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Stability of 5-methyltetrahydrofolate in frozen fresh fruits and vegetables

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Abstract

The stability of 5-methyltetrahydrofolate (5MTHF) in homogenized fresh fruits and vegetables representing samples for the USDA National Food and Nutrient Analysis Program was evaluated. Samples were homogenized in liquid nitrogen and 5MTHF was measured after 0, 2, 7, 30 days and then at 3-month intervals for a total of 12 months storage at −60 ± 5 °C, utilizing extraction by a tri-enzyme treatment, purification by strong anion-exchange solid-phase extraction, and quantification by reverse-phase HPLC. Method validation included analysis of a reference material and interlaboratory analysis of selected samples by HPLC and LC-MS. A canned spinach composite was assayed in each analytical batch to monitor inter-assay precision.

No change in 5MTHF content was detected in any of the samples after 12 months. Concentrations ranged from <10 μg/100 g in bananas to >100μg/100 g in spinach. Relative standard deviations were generally <7% within assay and <11% between assays.
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1. Introduction

The role of folate in reducing the risk of cardiovascular disease and neural tube defects is well recognized (Stanger, 2002). Naturally occurring folate comprises a group of mono- and polyglutamate derivatives of pteroic acid (4-[(pteridin-6-methyl)amino] benzoic acid) (folic acid). Tetrahydro-, dihydro-, formyl-, and methyltetrahydrofolates are the predominate naturally occurring folates in foods (Konings et al., 2001; Müller, 1993a, 1993b, 1993c), while folic acid is used for food fortification and in dietary supplements. Fruits and vegetables are a good source of naturally occurring folate, primarily 5-methyltetrahydrofolate (5MTHF) (Konings et al., 2001; Vahteristo, 1998; Vahteristo et al., 1997), which is the most bioavailable form of folate (Müller, 1993a).

Existing US food composition data for folate (United States Department of Agriculture, Agricultural Research Service, 2004) are derived from microbiological assay of total folate, whereby growth of a specific microorganism (*Lactobacillus casei* v. *rhamnosus*) is related to folate concentration (Eitenmiller & Landen, 1999, Chap. 11, pp. 454–457) and different vitamers are not

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distinguished. In contrast, high-performance liquid chromatography (HPLC) methods offer chemically definitive determination of individual folates (Konings, 1999; Pawlosky, Flanagan, & Pfeiffer, 2001). Konings et al. (2001) recently reported the folate composition of selected Dutch foods measured by HPLC, but such data are not available for the vast majority of foods consumed in the US.

The United States Department of Agriculture (USDA) National Food and Nutrient Analysis Program (NFNAP) is an ongoing project with the goal of updating and increasing the reliability of food composition data in the US National Nutrient Database for Standard Reference using a key foods approach and representative nationwide sampling (Haytowitz, Pehrsson, & Holden, 2000; Haytowitz, Pehrsson, & Holden, 2002; Pehrsson, Haytowitz, Holden, Perry, & Beckler, 2000; Perry, Beckler, Pehrsson, & Holden, 2001). Fresh produce is a major category of food being analyzed in the NFNAP, and folate is a key nutrient. Food samples are obtained from multiple outlets and must be composited prior to analysis. The large number of nutrients, foods, and laboratories involved in the NFNAP demand a practical and cost-effective sample handling scheme. The usual protocol involves shipping the foods to a central facility to be prepared, composited, homogenized, and distributed to multiple laboratories for analysis of various nutrients.

Homogenizing, freezing, and thawing of fresh fruits and vegetables disrupts cell membranes and releases endogenous enzymes that may oxidize, cause interconversions or otherwise alter the chemical composition of folates (Vahteristo, Lehikoinen, Ollilainen, & Varo, 1997). Resulting changes might increase during prolonged storage of samples prior to analysis and also vary with differences in food composition, oxygen availability, chemical environment, extent of heating, and forms of folate in the food. For example the presence of ascorbic acid increases the stability of folate while iron (Fe^{2+}) reduces stability, and large losses can occur during cooking and canning due to the water solubility of folates (Eitenmiller & Landen, 1999, Chap. 11, p. 418).

Maximum stabilization of nutrients in foods sampled for the NFNAP is accomplished by rapid processing in liquid nitrogen and storing homogenized samples at −60 ± 5 °C, under nitrogen, in darkness. Although folate in samples was typically analyzed within 2–3 weeks of preparation, knowledge of the stability under our long-term storage conditions was needed for flexibility in analytical schedules as well for verification that stored samples could be used for repeat analyses if necessary. Since 5MTHF comprises most of the folate in fruits and vegetables, the goal of this study was to evaluate the stability of 5MTHF in a representative range of fresh-frozen produce over time under the conditions of sample storage for the NFNAP.

2. Materials and methods

2.1. Samples

Seven fresh fruits and vegetables were selected to represent a range of matrices and folate concentration. Broccoli (*Brassica oleracea* var.*italica*), spinach (*Spinacia oleracea*), strawberries *(Fragaria X ananassa*), navel oranges (*Citrus sinensis*), red delicious apples (*Malus domestica*), bananas (*Musa X paradisiaca*), and russet potatoes (*Solanum tuberosum*) were studied. Two composites of each food were prepared from separate lots of 6.6–8.8 kg raw material purchased approximately 1 week apart at a major local grocery store (Kroger; Blacksburg, VA). Each lot was prepared and homogenized as follows. Immediately prior to homogenization the produce (except bananas) was rinsed thoroughly with distilled deionized (ddi) water, dried with a clean lint-free towel, trimmed of inedible parts (e.g. cores, stems) and damaged (e.g. moldy, bruised) areas. The peel was included in apple and potato composites. The fruit or vegetable was then cut into ∼1.25 cm pieces, quickly frozen in liquid nitrogen, and homogenized with a 6L Blixer food processor (Robot Coupe®, Ridgeland, MS, USA). The homogenized material was kept frozen in liquid nitrogen and dispensed among forty-eight 60 mL glass jars that were then sealed with Teflon®-lined caps, wrapped in aluminum foil, and stored at −60 ± 5 °C.

A control material was similarly prepared from canned whole-leaf spinach liquid and solids (no salt added) (Del Monte©; San Francisco, CA) except no liquid nitrogen was used during homogenization, and subsamples were stored at −75 ± 5 °C. A commercial reference material (BCR 485), a lyophilized mixture of sweet corn, tomatoes, and carrots developed by the European Commission, Institute of Reference Materials and Measurement (European Commission, Community Bureau of Reference, 1998) and supplied with an indicative value for 5MTHF, was purchased from RT Corporation (Laramie, Wyoming). The moisture content of BCR 485 was determined as weight lost upon drying a 2 g sub-sample at 103 ± 2 °C for 2 h at a pressure of 635 mmHg. All values for BCR 485 are reported on a dry weight basis.

2.2. Reagents and enzymes

Reagents and solvents were ACS reagent or HPLC grade. Potassium phosphate, 2-mercaptoethanol, and L-ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Acetonitrile, *o*-phosphoric acid (∼85%), sodium hydroxide, sodium chloride, and 2-octanol (laboratory grade) were purchased from Fisher Scientific (Pittsburgh, PA). 5-Methyltetrahydrofolic acid disodium salt was obtained from Sigma and the concen-

tration of 5MTHF was calculated from moisture content and purity (based on absorbance at 290nm) reported on the certificate of analysis. Protease type XIV (*Streptomyces griseus*, 5.7 units/mg) and α-amylase (*Aspergillus oryzae*, 56 units/mg) were also from Sigma. Rat plasma (non-sterile, adult male, with lithium/heparin anticoagulant) was obtained from Harlan Bioproducts (Indianapolis, IN). The plasma was centrifuged at 5500 rpm (7280 G) for 20 min at 4 °C, and the supernatant was recovered and tested to verify the absence of 5-methyltetrahydrofolate by assaying a 0.1 mL aliquot as described below.

2.3. Analysis of 5-methyltetrahydrofolate

Three aliquots of each composite were analyzed for 5MTHF immediately after preparation and again after 2, 7, and 30 days storage at −60 ± 5 °C in darkness. After 30 days, each composite was analyzed at approximately 60–90-day intervals for up to 12 months.

A modification of a previously reported tri-enzyme extraction and HPLC method with fluorescence and diode array detection (Doherty & Beecher, 2003) was used for quantitation of 5MTHF, as follows. Frozen samples were thawed in the sealed jars in a water bath at 25 ± 2 °C for 20 min immediately before analysis. After thorough stirring, an aliquot (2–6 g) was weighed into a 50 mL polycarbonate tube to achieve an estimated 5MTHF concentration of 80–180 ng/mL in the final dilution (below). Extraction buffer (10 mL; 1 M potassium phosphate, 10 mM ascorbic acid, 10 mM 2-mercaptoethanol, pH 6.0) was added and the sample was blended 2 min with a tissue homogenizer (Omni-Mixer®; Omni International, Warrenton, VA). The pH was adjusted to 6.0 with 4 M sodium hydroxide and α-amylase (20 mg) was added to each sample, followed by 3–5 drops *n*-octanol (anti-foaming agent) and incubation for 60 min at 37 °C with argon degassing during the first 15 min. Protease (1 mg) was added, incubated (3 h at 37 °C), then denatured by placing the tubes in a boiling water bath for 15 min then cooling in an ice bath for 5 min. Rat plasma (0.1 mL) was added to each sample as a source of folate deconjugase to convert folate polyglutamates to monoglutamates, followed by incubation for 14–18 h at 37 °C then boiling of tubes to inactivate the enzyme. Samples were centrifuged twice at 5500 rpm (7280 G) for 20 min. Supernatants from each sample were combined and filtered through Whatman 43® ashless filter paper in a Büchner funnel.

The extracts were further purified by strong anion exchange solid-phase extraction (SAX). Each SAX column (Extract Clean® 500 mg, Alltech, Deerfield, IL) was washed with 15 mL extraction buffer prior to applying the entire sample solution. The column was washed again with 15 mL extraction buffer, then 5MTFH was eluted with 6 mL of 1 M NaCl in 0.1 M potassium phosphate buffer (pH 6.0; 25% acetonitrile, 10 mM 2-mercaptoethanol, 10 mM ascorbic acid). Two to five drops *n*-octanol were added to each eluate, acetonitrile was evaporated with argon (30 min/50 °C), then each sample was diluted in volumetric flask with extraction buffer to between 10 and 50 mL to yield a target concentration within the HPLC calibration range (10–200 ng/mL) based on expected 5MTHF content. An aliquot of the solution (~1 mL) was transferred to an amber glass autosampler vial and routinely analyzed within 24 h, or the solution was held at ≤−70 °C and thawed and analyzed within 3 days.

HPLC was performed with a PE-Nelson (Norwalk, CT) system that included an LC Binary Pump 250, Advance LC sample processor ISS200, an LC oven 101, a diode array detector 235C, a fluorescence detector (model RF10-AXL; Shimadzu, Columbia, MD), and an Adsorbosphere™ HSC18 column, 150 mm × 4.6 mm × 3 μm ID (Alltech). The mobile phase was phosphate buffer (33 mM, pH 2.2)/acetonitrile, with a gradient from 0% to 30% acetonitrile at a flow rate of 0.8 mL/min: 100% buffer for 5 min, followed by linear gradient from 0% to 20% acetonitrile for 20 min, step to 30% acetonitrile for 5 min, then step to 100% buffer for 10 min. Column temperature was maintained at 28 ± 3 °C, and sample injections (200 μL) were made with an autosampler at room temperature (19–25 °C). Fluorescence data were recorded with emission and excitation wavelengths of 290 mm and 350 nm, respectively. Spectra were also captured on a diode array detector at 280 and 350 nm. 5MTHF was identified by comparison of the retention time and diode array spectral scan of the sample peak to that of a standard of similar concentration. Quantitation was based on fluorescence response. A calibration curve was constructed from data for 5MTHF standards at six concentrations ranging from approximately 10 to 200 ng/mL that were run in duplicate with each batch of samples. The curve followed a second order polynomial regression and was only accepted if the r^2 value was greater than 0.98.

2.4. Analytical quality control

Standardized homogenization protocols were used to prepare the composites. The homogeneity of each composite was verified by visual evaluation as well as analysis of moisture in aliquots taken from different points in the sub-sampling sequence for each homogenate. Two-gram aliquots sampled from throughout the dispensing sequence were vacuum dried for 3.5 h at 65 ± 5 °C at 635 mmHg to determine moisture content.

Accuracy of the 5MTHF assay was validated by analysis of BCR 485 reference material (lyophilized mixed vegetables) and independent analysis of aliquots of selected composites that were shipped (frozen, on dry ice) to a second laboratory [USDA Food Composition

Laboratory (FCL), Beltsville, MD], by HPLC and LC-MS using isotope dilution of added $^{13}C_5$-glutamyl-5MTHF (Pawlosky & Flanagan, 2001; Pawlosky et al., 2001).

For routine quality control, each composite was assayed in triplicate in each run, and BCR 485 and the canned spinach control composite were analyzed in each assay batch to monitor run-to-run precision.

2.5. Data analysis

5MTHF concentration in a given composite at the time it was prepared ($5MTHF_0$) was compared to 5MTHF concentration after the maximum storage time ($5MTHF_f$). Confidence intervals were calculated for the difference between 5MTHF in the test and control composites at each of time zero and final storage time ($\Delta 5MTHF_0$ and $\Delta 5MTHF_f$, respectively), as ±1.96 times the estimated standard error (SE). SE was a pooled estimate of variance calculated from the between- and within-assay variance. Because change in 5MTHF concentration in the test composites was the subject of study, between-assay analytical variance could not be separated from actual change in concentration; therefore, data for the first 39 assays of the canned spinach control material were used to estimate between-assay variance in the following calculation of SE for confidence intervals:

$$\sqrt{\frac{MSE_{test} + MSB_{controls}}{n_{test}} + \frac{SST_{controls}}{n_{controls}(n_{controls} - 1)}},$$

where MSE_{test} is the mean square error for the test composite data, $MSB_{controls}$ corresponds to the mean square between-assay for the control composite, $SST_{controls}$ is the total sum of squares for the control composite data, n_{test} is the number of replicates at the final storage time for the test composite, and $n_{controls}$ is the total number of control composite values. Parameters were calculated using the General Linear Model procedure (SAS, 2001). Because the formula is based on combining sum of squares, exact degrees of freedom are not available. The p value for any significant difference was determined from a z-normal table (Kirk, 1982).

3. Results and discussion

3.1. Quality control

3.1.1. Homogeneity

All composites were visually uniform among 2 g subsamples, though small pieces of varying size were observed throughout each composite. Moisture contents are reported in Table 1; differences among subsamples from the same composite were <0.5% in all cases.

3.1.2. Reference and control materials

Representative fluorescence chromatograms and diode array spectra for 5MTHF are shown in Fig. 1. Results for the canned spinach control composite and BCR 485 reference material are shown in Fig. 2. The assayed mean 5MTHF concentration in BCR 485 over the course of 15 months and 75 assay batches (Fig. 2(b)) was 205 μg/100 g, and all values were within the published tolerance limits for that material (European Commission, Community Bureau of Reference, 1998).

3.1.3. Precision and limit of quantitation

For samples with 5MTHF concentration greater than 5 μg/100 g the mean within- and between-assay relative standard deviations were 8–15% and 12–28%, respectively. Differences in final dilution and sample size necessary for foods with widely different 5MTHF contents and the resulting multiplication of variability in subsequent calculations to determine fresh weight concentration contribute to precision differences for different foods. These differences in assay precision may not be accounted for in some reported folate values obtained from other assay systems in which a particular food is analyzed in a single run, with a limited number of replicates. In these cases uncertainty of the reported mean folate concentration would be underestimated.

Given the multiple extractions, transfers, dilutions and other manipulation of samples, introduction of an internal standard (IS) at the beginning of the assay could substantially reduce overall analytical variability and

Table 1
Moisture content of composites (g/100 g)

Food	Composite I			Composite II		
	Mean (n)	SD	Range	Mean (n)	SD	Range
Spinach	89.83 (2)	0.05	89.80–89.87	89.77 (4)	0.08	89.70–89.88
Strawberries	91.52 (2)	0.10	91.45–91.59	91.62 (2)	0.17	91.50–91.74
Oranges	86.89 (4)	0.10	86.76–87.00	85.31 (2)	0.09	85.25–85.37
Potatoes	81.34 (2)	0.06	81.29–81.38	83.07 (2)	0.01	83.06–83.08
Bananas	74.12 (4)	0.15	74.00–74.33	73.33 (4)	0.23	73.01–73.56
Apples	84.24 (2)	0.06	82.20–84.28	86.43 (2)	0.01	86.42–86.43
Broccoli	90.24 (2)	0.04	90.22–90.27	88.23 (4)	0.13	88.12–88.35
Canned Spinach (control)	92.43 (4)	0.13	92.26–92.54	n/a	n/a	n/a

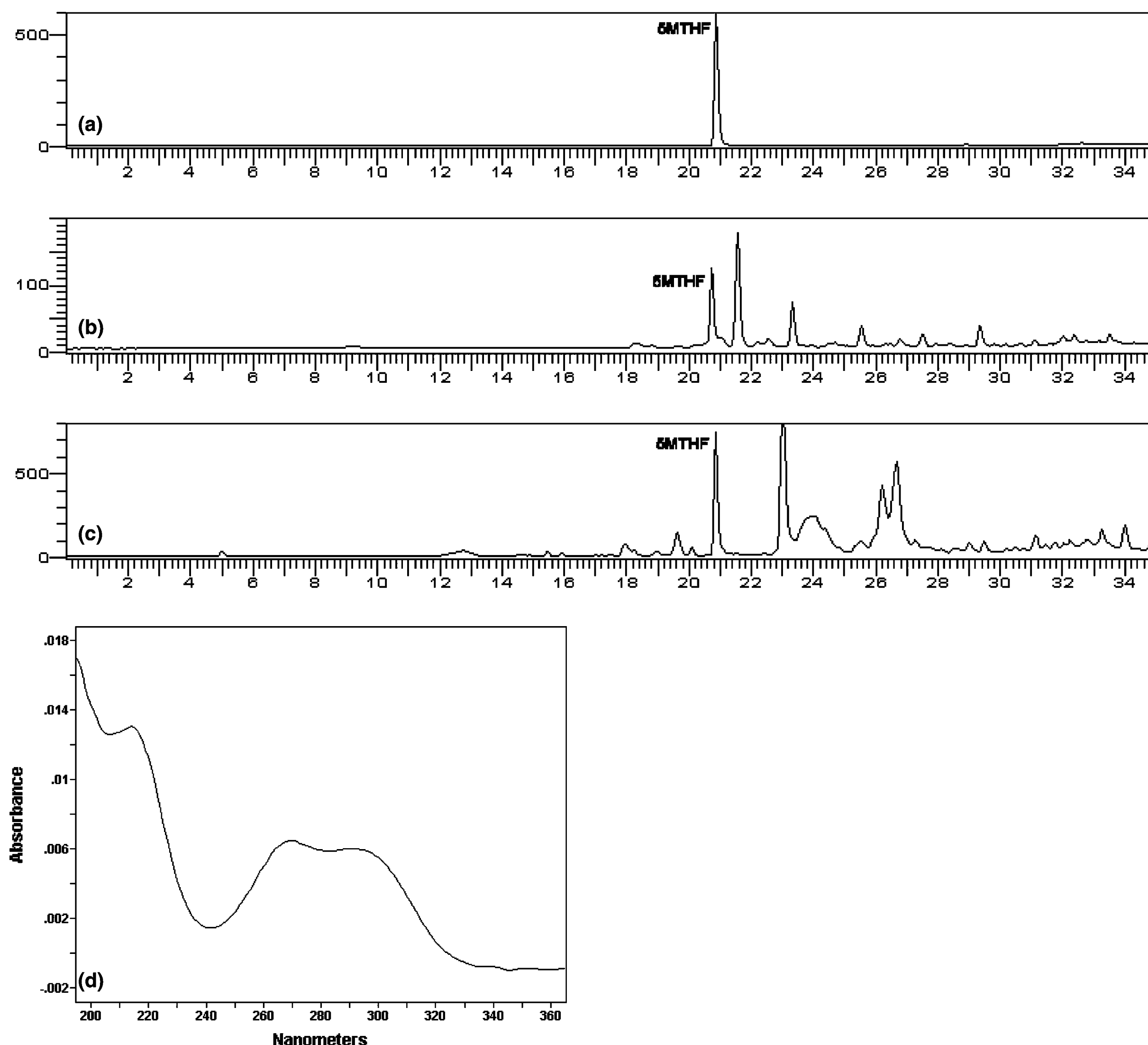

Fig. 1. Representative fluorescence chromatograms and diode array spectra for 5-methyltetrahydrofolate (5MTHF) in a standard and selected samples. (a) Fluorescence chromatogram for 53.7 ng/mL standard of 5MTHF; (b) fluorescence chromatogram for strawberries; (c) fluorescence chromatogram for broccoli; (d) Spectral scan from 200 to 360 nm for 5MTHF standard taken at the maximum of the 5MTHF peak.

improve the quality of folate results. However, no suitable IS has been identified.

The practical limit of quantitation (LOQ) was ~3 μg/100 g and depended on the sample matrix and folate content. With a clear baseline (e.g. orange juice), the LOQ could be as low as 1 μg/100 g, but if there are interfering compounds the amount of 5MTHF must give a much higher response for quantitation. Oranges are an example of a food with background interference but 5MTHF content high enough to permit quantitation, whereas bananas are a sample with a low 5MTHF content as well as interference.

3.1.4. Inter-laboratory analyses

Table 2 summarizes results for 5MTHF in composites that were also assayed by HPLC with fluorescence detection (HPLC-FD) and LC-MS at another laboratory (FCL). Results were in excellent agreement between laboratories based on the limited number of replicate assays, with only slight differences in values for broccoli and strawberries observed by both fluorescence and MS detection. Interestingly, although other researchers have found erroneously high values using HPLC-FD quantification of 5MTHF in broccoli (Freisleben, Schieberle, & Rychlik, 2003), our results for broccoli were closer to the lower concentrations verified by those authors using stable isotope dilution mass spectrometry, and no significant difference was observed between HPLC-FD and LC-MS values determined within the same laboratory in our study (Table 2). While caution should be exercised in comparing different samples of a food due to inherent variability in

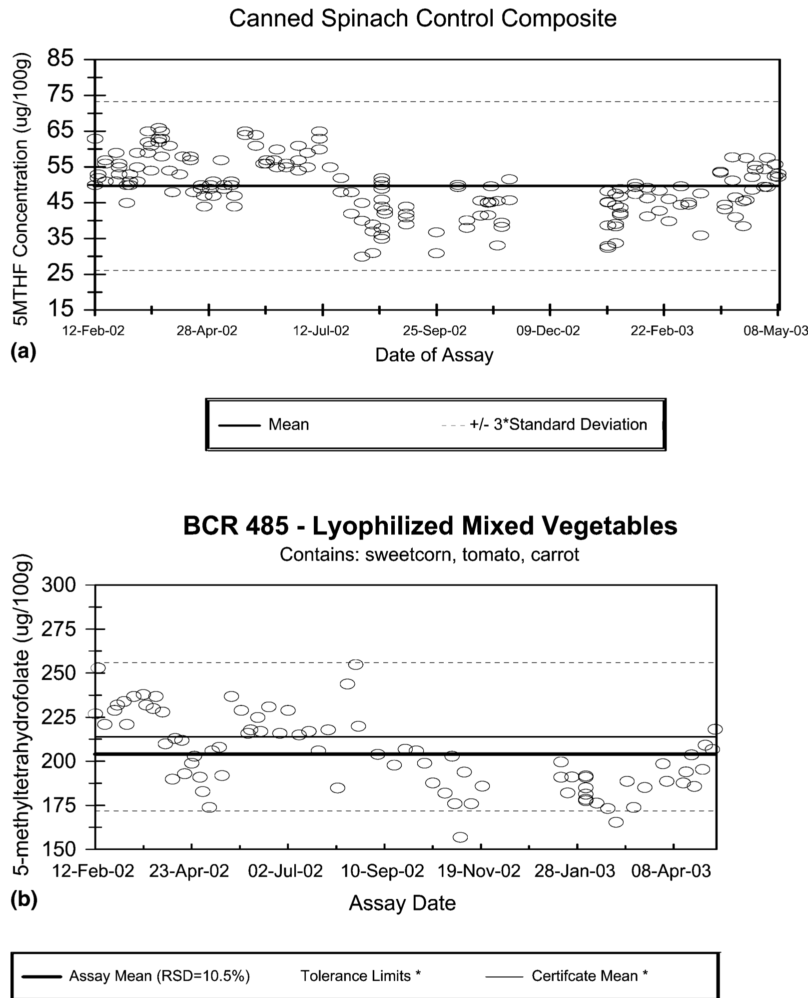

Fig. 2. Results for canned spinach control composite (a) and BCR 485 reference material (b).

Table 2
Results for interlaboratory analysis of 5-methyltetrahydrofolate (5MTHF) in selected samples[a]

Sample	VT 5MTHF (μg/100 g) mean (SE)	FCL 5MTHF (μg/100 g) mean (SE)	FCL 5MTHF (μg/100 g) mean (SE)
	HPLC-FD	HPLC-FD	LC-MS
BCR 485 (freeze-dried mixed vegetables) reference material[b]	204 (2.4)	196 (3.3)	na
Spinach (composite I)	89 (2.8)	75 (6.1)	89 (3.4)
Spinach (composite II)	57 (1.7)	57 (2.4)	na
Strawberries (composite I)	21 (1.1)	28 (0.2)	24 (0.1)
Strawberries (composite II)	14 (0.6)	29 (1.6)	22 (1.3)
Broccoli (composite II)	40 (1.6)	28 (0.3)	34 (0.9)
Oranges (composite II)	23 (1.4)	20 (0.9)	na
Potatoes (composite II)	9 (0.9)	10 (0.3)	5 (0.9)

[a] VT, Virginia Tech; FCL, USDA Food Composition Laboratory; HPLC-FD, Quantitation by HPLC with fluorescence detection; LC-MS, Quantitation by liquid chromatography-isotope dilution mass spectrometry; na, not assayed; SE, standard error.
[b] Indicative 5MTHF mean and tolerance limits: 214 ± 42 μg/100 g (European Commission, Community Bureau of Reference, 1998).

nutrient concentrations, the analytical observations of Freisleben et al. (2003) suggest that in routine assay by HPLC-FD it is advisable to confirm values using MS if a particular food is found to have a nutritionally significant 5MTHF content.

Table 3
5-Methyltetrahydrofolate (5MTHF) content of fruit and vegetable composites thawed by two different methods

Food	Composite	Thawed overnight[a] 5MTHF (μg/100 g) mean (SE)	Quickly thawed[b] 5MTHF (μg/100 g) mean (SE)
Spinach	I	74[c] (1.3)	113[c](2.6)
Oranges	I	28 (1.3)	26 (2.0)
Strawberries	I	27 (1.0)	27 (0.5)
Broccoli	II	46 (1.0)	47 (1.5)
Apples	I	4 (0.3)	3 (0.3)

[a] 15–18 h in refrigerator (2–8 °C), followed by 20 min at room temperature (20–25 °C); SE, standard error.
[b] 20 min in 25 ± 2 °C water bath.
[c] Means are significantly different ($p = 0.0002$).

3.1.5. Effect of thawing conditions

Since early assays indicated possible loss of 5MTHF in samples thawed at 2–8 °C [15–18 h, followed by 20 min at room temperature (20–25 °C)] versus quickly thawing at room temperature immediately prior to analysis (20 min at 25 ± 2 °C), the latter was adopted as the standard method for the remainder of the study. Data from any samples thawed overnight were excluded from the evaluation of 5MTHF stability over time. However, several of the composites were later assayed

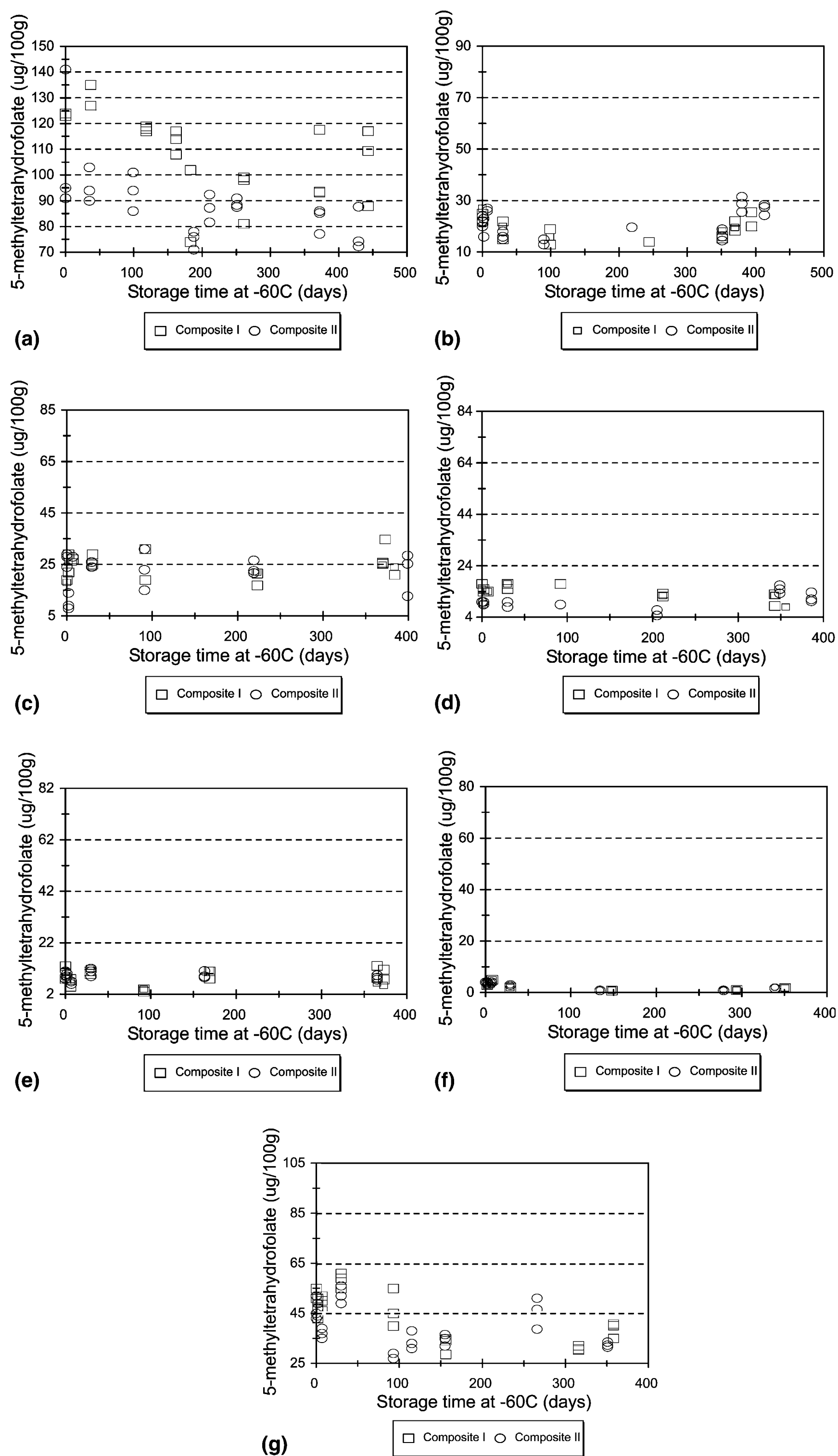

Fig. 3. 5-Methyltetrahydrofolate (5MTHF) concentration in homogenized fruit and vegetable composites stored at −60 ± 5 °C: spinach (a); strawberries (b); oranges (c); potatoes (d); bananas (e); apples (f); broccoli (g).

in a controlled comparison of thawing methods to further evaluate the impact of this step on 5MTHF content.

While thawing method had no effect on 5MTHF ($p > 0.3$) in apples, strawberries, oranges, and broccoli, 5MTHF was markedly higher in frozen fresh spinach samples that were quick-versus overnight-thawed (113 and 74 μg/100 g, respectively; $p = 0.0002$) (Table 3). These results were confirmed by independent experiments at a second laboratory (FCL) and suggest lability of 5MTHF in some but not all food matrices at refrigeration temperature. It is possible that comminution of the food releases endogenous enzymes that degrade 5MTHF, and that there is some activity at 2–8 °C, with spinach but not all foods containing such enzymes. Further experiments are necessary to clarify the interaction between storage temperature and matrix effects in different foods. Though further studies are necessary to determine the mechanism for the observed loss of 5MTHF folate, our results here suggest that "quick" thawing should be used routinely for reliable analysis of 5MTHF in fresh-frozen fruits and vegetables.

3.2. Stability of 5MTHF in fresh-frozen fruits and vegetables

Fig. 3 shows 5MTHF concentration as a function of storage time in each of the seven fruits and vegetables studied. No change in 5MTHF could be detected in any of the composites after 12 months using a 95% confidence interval for the difference in 5MTHF concentration between the test and control samples (Table 4). We conclude that fresh fruits and vegetables may be homogenized and stored frozen under the conditions of the present study for up to 12 months with no loss of 5MTHF beyond the limits of analytical variability.

Table 4
95% Confidence intervals (CI) for the difference between test composite and control composite mean 5MTHF concentration at time zero and final storage time[a]

Composite	Final storage time at −60 ± 5 °C (days)	Time zero CI (lower limit, upper limit)	Final storage time CI (lower limit, upper limit)
Spinach I	443	53.4, 82.8	35.0, 64.4
Spinach II	429	38.0, 69.5	7.0, 38.5
Strawberries I	394	21.0, 40.2	22.7, 41.8
Strawberries II	413	23.8, 42.7	18.8, 37.7
Oranges I	373	26.9, 46.6	10.4, 30.1
Oranges II	399	17.7, 38.8	22.7, 43.8
Broccoli I	358	−8.5, 11.7	6.2, 26.4
Broccoli II	351	−1.3, 18.5	13.4, 33.1
Bananas I	373	35.8, 54.7	37.8, 56.7
Bananas II	365	36.1, 54.5	37.1, 55.5
Potatoes I	343	29.6, 48.2	38.0, 56.5
Potatoes II	386	36.1, 54.4	34.1, 52.4
Apples I	351	43.1, 61.4	44.1, 62.4
Apples II	339	42.1, 60.4	44.1, 62.4

[a] See text for description of statistical analysis.

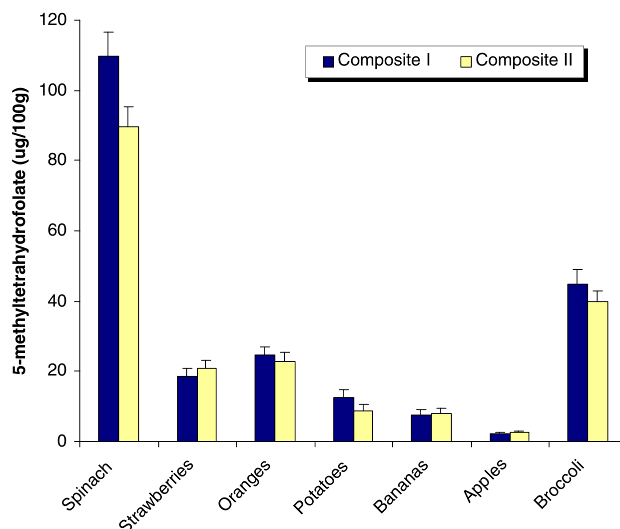

Fig. 4. Mean 5-methyltetrahydrofolate (5MTHF) content of assayed fruit and vegetable composites, with 95% confidence intervals.

Fig. 4 summarizes the overall 95% confidence interval for the mean 5MTHF content of each composite. Between-composite differences in 5MTHF were evident for spinach, broccoli, and potatoes, but only spinach exhibited a remarkable difference between the two composites. For each food the two composites were collected from the same retail outlet 2 weeks apart; therefore these data illustrate natural variation in folate content that may exist among specific lots of a given food purchased in the manner of a typical consumer. More variation might be expected among samples from different seasons, growing locations, varieties, as well as pre- and post-purchase storage conditions prior to consumption.

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