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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.

Keywords: Folate; 5-Methyltetrahydrofolate; High-performance liquid chromatography; Pteroylglutamic acid; Fruit; Vegetables; Food composition; Stability; Sample preparation; Spinach; Broccoli; Strawberries; Bananas; Potatoes; Apples; Oranges

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-