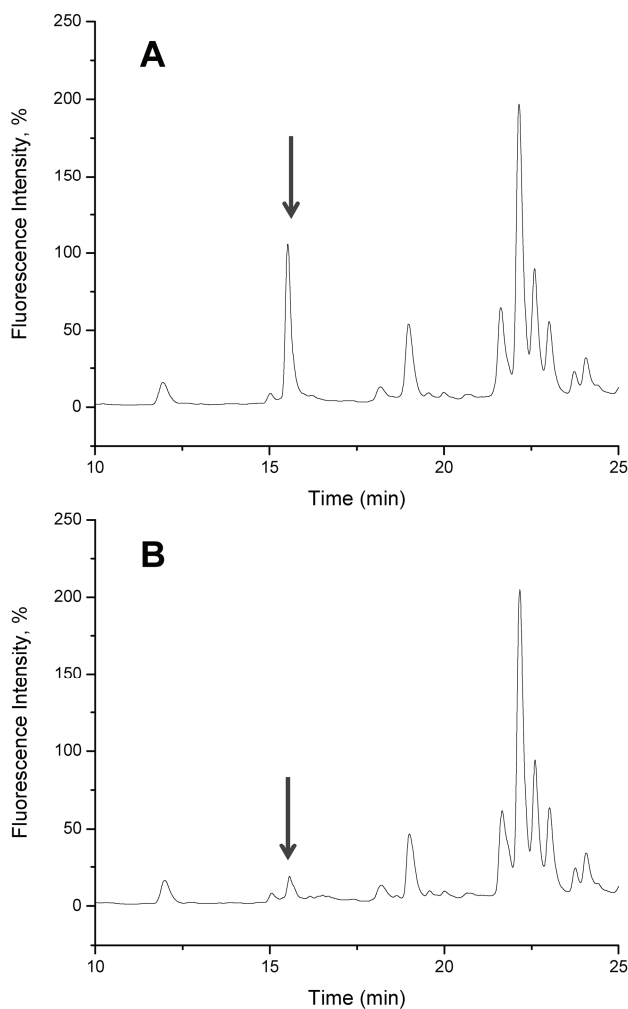
From HPLC-MWD/FLD analysis, the concentration of folates in the beer, liquor, wine, and juice samples was determined using an external standard calibration curve in which the peak area was plotted against concentration (ng/mL). A total of seven calibration standards were used, ranging in concentration from 26,000 ng/mL to 0.1 ng/mL. An example chromatogram of a 5MTHF standard



**Fig. 1.** Sample chromatogram of 5MTHF standard (950 ng/mL) analyzed by HPLC-FLD ( $\lambda_{\text{excitation}} = 290 \text{ nm}$ ,  $\lambda_{\text{emission}} = 360 \text{ nm}$ ). Retention time is 15.5 min.

(at 950 ng/mL) is shown in Fig. 1. The amount of each folate was calculated in the free acid form. Standards of each folate, whose concentrations had been previously determined by UV-Vis analysis as described above, were analyzed by HPLC and the concentrations were determined using the external standard calibration curves. The two modes of concentration determination had a minimum agreement of 90%. The limit of detection was determined to be three times the standard deviation of the lowest standard that was analyzed four times. The limit of quantification was determined by multiplying this same standard deviation by seven. Blank samples were included in the analysis to account for any endogenous folate found in the enzyme preparations or reconstituted rat plasma.

For quantitation of methyl-*p*ABA by LC-MS/MS, a six point calibration curve was prepared by plotting the ratio of the analyte response ( $m/z$  152 to  $m/z$  93 transition) to the IS ( $m/z$  158 to  $m/z$  99 transition) against the amount of analyte injected. Second order regression with a 1/x weighting was used to fit the calibration standards. Calibration curves were prepared in triplicate and were run before, during, and at the end of each set of samples.



**Fig. 2.** Sample chromatograms of Beer 2 spiked with 568 ng/mL 5MTHF (panel A) and before spiking (panel B). At this spiking level, 5MTHF was quantitatively recovered (mean 95%) with good precision (CV of 5.7%).

## Method validation

To determine the accuracy of the method, the recovery of known amounts of 5MTHF added to two different types of beer in triplicate was determined (example chromatograms shown in Fig. 2, panels A and B). The spiked samples were treated with the same enzyme preparations and heat treatments before clean up with SAX SPE as described above<sup>2</sup>. The recovery of the spiked folates was determined in the following manner:

$$100\% * [C_{\text{spike}} - C_{\text{control}}] / C_{\text{total folates added}}$$

## RESULTS AND DISCUSSION

### Chromatographic separation

The folates THF, 5MTHF, 5,10-CH<sub>2</sub>-THF, 5,10-CH=THF, and FA were all easily separated, but PteGlu<sub>3</sub> and 5FTHF co-eluted on the HPLC-MWD/FLD chromatographic system. However, since none of the beer samples were expected to have significant PteGlu<sub>3</sub> concentrations, this separation was not optimized.

### Folate deconjugation

Folate conjugase from rat plasma has the highest reported degree of conversion of PteGlu<sub>3</sub> to FA<sup>1</sup> at 99.4% at 37°C at pH 6.0. Although the pH of the beer samples were checked before addition of the enzymes, the alcohol content of the beer may have inhibited the activity of the enzymes as there was no FA recovery from the PteGlu<sub>3</sub>. Thus, the only folates that were measured by this analysis were in the monoglutamyl form.

### Method validation

The method was validated by adding spikes to triplicate samples at a concentration of 568 ng/mL of 5MTHF (level one) and 57 ng/mL of 5MTHF (level two) for two different types of beer. One of these beers (B1) was hypothesized to have low folate content because it was filtered multiple times before bottling, whereas the other beer sample (B2) was bottle-conditioned with yeast. The peak area of 5MTHF at spike level one (568 ng/mL) could be analyzed by both the MWD and FLD and the recovery results are shown in Table I.

Briefly, the recovery of 5MTHF at 568 ng/mL added to B1 was 104% (CV of 7.1% for n = 3) and the recovery was 126% (CV of 12% for n = 3) for B2 when the signal

produced by the MWD was utilized for analysis. The peak areas of spike 2 (57 ng/mL) were below MWD detection limits. When the signal produced by the FLD was utilized for recovery analysis, the recovery of 5MTHF (568 ng/mL) was 95% (CV of 5.7% for n = 3) for B1 and 100% (CV of 1.2% for n = 3) for B2. When 57 ng/mL of 5MTHF was added, the spike recovery was 101% (CV of 8.6% for n = 2) for B1 and the recovery was 85% (CV of 4.2% for n = 2) for B2. Overall recoveries of 5MTHF by MWD signal analysis at 568 ng/mL were as follows: 115% (CV of 14% for n = 6 samples) and by FLD signal analysis, mean recovery was 98% (CV of 5.8% for n = 6 samples). The recovery of samples spiked with 57 ng/mL could not be determined using MWD signals because these signals were below method detection limits. However, when the fluorescence intensity was used, the recovery of the spike averaged 93% (CV of 11% for n = 4 samples) for the 57 ng/mL spike. The FLD signal was therefore used for all 5MTHF quantification of beer, wine, and liquor samples because it was more accurate and precise than the MWD signal.

### 5MTHF concentrations in beer by HPLC-FLD

**Domestic beers.** Folate concentrations per bottle of domestic beer are shown in Table II. Only two beers had non-detectable levels of 5MTHF. Total 5MTHF in nine other beers ranged from 2.8 to 24.2 µg, or roughly 0.7 to 6.1% of the US RDA of folate, which is 400 µg per day.

**Imported beers.** Folate concentrations per bottle of imported beers are shown in Table III. Folate concentrations in these beers were significantly less than the domestic beers that were analyzed and some contained no detectable folate. The seven remaining beers that were analyzed had total 5MTHF concentrations of 2.2 to 7.8 µg, or roughly 0.5 to 1.9% of the US RDA for folate. This data begs the question of the extent to which folate is lost during shipping and longer term storage of beers. Future work will include forced aging tests from a variety of canned or bottled beers in different storage conditions.

**Other beverages.** Samples of orange juice, red wine, vodka, and whiskey were included in this analysis (Table IV). Only orange juice contained significant amounts of 5MTHF per 240 mL serving (24.9 µg or 6.2% of the US RDA). The package label of the orange juice claimed that each serving contained 8.5% of daily folate requirements. The total folate content in orange juice was likely quantified using the *L. casei* microbiological assay. The accuracy of the microbiological assay has been questioned for red blood cell folate analysis. Results of folate analysis by HPLC or GC methods versus the microbiological assay have not compared well in previous reports<sup>9,10</sup>. It is important that this difference in methodology be highlighted and that the reported folate concentrations in orange juice cannot be directly compared with the analysed values in the present study.

### Folate concentrations by LC-MS/MS analysis

Beer, wine, orange juice, and liquor samples were analyzed for folate content by LC-MS/MS by measuring endogenous *p*ABA levels before hydrolysis and then treating a second aliquot with 12.1 N HCl and heat to deter-

**Table I.** Method validation results from HPLC-MWD/FLD analysis.<sup>a</sup>

Beer	5MTHF added (ng/mL)	MWD		FLD	
		Mean recovery (%)	CV (%)	Mean recovery (%)	CV (%)
Beer 1	568	104	7.1	95	5.7
Beer 1	57	0	...	101	8.6
Beer 2	568	126	15	100	1.2
Beer 2	57	0	...	85	4.2

<sup>a</sup>The peak areas of spike 2 (57 ng/mL) were below detection for the MWD, so only the FLD signal was used for peak identification and quantitation. Spiking concentration, mean percent recovery, and percent coefficient of variation are provided for each level.

**Table II.** Folate concentrations per bottle in selected domestic beers.<sup>a</sup>

Domestic beers	Folate (µg)/bottle	% of RDA (400 µg)
Alcohol-free lager	8.3	2.1
Pale ale	ND	...
Malt liquor	6.7	1.7
Sterile-filtered lager	2.8	0.7
Pasteurized low carbohydrate lager	9.8	2.4
Pasteurized lager	3.4	0.8
Red ale	13.5	3.4
Light lager 1	5.3	1.3
Light lager 2	8.0	2.0
Lager	4.7	1.2
High-strength lager	11.7	2.9
India Pale Ale 1	11.1	2.8
Pale ale	10.2	2.5
Wheat beer	ND	...
Brown ale	17.3	4.3
Hefeweisen	14.1	3.5
Pale ale	16.3	4.1
India Pale Ale 2	24.2	6.1

<sup>a</sup> All beers were in 355 mL bottles except for India Pale Ale 2, which was 651 mL.

mine total folate concentrations. The results from this analysis appear in Table V. Orange juice had a folate concentration (expressed throughout in terms of 5MTHF, µg) of 19 µg, or 4.8% of the US RDA. This value is 76% of the determined value by HPLC-FLD. The negative control samples of whiskey and vodka contained no measurable amounts of folate, but the red wine sample had a reported 5MTHF concentration of 26 µg (6.5% of the US RDA). It is hypothesized that this high concentration is due to the hydrolysis of polyphenolic compounds in the wine sample. The beer samples, which also contain polyphenolic compounds, also had much higher reported folate concentrations compared to the HPLC-FLD analysis. Domestic beer samples had total folate concentrations (from determined *p*ABA levels) ranging from 31.6 to 68.8 µg of folate (7.9 to 17.2% of the US RDA). Imported beer samples had total folate concentrations ranging from 29.2 to 85.6 µg of folate (7.3 to 21.4% of the US RDA). Beers from microbreweries had folate concentrations ranging from 52.4 to 283.2 µg of folate (13.1 to 70.8% of the US RDA).

The LC-MS/MS method that we describe was developed for the analysis of red blood cell folate by measuring the central moiety, *p*ABA, of folate. Mammals are incapable of synthesizing folate and must get an adequate and constant supply from the diet. In whole blood samples, the *p*ABA that is measured could only come from folate or the eponymous drug. Plants, yeast, and bacteria, however, must synthesize folate to survive. The *p*ABA moiety of folate is synthesized in the shikimate pathway. This pathway is important for the synthesis of not only *p*ABA and other aromatic amino acids, but also for the production of secondary metabolites, including tannins, flavonoids, and some alkaloids. Thus, the HPLC-FLD results are more specific because only the 5MTHF compound is analyzed. The LC-MS/MS results are unreliable because they result from the hydrolysis of any number *p*ABA-containing compounds contained within the beverage samples. Wine and beer are known to contain polyphenolic compounds. Hydrolysis of these compounds could thus interfere with

**Table III.** Folate concentrations per bottle in selected imported beers

Imported beers	Folate (µg)/bottle	% of RDA (400 µg)
Pale ale	6.9	1.7
Lager (green glass)	3.9	1
Lager (green glass)	ND	...
Lager (flint glass)	4.4	1.1
Ale (flint glass)	ND	...
Lager	7.8	1.9
Lager	3.6	0.9
Lager	2.5	0.6
Stout	12.7	3.2
Oatmeal stout	ND	...
Chocolate stout	ND	...
Ale	ND	...
Stout	ND	...

**Table IV.** Folate concentrations in juices, wine, and liquors.

Beverage	Folate (µg)/serving	% of RDA (400 µg)
Orange juice	24.9	6.2
Red wine	ND	...
Vodka	ND	...
Whiskey	ND	...

**Table V.** Folate concentrations in beer, juice, wine, and liquor samples by LC-MS/MS.

Beverage	Folate (µg)/bottle	% of RDA (400 µg)
Pale ale	33.6	8.4
Lager (green glass)	68.4	17.1
Lager (green glass)	58.0	14.5
Lager (flint glass)	47.6	11.9
Ale (flint glass)	29.4	7.4
Lager (green glass)	85.6	21.4
Alcohol-free beer	41.7	10.4
India Pale Ale	75.6	18.9
Pale ale	131.8	33.0
Pale ale	92.1	23.0
Wheat beer	82.3	20.6
Brown ale	52.3	13.1
Lager	35.6	8.9
Lager	29.1	7.3
Hefeweisen	123.1	30.8
Pale ale	58.4	14.6
Stout	40.6	10.2
Oatmeal stout	69.8	17.5
India Pale Ale	283.1	70.8
Malt liquor	38.6	9.7
Chocolate stout	70.0	17.5
Strong lager	37.8	9.5
Ale	32.9	8.2
Stout	42.1	10.5
Sterile-filtered lager	34.8	8.7
Pasteurized low carb lager	31.4	7.9
Pasteurized lager	72.0	18.0
Red ale	68.9	17.2
Light lager	63.4	15.8
Lager	46.9	11.7
Orange juice	19.0	4.8
Red wine	26.0	6.5
Vodka	0.0	0.0
Whiskey	0.0	0.0

folate analysis. These significantly higher concentrations in total folate by LC-MS/MS versus the results obtained from HPLC-FLD may be indicative of hydrolyzed polyphenolic compounds. Only the folate results from the HPLC-FLD analysis should be considered.

## Comparison of the present values for folate in beer with those previously reported

Walker et al.<sup>7,8</sup> reported values for total folate in beers ranging between 47 and 125 µg/L while Pietercelie et al.<sup>5</sup> claimed that 14% of refermented Belgian beers contained more than 200 µg/L, 14% had 150–200 µg/L, 47% contained 100–150 µg/L and the remainder featured 50–100 µg/L. The latter authors drew attention to the great variability between measured levels in samples of the same beers. Savage et al.<sup>6</sup> reported values for total folate analyzed by a chemiluminescence assay in home-brewed Zimbabwean beers, Zimbabwean commercial beers, and European and American drinks. Folate content in home-brewed beers had approximately 110 µg/L, whereas commercial beers had total folate content that ranged from 52 to 328 µg/L. Analyzed wines and spirits had less than 9 µg/L total folate. Analyzed European beers had folate content of 30 to 64 µg/L, American wine had <0.01 µg/L, and spirits (including gin, vodka, and whiskey) had 13 µg/L or less. We find levels of folate rather lower than these earlier reports, ranging from approximately 8 through to slightly less than 50 µg/L. The earlier reports were made using a microbiological assay for folate. We have not so far had the opportunity to compare the same beers for folate as determined by the presently reported method and the microbiological assay.

Additionally, it is important to note that in this study, the recovery of the deconjugation of pteroyltriglutamate to folic acid was not optimized. It was suggested that the presence of the alcohol in the beer and wine samples interfered with the enzyme function. As such, only 5MTHF in the free monoglutamyl form was measured. Any intracellular folate (i.e. polyglutamyl folate) was not measured. For this reason, the folate content in beers analyzed in this study may have lower folate content than previously reported. Other investigators have measured folate content in samples by the microbiological assay or by the chemiluminescence assay. The accuracy of these assays has been questioned for red blood cell folate analysis.<sup>9,10</sup> In this report, we have validated our HPLC-FLD method by showing that the recovery of the 5MTHF spike is quantitative and precise. For the analysis of monoglutamyl folate in beverages, this method is robust and accurate.

Further work is needed to explain the reasons for differences in the level of folate in different beers. Lower levels in imported beers may reflect the instability of folate in beer, but this remains to be explored.

## ACKNOWLEDGEMENT

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