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Original Article

A differential assay of folic acid and total folate in foods containing enriched cereal-grain products to calculate μg dietary folate equivalents (μg DFE)

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Abstract

A method was developed to differentiate folic acid from total folate in enriched cereal products. By elimination of the protease and conjugase digestion steps from the trienzyme digestion used to measure total folate, folic acid can be directly quantified by the microbiological assay using *Lactobacillus casei* (*ssp. rhamnosus*) ATCC 7469. Determination of total folate by the trienzyme digestion together with assay of folic acid by the procedure reported here allows calculation of food folate and μg dietary folate equivalents (μg DFE). Recovery studies conducted at five concentration levels in a non-fortified wheat flour gave a mean recovery of 95.5 (RSD% 9.7) for folic acid and 98.5 (RSD% 5.0) for total folate. Recoveries from a variety of enriched cereal foods ranged from 88% to 99% for the folic acid extraction and 90% to 96% for the trienzyme digestion. Analysis of SRM 1846 Infant Formula ($n = 11$) gave means ± S.D. of 123.5 ± 6.9 μg/100 g of folic acid and 136.6 ± 6.7 μg/100 g of total folate (reference concentration value = 129 ± 28 μg/100 g). Intermediate precision determined as intra-laboratory reproducibility(r) gave RSDr % values of 4.32 for the folic acid assay and 3.15 for the total folate assay. Microgram DFE values were determined for several enriched cereals by calculating food folate as the difference between total folate and folic acid and the formula μg DFE = μg food folate + (1.7 × μg folic acid). The differential assay of folic acid and total folate is a simple and accurate procedure to determine μg DFE in enriched cereal-grains or foods containing enriched flour.

Keywords: Analysis; Folic acid; Total folate; μg DFE; Food folate

1. Introduction

Fortification of cereal-grain products with folic acid began in the United States on January 1, 1998 (Department of Health and Human Services, Public Health Service and Food and Drug Administration (DHHS/PHS), 1996). The current fortification level is 140 μg/100 g product. For specific products, the required levels specified in the standard identities reflect the fortification level plus the amount of native, food folate provided by the unfortified product (Department of Health and Human Services, Public Health Service and Food and Drug Administration (DHHS/PHS), 1996). Thus, for enriched white flour, the minimum amount of folate plus folic acid in the product is 700 μg/lb or 156 μg/100 g, reflecting approximately 16 μg/100 g of native food folate per 100 g of product.

Shortly after initiation of the folic acid fortification program, the Institute of Medicine, National Academies of Science, published the dietary reference intake report on recommendations for folate intake (Institute of Medicine, 1998). The μg dietary folate equivalent (μg DFE) was established to adjust for differences in bioavailability of folic acid which is considerably more available than food folate (Sauberlich et al., 1987; Cuskelly et al., 1996; Wei et al., 1996; Pfeiffer et al., 1997a; Gregory, 1997). Folic acid

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taken with food was determined to be 85 percent bioavailable compared to 50 percent bioavailability for food folate (Gregory, 2004). Therefore, folic acid taken with food is 1.7 times (85/50) more available (DHHS/PHS, 1996). To adjust for this difference, the μg DFE was set according to the following relationship:

1 μg DFE
= 1 μg food folate
= 0.6 μg folic acid taken with meals.

Therefore, μg DFE = μg food folate
+ (1.7 × μg folic acid).

In order to calculate the amount of μg DFE present in a food, the analytical method must be capable of differentiating folic acid from folates. Such differentiation can be accomplished by LC or LC combined with mass spectrometry methods; however, few of these methods are in routine use in food analysis programs due to complexity, cost and time intensiveness.

Currently, a widely accepted method for determination of total folate is microbiological assay by *Lactobacillus casei* (*ssp. rhamnosus*) ATCC 7469. A common extraction uses a trienzyme extraction with α-amylase and a broad spectrum protease (Pronase®) to liberate food folates from non-specific binding by carbohydrates and proteins and by conjugase to remove glutamic acid residues from γ-glutamyl folates with three or more glutamic acid residues (DeSouza and Eitenmiller, 1990; Martin et al., 1990). Deconjugation of folate is necessary since *L. casei* (*ssp. rhamnosus*) ATCC 7469 will not respond to poly γ-glutamyl folates with more than three glutamic acid residues (Goli and Vanderslice, 1992). Since its introduction, the trienzyme extraction has been documented by many research groups as a sound method to extract folate from most food matrices. Tamura et al. (1997) suggested that food composition databases be revised with methodology incorporating the trienzyme extraction. Pfeiffer et al. (1997b) applied the extraction to LC quantitation of folate in cereal-grain products and concluded that the trienzyme extraction was applicable to both LC and microbiological assays. They further stated that the trienzyme extraction should be used for folate assay of cereal-grain products. The trienzyme extraction was adopted into routine analysis at the Atlanta Center for Nutrient Analysis, US Food and Drug Administration, several years ago for assay of FDA Total Diet Market Basket samples.

More recent work by Rader et al. (1998, 2000) documented the efficacy of the trienzyme extraction for analysis of total folate in fortified and non-fortified cereals. Modifications proposed by Rader et al. (1998, 2000) were incorporated into a collaborative study on the trienzyme procedure completed by AOAC International (AOAC) and the American Association of Cereal Chemists (AACC) (DeVries et al., 2001). The method was accepted Official First Action Status by AOAC (Devries et al., 2005) and First Approval Status by AACC (AACC, 2000).

Based upon wide acceptance of the microbiological method for total folate in foods, the purpose of the current research was to modify the AACC method to allow direct microbiological assay of folic acid in cereal-grain products. The determination of folic acid and determination of total folate by the AACC method provides necessary analytical data to calculate μg DFE.

2. Materials and methods

2.1. Samples

Non-enriched and enriched cereal-grain products were collected through the United States Department of Agriculture National Food and Nutrient Analysis Program (USDA/NFNAP). The NFNAP sampling program ensures nationwide, representative food samples for nutrient analysis. Some prepared food samples containing enriched flour were purchased in the Atlanta, GA area. USDA samples were composited at Virginia Polytechnic Institute and State University, Food Analysis Laboratory Control Center (FALCC) according to NFNAP protocols. Full details of the NFNAP sampling program that assures a representative sample are available (Pehrsson et al., 2003). Subsamples were shipped frozen to the University of Georgia and held frozen at $< -40\,^{\circ}C$ until assayed. Folic acid and total folate analyses were completed within 1 month of sample receipt.

2.2. Total folate and folic acid analyses

Total folate was assayed by AOAC Method 2004.05 (DeVries et al., 2005) (AACC Method 86–47, AACC, 2000). The procedure involves autoclaving 0.25–1.0 g of sample for 15 min in 20 mL of 0.1 M phosphate buffer, pH 7.8, containing 1% ascorbic acid plus water to give 50 mL. Following autoclaving and cooling, an additional 10 mL of buffer is added to the digest. The trienzyme digestion is completed by adding 1.0 mL of Pronase® (Calbiochem, #53702, San Diego, CA) solution (2.0 mg/mL in water) and digesting for 3 h at 37 °C. Following the Pronase® digestion, the digest is heated for 3 min at 100 °C, cooled and digested with α-amylase (Fluka, #10065, St Louis, MO) (1.0 mL of a 20 mg/mL solution in water) for 2 h at 37 °C. Conjugase digestion is then completed by adding 4 mL of chicken pancreas conjugase (Difco, #245910, Sparks, MD) solution (5 mg/mL in water) and holding at 37 °C for 16 h. After digestion, the digest is heated at 100 °C for 3 min, cooled, adjusted to pH 4.5 with HCl, taken to volume with water and filtered through ashless filter paper. For further dilution, an aliquot of the filtrate plus an equal volume of the pH 6.8 buffer is taken to final volume with adjustment to pH 6.8, if necessary. In our procedure to directly assay folic acid, the Pronase® and conjugase digestion steps are eliminated. Assay is by standard