FOLIC ACID: CONSIDERATIONS REGARDING FOOD VALUES IN DATABASES.

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The need for current and validated databases for nutrients in foods is very apparent as dietary guidance increasingly focuses on specific nutrients or classes of nutrients and their roles in reducing risk of chronic illness. Folate is a critically important micronutrient because increased intakes of this B vitamin by some women may reduce their risk of a neural tube birth defect-affected pregnancy (DHHS/PHS, 1992).

On March 5, 1996, FDA finalized regulations mandating the fortification of a wide range of enriched cereal-grain products with folic acid. FDA took this action to assist women of childbearing age in meeting the Public Health Service recommendation that they consume 400 ：g folate per day to reduce their risk of having a pregnancy affected with a neural tube birth defect (FDA, 1996a, 1996b, 1996c).

USDA database values for folate in foods played an important role as FDA developed its fortification proposals (FDA, 1993a, 1993b). FDA used Nationwide Food Consumption Survey (NFCS) data along with the folate composition data base that provided specific information on folate content of foods and food ingredients to estimate "usual" daily folate intakes from all sources. The agency then estimated the potential impact of various fortification options by altering the folate composition of selected foods and ingredients in the data bases and recalculating intakes. For example, the agency recalculated intake estimates to determine potential intake levels if, for example, all cereal-grain products were fortified with 70 ：g folic acid/100 g or with 140 ：g folic acid/100 g, etc. (Crane et al., 1995). The availability of ingredient data allowed FDA to estimate potential effects on folate intakes that could result from fortifying the flour consumed in breads, rolls, buns or cake as well as the flour consumed as a component of, for example, a frozen dinner (e.g., in pizza crust, noodles, breadings, etc.)

The validity of these estimates is dependent upon the accuracy of the folate data in the data bases. Two potential sources of underestimation in the calculation of intakes were noted in FDA's fortification documents: (1) There is general agreement that methods currently used for folate analysis are unsatisfactory and very likely underestimate the folate content of foods. Comparisons of newer methods of sample preparation with older methods have consistently revealed underestimates in the range of 20 to 50 percent for some products; and (2) Manufacturers frequently add overages of nutrients such as folic acid to products to ensure that the product contains at least the amount of the nutrient shown on the label. Because, in updating food composition data bases, label values were used to define the folate composition of some fortified foods (e.g., ready-to-eat cereals, dietary supplements), the values that the agency used are likely to understate the actual folate content of such foods.

January 1, 1998 was the full compliance date for the new fortification regulations (Table

1) (FDA, 1996b). This deadline again focused attention on the need for validated methods for folate analysis. Difficulties with traditional methods of analysis include incomplete release of folates from food matrices and incomplete hydrolysis of folylpolyglutamates prior to quantification (Gregory et al., 1989; Tamura, 1990; IOM, 1998).

There is currently no official method for the analysis of folate in foods. Association of Official Analytical Chemists (AOAC) official method 992.05 (Folic Acid Pteroyl-monoglutamic Acid in Infant Formula) which uses Lactobacillus casei (L. rhamnosus) (AOAC, 1995), is a microbiological method that was developed for analysis of folate in infant formula. This method proved to be a suitable starting point from which to develop a method for use with cereal-grain products. While AOAC official method 992.05 was not intended for measurement of total folate, the method can measure folate indigenous to infant formula ingredients other than added folic acid because it includes the deconjugation step with chicken pancreas conjugase (Eitenmiller and Landen, 1995). Additional extraction steps that are needed include the use of "-amylase and protease. Use of extractions employing these enzymes has been shown to increase the yield of folates from high starch or glycogen-rich foods and from high protein foods ("-amylase, and protease, respectively)(DeSouza and Eitenmiller, 1990).

We undertook studies to determine the pre-fortification levels of folate in enriched cereal-grain products that would be subject to the new regulations. We also needed to identify and validate modifications of AOAC official method 992.05 that were expected to lead to a method suitable for a collaborative study for the determination of folates in cereal-grain products. The process of validating the microbiological assay for cereal-grain products included determining optimal conditions for use of the enzymes, optimal pH, the response of the assay microorganism Lactobacillus casei to calibrants other than folic acid, recoveries of calibrants added to food samples, analysis of a folate-containing Standard Reference Material, and analysis of folate-containing samples from the check sample program of the American Association of Cereal Chemists. Representative cereal-grain products were then analyzed by the single enzyme (i.e., conjugase only) procedure and by the tri-enzyme assay to gain insight into the extent to which the former methodologies may have underestimated the folate content of cereal-grain products.

Analyses of breads, flours, corn grits and meals, rice, macaroni and noodle products were included in our studies. Fifty-seven such products were analyzed by the single enzyme assay and 37 of the products were assayed by the new tri-enzyme methodology. With respect to necessary validation steps, we found that treatment of digests with 20 mg of the chicken pancreas preparation for 4 hours was sufficient for the cereal-grain products we studied. Other aspects of the validation procedure, including recovery studies of folate added to cereal-grain foods, use of other calibrants, analysis of folate in an infant formula Standard Reference Material and analysis of folate in check samples from the American Association of Cereal Chemists, are described by Rader et al. (1998). It is important to recognize that the conditions we

identified for cereal-grain products may not be adequate for all types of foods. As Dr. Gregory emphasized earlier in this symposium, optimal conditions must be carefully identified for specific food types. While we optimized conditions of extraction for cereal-grain products, we do not know whether these conditions will be adequate for all types of foods. In this area alone, considerable work remains to be accomplished.

Levels of folate in 24 enriched bakery products, wheat flour, corn grits, corn meals, rice, macaroni and noodle products are listed in Tables 2 and 3. All of these products were analyzed by both the single-enzyme and tri-enzyme procedures. Of the 11 products listed in Table 2, 8 showed significantly increased levels of folate when assayed by the tri-enzyme procedure. Four (4) of 13 products listed in Table 3 showed significantly increased levels of folate when assayed by the tri-enzyme procedure. Two (2) of the enriched macaroni products (i.e., trio shells, large shells) were found to be fortified with folic acid at levels consistent with the March 5, 1996 regulations. The remainder of the products were not fortified at the time of product collection and analysis.

We also measured levels of folate in a wide range of ready-to-eat breakfast cereals and other breakfast foods. A sample of the results is shown in Table 4. The majority of these products identified an enriched cereal-grain component (e.g., enriched flour, enriched rice) in their ingredient lists. Many of these foods were fortified with folic acid. Results obtained by the single enzyme and tri-enzyme assays were not significantly different for these products.

At the time of sample collection for our studies, some cereal-grain foods were fortified with 10 to 100% of the Daily Value for folic acid (i.e., 40 to 400 ：g folate/serving). We compared label declarations for folate with the values found on analysis for the products listed in Table 5. Of particular interest was the finding that a wide variety of breakfast substitutes, broadly including foods such as frozen waffles, frozen pancakes, toaster pastries, granola bars, powdered instant breakfast beverages, breakfast bars, cereal bars, health bars, etc. were fortified with folic acid at levels up to 25 to 35% of the DV in some cases. These products make up about 20% of the total market for breakfast foods. In addition, products such as cereal bars, health bars, and granola bars are also eaten as snack foods. Consumption of such foods may contribute significantly to total daily folate intake. We found that the analyzed values for folate were 100 to 189% of the label declarations for a sampling of such products. The high values found may represent manufacturer's excess addition of folic acid in addition to endogenous folate in the products. The microbiological assay does not distinguish between "added" folate and "endogenous" folate in a food. As noted above, "overages" of nutrients are typically added to fortified foods. Because the microbiological assay measures "total folate" from all sources, it is not possible to quantify the "endogenous" component of folate in a fortified cereal-grain product and the "fortified" component in the product separately.

The essentiality of a tri-enzyme digestion has been clearly demonstrated by the work of

Pfeiffer et al. (1997) and Tamura et al. (1997). The work described here with a wide range of cereal-grain products has confirmed the need for tri-enzyme treatment during preparation of cereal-grain products for analysis of folate. All of these studies are in agreement that traditional extraction and conjugase treatments are not appropriate for the analysis of total folates from cereal-grain products.

The results obtained with the tri-enzyme procedure were significantly higher than results obtained with conjugase treatment alone for more than 30% of the cereal-grain products we examined. The results in Tables 2 and 3 indicate that the extent of differences between results obtained by the single enzyme and tri-enzyme methods varied from product to product. There is currently no way to predict with certainty how a specific product will respond to different extraction procedures. However, it is clear that failure to use a tri-enzyme extraction will lead to significant underestimations of folate content for many products.

With implementation of the new regulations, food composition tables will no longer be accurate for folate content of enriched cereal-grain products. While one might assume that it is possible to estimate with a high degree of confidence the folate content of an enriched cereal-grain product based upon the levels of folic acid required by the regulations, this is much more difficult in practice. This is because a manufacturer may add overages of folic acid of unknown magnitude to assure that the folate content of the food does not fall below the label declaration and because the food may also contain an unknown amount of endogenous folate to which the fortificant is added. In comparing "old" and "new" folate data, therefore, it is important to recognize that differences will derive from improved methods of analysis as well as from real changes in composition due to fortification. Thus, it is not possible to estimate current or "new" food folate values from "old" food folate data with a high degree of confidence.

To resolve these and other uncertainties, more attention needs to be focused on careful analysis of an increasing number and types of foods. It is likely that at least for the immediate future, new data on folate in cereal-grain foods will be more complete than new data for folate in other food categories. Additional attention must be directed toward validating methods for folate in those food categories with little or inadequate data (e.g., fruits, dairy products, vegetables, meat and poultry). It is also important to clearly identify fortified and unfortified foods in new data bases, and to recognize new classes of previously unfortified foods.

Issues regarding the folate fortification program will continue to draw wide attention. Issues that require further discussion and data gathering include: (1) needs to collaboratively test improved methods for folate analysis in enriched cereal-grain products; (2) needs to develop and collaboratively test methods for analysis of naturally-occurring folates in food groups for which current data are limited or unsatisfactory; (3) questions of how to use current food folate data in the context of an evolving data base; and (4) questions of how to update data bases so that they accurately reflect the total folate content of newly-fortified enriched cereal-grain

products as well as other important food sources of folate. A meaningful evaluation of the effectiveness of the folate fortification program cannot be made until current and much more accurate data on the total folate content of foods are available in the next generation of food composition databases.

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Table 1. Examples of fortification requirements for addition of folic acid to enriched cereal-grain products in the U.S.

STANDARDIZED PRODUCT	Folic acid mg/lb	Folic acid µg/100 g
§136 - BAKERY PRODUCTS		
Enriched bread, rolls, buns	0.43	95
§137 - CEREAL FLOURS & RELATED PRODUCTS		
Enriched flour	0.7	154
Enriched self-rising flour	0.7	154
Enriched corn grits	0.7-1.0	154-220
Enriched corn meals	0.7-1.0	154-220
Enriched farina	0.7-0.87	154-192
Enriched rice	0.7-1.4	154-308
§139 - MACARONI & NOODLE PRODUCTS		
Enriched macaroni	0.90-1.2	198-264
Enriched non-fat milk macaroni	0.90-1.2	198-264
Enriched noodle products	0.90-1.2	198-264

Table 2. Current levels of folate in enriched cereal-grain products measured after conjugase (mono-) and tri-enzyme digestions.

Product	Folate, μg/100 g			
	Mono-	Tri-	%	P
Enriched bread, rolls & buns				
White pita bread	23.8	30.3	127	<0.005
Whole wheat bread	62.9	60.7	97	NS
Enriched flour				
Wheat flour	26.1	33.1	127	<0.05
Wheat flour	24.8	32.2	130	<0.05
Baking mix	19.8	24.8	125	<0.05
Hot roll mix	33.5	38.6	115	NS
Enriched corn grits				
Instant grits	25.2	28.7	115	<0.05
Quick grits	27.5	31.5	115	NS
Enriched corn meals				
Yellow corn meal	32.6	37.5	115	<0.05
Yellow corn meal	25.3	29.2	115	<0.05
White corn meal	19.7	26.1	133	<0.05

Values are means of 2-3 independent determinations per product. Percent differences in results obtained by the single enzyme (mono-) and tri-enzyme (tri-) digestion procedures were calculated as follows: % =((Tri/Mono) x 100). P by t-tests. NS, not significant.

Table 3. Current levels of folate in enriched cereal-grain products measured after conjugase (mono-) and tri-enzyme digestions.

Product	Folate, ∶g/100 g			
	Mono-	Tri-	%	P
Enriched rice				
Medium grain rice	20.1	25.6	127	<0.1
Long grain inst rice	28.0	28.3	101	NS
Long grain wild rice	50.7	59.4	117	NS
Whole grain brown rice	23.7	31.3	132	<0.05
Enriched macaroni products				
Pasta	27.6	32.5	113	NS
Shells	31.4	39.6	126	<0.05
Thin spaghetti	35.1	39.0	111	NS
Thin spaghetti	35.6	37.8	106	NS
Trio shells	181.5	211.7	117	NS
Large shells	179.9	203.5	113	NS
Enriched noodle products				
Egg noodles	42.9	45.2	105	NS
Egg noodles w/o yolk	28.3	32.9	113	<0.1
Noodles & sauce	83.2	80.4	97	NS

Values are means of 2-3 independent determinations per product. Percent differences in results obtained by the single enzyme (mono-) and tri-enzyme (tri-) digestion procedures were calculated as follows: % =((Tri/Mono) x 100). P by t-tests. NS, not significant.

Table 4. Folate in ready-to-eat cereals measured after conjugase (mono-) and tri-enzyme digestions.

Product	Folate, μg/100 g			
	Mono-	Tri-	%	P
Breakfast cereals				
Whole wheat cereal	44.2	47.3	107	NS
Toasted rice cereal	397.6	450.1	113	NS
Whole grain oat cereal	416.6	419.5	101	NS
Bran cereal	629.3	677.8	108	NS
Other breakfast foods				
Whole grain wheat waffles	67.8	72.2	106	NS
Waffles	158.1	143.9	91	NS
Cereal bar,blueberry	278.7	295.2	105	NS

Values are means of 3-4 independent determinations per product. Percent differences in results obtained by the single enzyme (mono-) and tri-enzyme (tri-) digestion procedures were calculated as follows: % = ((Tri/Mono) x 100). P by t-tests. NS, not significant. The whole wheat cereal product was unfortified. Other products were fortified with folic acid at 40 to 400 μg/serving.

Table 5. Consistency between label values and analyzed values for folic acid-fortified foods.

Product	Folate			
	%DV /srv	µg/srv	Analyzed µg/srv	% of label value
Rice cereal snack	10	40	40	100
Toaster tart	10	40	58	145
Whole grain waffles	10	40	53	133
Waffles	20	80	107	120
Fruit-flavored drink mix	20	80	96	120
Toasted rice cereal	25	100	119	119
Whole grain oat cereal	25	100	125	125
Wheat & corn bran cereal	25	100	189	189
Multigrain cereal bar	25	100	115	115
Toasted corn,oats,wheat & rice cereal	100	400	398	100

Information on folic acid content obtained from product labels was compared with results obtained by analysis of the products. Values for folate on food labels are declared as the percent of the Daily Value (DV) that is present in one serving of the food. The DV for folate is 400 µg. Srv size, serving size. % of label value was calculated as follows = ((Analyzed/declared) x 100).