ELSEVIER

Food Chemistry 70 (2000) 275–289

Food Chemistry

www.elsevier.com/locate/foodchem

Rapid communication

Total folate in enriched cereal-grain products in the United States following fortification

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Received 24 September 1999; received in revised form 30 January 2000; accepted 30 January 2000

Abstract

The full compliance date for mandatory folic acid fortification of enriched cereal-grain products in the United States was 1 January, 1998. There is currently a great interest in determining the effectiveness of this measure, which was instituted to increase the folate intakes of women of child-bearing age to reduce their risk of having a pregnancy affected by a neural tube birth defect. We surveyed 83 enriched cereal-grain products that are required to be fortified with folic acid under the new regulations and an additional 79 foods that contain enriched cereal-grain ingredients or that are currently fortified with folic acid. Products were collected and analyzed between February 1998 and April 1999. Total folate was determined by microbiological assay using a tri-enzyme digestion. We compared analyzed values for total folate with amounts required by Federal regulations and/or with label declarations of folate content. For many enriched cereal-grain products, there were significant differences between amounts of folate found on analysis and amounts required by Federal regulations. In part because of this, label declarations of folate content were also in error. The high values found in some enriched cereal-grain products may represent manufacturers' averages as well as the presence of higher-than-expected levels of endogenous folates. These results indicate that reliable food composition databases cannot be developed without extensive new data on the actual concentrations of folate in recently fortified enriched cereal-grain products as well as in products containing enriched cereal-grain ingredients. Reliance on older data bases or on compositional information that has a weak analytical underpinning will lead to unsound estimates of folate intake, and hence, of the potential impact of the new fortification program. Published by Elsevier Science Ltd.

Keywords: Folates; Enriched cereal-grain products; Fortification

1. Introduction

The United States Food and Drug Administration (FDA) recently finalized regulations mandating the fortification of enriched cereal-grain products with folic acid. This action was taken to assist women in increasing their folate intake to reduce their risk of having a pregnancy affected by a neural tube birth defect (Food and Drug Administration, USA [FDA], 1996a,b,c).

There is currently intense interest in assessing the effects of this fortification program. In time, measurement of changes in the prevalence of neural tube birth defects in the United States may enable the effects of fortification with folic acid to be quantified. Such data will not be available for several years, however. In the interim, other approaches are being used to estimate the potential effects of fortification. These include measurement of effects of short-term feeding of fortified foods on indices of folate status (Malinow et al., 1998), measurement of indices of folate status in individuals before and after implementation of the fortification (Jacques, Selhub, Bostom, Wilson & Rosenberg, 1999), and estimation of current folate intakes based on updated food consumption surveys (Lewis, Crane, Wilson & Yetley, 1999).

The validity of estimates used to determine the potential impact of the fortification program is dependent upon the accuracy of folate data in national data bases. The inadequacy of methods for the analysis of food folates and the addition of overages of nutrients to fortified foods were recognized as two potential sources of underestimation in FDA's original intake estimates (FDA, 1996a, b). There is general agreement that methods traditionally used for folate analysis underestimate the folate content of foods because such methods result in

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0308-8146/00/$ - see front matter Published by Elsevier Science Ltd.
PII: S0308-8146(00)00116-3

incomplete release of folates from food matrices and incomplete hydrolysis of folylpolyglutamates prior to quantification (Institute of Medicine [IOM], 1998; Gregory, 1989; Tamura, 1990, 1998; Rader, Weaver & Angyal, 1998). In addition, manufacturers may add overages of nutrients such as folic acid to fortified products to ensure that the product contains at least the amount of the nutrient shown on the label throughout the shelf-life of the product. Because, in updating food composition data bases, label values rather than analytical values were used to define the folate composition of some fortified foods (e.g., ready-to-eat cereals, dietary supplements), the agency recognized that the values it used were likely to understate the actual folate content of such foods (FDA, 1993a, b, 1996a, b).

The weakness of folate food composition data was also recognized in the Third Report on Nutrition Monitoring in the United States (Life Sciences Research Office [LSRO], 1995). In evaluating assay methods and quality of food folate composition data for use in assessing dietary intakes, the report rated the assay methods as ''conflicting' and the data quality as ''variable'. The report commented further that recent findings suggested that traditional methods were not acceptable for the assay of complex foods and main dishes, and that research was needed on the development and validation of the methodology (LSRO). Improved methodology for analyzing folate in food and blood samples was identified as the most critical need for the further study of folate (LSRO).

The 1 January 1998 full compliance deadline for folic acid fortification focused attention on the critical need to estimate dietary folate intakes for purposes of nutrition monitoring and food safety evaluations. This in turn re-emphasized the importance of validated methods for analysis of folates in foods.

As part of its responsibility for the safety of the food supply, the FDA is interested in continually monitoring both the effectiveness and safety of fortification practices. In 1997, we undertook studies to determine pre-fortification levels of folate in enriched cereal-grain products that were subject to the new regulations. At that time, we addressed methodological problems by identifying and validating modifications of AOAC official method 992.05 (Association of Official Analytical Chemists [AOAC], 1995a, b) that were expected to lead to a microbiological method suitable for a collaborative study for the determination of folates in cereal-grain products (Rader et al., 1998). The method is the subject of a collaborative study sponsored by the Association of Official Analytical Chemists International (AOAC) and the American Association of Cereal Chemists (AACC), with results expected before the end of 2000.

We now report the results of analysis, by the method above, of more than 150 cereal-grain and related products for total folate. Some of these products are required to be enriched with folic acid under the new regulations, others contain enriched cereal-grain ingredients, and a third group consisted of products that have been traditionally fortified with folic acid. A number of unenriched products were also analyzed for comparative purposes. We have also compared analyzed values for total folate with amounts required by Federal regulations and/or with product label declarations of folate content, since these two sources of information are frequently used to impute values for folate in databases.

Analysis of all folic acid-fortified products and all products containing an enriched cereal-grain component in the marketplace is not realistic, since thousands of such products are available and product formulations are subject to change. In providing an overview of the fortification program in its first year, however, these survey data give an indication of the consistency between the folate content of newly fortified enriched products and Federal regulations and/or between folate content and product labels. The new data obtained in the present study also assist in identifying limitations in the approaches currently used to determine the impact of the fortification program. New data on the folate content of foods are also expected to contribute to the development of improved databases on the content of folate in enriched cereal-grain products. Updated food composition data, when combined with food intake data, can then be used to generate improved estimates of the potential impact of the folic acid fortification program.

2. Materials and methods

2.1. Reagents

Preparation of reagents has been described in detail previously (Angyal, 1996; Rader et al., 1998). Agar culture medium, culture suspension medium, and folic acid *Lactobacillus casei* assay medium were obtained from Difco Laboratories (Detroit, MI, USA).) The test micro-organism, *L. casei* subsp. *Rhamnosus* (ATCC No. 7469) (American Type Culture Collection, Rockville, MD, USA) was stored at refrigerator temperatures and maintained through weekly transfer on agar maintenance medium as previously described (Rader et al., 1998).

2.2. Standard solutions

Folic acid was obtained from the United States Pharmacopeia (Rockville, MD, USA). Water content was determined using a Mark I moisture analyzer (Denver Instrument Company, Arvada, CO, USA) and found to be 7.67%. Folic acid was dissolved in deaerated 0.1 M phosphate buffer, pH 7.0. A portion was taken immediately

for spectrophotometric determination of the concentration (Blakley, 1969; Pfeiffer, Rogers & Gregory, 1997). The working standard solution was usually 0.2 ng/ml (wt/vol). Suitable volumes of the stock solution (100 μg/ml) were diluted with water to obtain desired concentrations. Final volumes contained 2 ml 1% (wt/vol) ascorbic acid (1.42% (wt/vol) Na_2HPO_4 buffer (pH 6.7±0.1) per ml. The working standard solution was used to construct a five-point standard curve using the following volumes (ml): 1, 2, 3, 4, 5 (i.e. 0.02–0.10 ng/ml).

2.3. Enzyme preparations

HPLC water was used for all enzyme preparations. Chicken pancreas conjugase (Difco Laboratories, Detroit, MI, USA; No 0459-12), α-amylase (Sigma Chemical Co., St. Louis, MO, USA; No A-0273) and protease (Sigma Chemical Co., St. Louis, MO, USA; Pronase E, No. P-5147) was prepared as described previously (Rader et al., 1998).

2.4. Foods

The choice of cereal-grain products to be analyzed was based on a review of market data. The top-ranked sellers in the major enriched cereal-grain categories covered by the fortification regulations (i.e. enriched breads, rolls and buns, enriched flour, enriched corn grits, enriched corn meals, enriched farina, enriched rice, enriched macaroni and enriched spaghetti and noodle products) were identified from 1998 Nielsen data (Nielsen, 1998). In most categories, store brands were the top sellers. Cereal-grain products were purchased locally between February 1998 and April 1999. All products were purchased from a single lot. Whole packages of the product were composited, resulting in composites of 1–2 or more pounds.

We calculated the amounts of folate expected to be found in the products by reference to Federal regulations and/or to information provided on product labels as described below.

2.4.1. Declaration of folic acid on food labels

Folate must be declared on food labels only when it is added to foods as a nutrient supplement or when a claim is made about it (e.g., a claim of ''good source of folate' on a breakfast cereal). Folate does not need to be declared if it is required or permitted by a food standard and the standardized food is included as an ingredient in another food. When declared, the amount of folate present in a food is expressed as a percentage of the daily value (% DV) per serving of the food, in accord with the reference daily intake (RDI) value for folate established in Federal regulations. RDI values are listed in Federal Regulations [§101.9(c)(8)(iv)]. For folate, this amount is 400 μg.

2.4.2. Standardized products

Specifications for standardized enriched cereal-grain products are found in the US Code of Federal Regulations at 21 CFR Parts 136, 137 and 139. Requirements are expressed as amounts of folic acid in mg/pound that are required to be added to specific enriched cereal-grain products. The amounts also apply to cross-referenced standards of identity for enriched self-rising flour, enriched non-fat milk macaroni products, enriched vegetable macaroni products and enriched vegetable noodle products.

2.4.3. Non-standardized products

In addition to the specific enriched cereal-grain products mentioned above, the food additive regulation for folic acid (§172.345; FDA, 1996c) allows the addition of folic acid to a limited number of non-standardized foods. These include breakfast cereals, infant formulas, foods for special dietary use and meal replacement products. With respect to breakfast cereals, the levels of folic acid added may not exceed 100% of the daily value (DV) per serving (i.e. 400 μg/serving). The food additive regulation for folic acid (CFR §172.345) was amended to include fortified grits among the non-standardized foods to which folic acid may be added. The level of addition is the same as that permitted in the original standard for enriched corn grits.

2.5. Sample preparation

Samples were ground in a Waring blender or small coffee grinder. Preparation was carried out under subdued light. Care was taken to minimize contact with air by flushing with nitrogen during sample preparation and by storing the ground samples under nitrogen at room temperature or frozen in tightly sealed glass bottles.

2.6. Digestion procedure

Test portions of the composites equal to about 0.25 to 1.0 g dry solids and containing about 1 (μg folic acid were placed in 125 ml Erlenmeyer flasks containing 10 ml buffer [1.42% (wt/vol) Na_2HPO_4 and 1% (wt/vol) ascorbic acid, pH to 7.8 with 4 N NaOH] and mixed thoroughly. A standard containing 1 μg folic acid/ml was analyzed with each set of samples. An additional 10 ml of buffer was added and 0.1 ml octanol was added as an antifoaming agent. The flask was covered with a small beaker, autoclaved for 15 min at 121–123°C, cooled and an additional 10 ml buffer was added.

2.7. Tri-enzyme procedure

Four milliliters of conjugase preparation and 1 ml of α-amylase preparation (Rader et al., 1998) were added to each flask . Flasks were covered and incubated for 4 h at 37°C. After 4 h, 1 ml of the protease enzyme was

added and the flasks were incubated overnight at 37°C (Rader et al., 1998). The enzymes were inactivated by autoclaving the sample and standard for 3 min at 100°C, followed by cooling. The samples and standard were adjusted to pH 4.5 with HCl, diluted to a final volume of 100 ml with water and filtered. An aliquot of the clear filtrate was diluted to a final volume such that the folate concentration was about 0.2 ng/ml. The final volume of each solution to be assayed contained $\geqslant 10$ ml of pH 6.7±0.1 buffer (1.42% (wt/vol) Na_2HPO_4 and 1% (wt/vol) ascorbic acid; pH adjusted to 6.7±0.1 with 4 N NaOH).

2.8. Assay

Total folates were determined by microbiological assay using a modification of AOAC official method 992.05 (AOAC, 1995a,b; Angyal, 1996). *L. casei* (ATCC 7469) was the assay micro-organism. A minimum of three independent analyses (i.e. sample weighing, digestion, microbiological assay) were carried out for each food sample. Twelve tubes containing 4 different amounts of extract (i.e. 1–4 ml) in triplicate were used to assay each test sample. The assay buffer consisted of 1 g ascorbic acid (reagent grade) dissolved in 100 ml 1.42% (wt/vol) Na_2HPO_4. The pH was adjusted to 6.7±1 with 4 N NaOH. Growth was read after 22 h of incubation at 37°C in a Bausch and Lomb Spectronic 20 spectrophotometer equipped with a flow cell. The assay tube reading apparatus consisted of a Gilson escargot fractionator Model 222 sample changer modified with an air-agitator system and connected to the spectrophotometer and a printer and computer. Enzyme blank preparations were assayed for folic acid contribution at different dilution levels similar to those used to dilute test preparations containing different levels of folate. Negligible responses were observed for dilutions corresponding to those used for products containing the lowest level of folic acid. For this reason, blank corrections were deemed unnecessary.

An eight-point fourth degree polynomial regression plot and a computer program designed according to the official AOAC protocol were used to calculate ng folate/ml extract and μg folate/100 g sample. Assay results were considered acceptable if the assay met the AOAC criteria for microbiological assays (i.e. assays with less than two-thirds of the tubes (67%) within the acceptable range were not used to determine the folate content of product samples).

2.9. Analysis of reference material and check samples

Standard Reference Material SRM 1846, a spray-dried milk-based infant formula that is intended primarily for use in validating methods for determining proximates, minerals and certain vitamins in infant formula and similar matrices was used as an in-house quality control material. The mass fraction value for folate in SRM 1846 of 1.29±0.28 mg/kg was determined by microbiological assay (Sharpless et al., 1997). Cereal-grain samples from the Vitamin, Mineral and Proximate Check (VMP) series were obtained from the American Association of Cereal Chemists International Check Sample Service (AACC, St. Paul, MN, USA). Portions of SRM 1846 and/or AACC check samples were analyzed with each set of analyses. Preparation of these samples was the same as that described above for foods.

3. Results

3.1. Analysis of reference material 1846 and AACC check samples

SRM 1846 was assayed repeatedly throughout the study. Values (mean±S.D.) found for folate in SRM 1846 were 1.41±0.163 mg/kg ($n = 39$) vs the mass fraction value of 1.29±0.28 mg/kg determined by microbiological assay for this material. Percentage coefficient of variation [% CV = (S.D./mean)×100)] was 11.6%. We also analyzed six cereal-grain check samples from the AACC repeatedly throughout the study (Table 1). In all cases, results obtained by the tri-enzyme method fell within the range of values reported for the check samples. With the exception of results for % CV for AACC sample VMP-4, analyzed values and % CVs agreed well with those reported by AACC.

3.2. Presentation of results

Results for the enriched cereal-grain products are grouped according to the applicable Federal standard. Analytical results are expressed as "μg total folate/serving'.

Table 1
Values for folate in vitamin, mineral and proximate (VMP) check samples from the AACC determined by the tri-enzyme procedure[a]

Sample	Tri-enzyme procedure			AACC values[c]		
	Folate, mg/100 g	(*N*)	% CV[b]	Folate, mg/100 g	(*N*)	% CV
VMP-3	0.446±0.071	(9)	15.9	0.385±0.062	(13)	16.1
VMP-5	1.399±0.151	(13)	10.8	1.508±0.168	(12)	11.1
VMP-6	0.518±0.098	(9)	18.9	0.443±0.036	(13)	8.1
VMP-2	1.024±0.063	(9)	6.2	0.956±0.056	(11)	5.9
VMP-3	1.278±0.162	(6)	12.7	1.395±0.143	(9)	10.3
VMP-4	0.976±0.088	(14)	9.0	0.864±0.319	(12)	36.9

[a] Values are means±S.D. of (*N*) determinations.

[b] Coefficients of variation (% CV) were calculated as [(SD/mean)×100]. Overall CV for the tri-enzyme procedure was 12.3±4.6%.

[c] AACC values were determined by laboratories subscribing to the AACC check sample program.

For comparison with fortification levels specified in Federal regulations, the amount specified in the regulation was converted to μg/100 g and the analyzed values are recalculated as "μg folate/100 g'. Amounts required by specific regulations are shown at the bottom of the Tables. Analytical values are also accompanied by information from the product label (e.g. %DV). Within classes of products in which a range of "% DV' values was found on the labels, the products are listed in decreasing order of the "% DV' stated on their labels, and hence, in decreasing order of the (g folate/serving expected to be found in the food.

Comparisons of the consistency between label declarations and/or requirements of the Federal standards were made by means of the following calculations:

(a) % of label value = [(analyzed amount/amount declared on label) × 100].
(b) For enriched bread, rolls and buns and for enriched flour: % of regulation = [(analyzed amount per 100 g/amount required by regulation) × 100].
(c) For enriched farina, enriched rice, enriched macaroni and enriched noodle products, Federal regulations provide a range for the addition of folic acid (i.e. a minimum and a maximum value). Analyzed values falling within the limits defined in the regulations are identified in Tables 3–10 as "in range'. When analyzed values fell above the maximum values or below the minimum values in the ranges, the analyzed values are expressed as a percent of the maximum value (i.e., "___% of max') or as a percentage of the minimum value (i.e. "___% of min') of the range, respectively.

3.2.1. Bakery products-enriched bread, rolls and buns (Table 2)

Mean analyzed values ranged from 131 to 191 μg/100 g or 138–201% of the amount stated in the regulation. Labels of enriched products in this category stated that products contained 4–20% of the DV for folate (i.e., 16–80 μg/serving). Statements of folate content were present on labels of 9 products. The label statements understated the amounts of folate present in 8 of the 9 products. Levels of folate in whole wheat breads, for which there is no enrichment standard, were markedly lower than those found in enriched breads.

3.2.2. Cereal flours and related products

3.2.2.1. Enriched flours (Table 3). Mean analyzed values ranged from 33 to 229 μg folate/100 g flour or 21–149% of the amount stated in the regulation. In three cases in which information on % DV was included on product labels, the analyzed values were significantly higher than label declarations. Six baking mixes in which enriched flour was the first- or second-listed

Table 2
Total folate in enriched bread, rolls and buns[a]

Bakery products	Label information Folate			Analyzed values[b] Folate		Folate	
	% DV	Serv g	μg/serv	μg/serv	μg/100 g	% of label	% of regul value[c]
Enriched bread, rolls & buns							
Sourdough baquettes	20	57	80	79±11	139±19	99	146
Potato hot dog rolls	10	53	40	88±11	167±21	221	176
French loaves	10	57	40	84±6	148±11	211	156
Buttertop bread	6	28	24	52±8	186±30	217	196
Sandwich bread	6	26	24	46±4	177±17	192	186
Split-top rolls	6	32	24	42±5	131±16	175	138
White bread	6	25	24	35±5	140±9	145	147
Italian bread	4	32	16	61±11	191±34	381	201
White bread	4	26	16	49±5	188±20	306	198
Potato rolls	–	53	N/A	76±12	143±23	N/A[d]	150
Sourdough rolls	–	43	N/A	71±3	167±8	N/A	176
Whole wheat and multigrain breads (unenriched)							
100% whle wht bread	4	43	16	31±1	71±3	188	N/A
100% whol wht bread	0	43	0	35±6	81±14	N/A	N/A
100% whle wht bread	0	43	0	24±5	55±11	N/A	N/A
Multigrain bread*	–	34	N/A	20±3	58±6	N/A	N/A

*enr flour in ingredient list

[a] Abbreviations: DV, daily value for folate, 400 μg; wht, wheat; srv, serving size; regul, amount specified in regulation, 95 μg/100 g product.
[b] Analyzed values are means ±S.D. of 3 or more independent determinations by the tri-enzyme procedure.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label) × 100], % of regulation = [(analyzed amount per 100 g/amount specified in Federal regulations) × 100].
[d] N/A, not applicable.

Table 3
Total folate in enriched flours, enriched flour-containing baking mixes and other flours[a]

Enriched and unenriched flours	Label information Folate			Analyzed values[b] Folate		Folate	
	% DV	Serv g	μg/serv	μg/serv	μg/100 g	% of label value[c]	% of regul
Enriched flours							
All-purpose flour	10	30	40	69±9	229±31	173	149
All purpose flour	10	30	40	68±16	229±53	170	149
Flour	10	40	40	66±3	220±11	165	143
All-purpose flour	–	30	N/A[d]	61±4	204±13	N/A	132
All-purpose flour	–	31	N/A	61±12	197±39	N/A	128
Wheat flour	–	30	N/A	52±3	174±11	N/A	113
Wheat flour	–	30	N/A	23±6	77±20	N/A	50
Wheat flour	–	30	N/A	10±0.2	33±1	N/A	21
Enriched flour-containing baking mixes							
All-purpose baking mix	10	40	40	71±10	178±26	178	N/A
All-purpose baking mix	10	40	40	50±11	125±28	125	N/A
Bread mix, stngrdwheat	–	43	N/A	93±20	217±44	N/A	N/A
Bread mix, wheat	–	43	N/A	54±10	125±23	N/A	N/A
Bread machine mix, white	–	45	N/A	43±3	96±7	N/A	N/A
Bread machine mix, white	–	40	N/A	32±5	80±12	N/A	N/A
Unenriched flours							
Whole grain soy flour	–	45	–	136±12	302±27	N/A	N/A
Whole wheat flour	–	35	–	31±7	88±20	N/A	N/A
Whole wheat flour	–	35	–	31±8	89±23	N/A	N/A

[a] Abbreviations: DV, daily value for folate, 400 μg; serv, serving size; regul, amount specified in regulation, 154 μg/100 g product.
[b] Analyzed values are means ±S.D. of 3 or more independent determinations by the tri-enzyme procedure.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label) × 100]; % of regulation = [(analyzed amount per 100 g/amount specified in regulations) × 100].
[d] N/A, not applicable.

Table 4
Total folate in enriched corn grits and enriched corn meals[a]

Enriched corn grits and corn meals	Label information Folate			Analyzed values Folate[b]		Folate	
	% DV	Serv g	μg/serv	μg/serv	μg/100 g	% of label value[c]	Regulation % of max or min
Corn grits							
White hominy	15	41	60	99±14	240±35	165	109% of max
White hominy	15	41	60	90±20	219±50	150	In range
White hominy	15	40	60	13±4	32±10	21	21% of min
Quick grits	10	37	40	134±17	361±47	334	164% of max
Quick grits	10	37	40	64±10	173±27	160	In range
Instant grits	10	28	40	62±10	220±34	155	In range
Corn meals							
Yellow corn meal	10	27	40	104±8	387±29	263	176% of max
Yellow corn meal	10	30	40	86±8	288±28	216	131% of max
Yellow corn meal	10	30	40	85±12	282±40	213	128% of max
White corn meal	10	30	40	82±8	272±25	205	124% of max
Yellow corn meal	10	27	40	49±2	181±8	190	115% of max
Yellow corn meal	—	28	N/A[d]	39±7	139±24	N/A	90% of min
Yellow corn meal	–	28	N/A	23±2	83±6	N/A	54% of min
Blends of enriched corn meal and wheat flour							
White corn meal & enr wheat flour	10	30	40	82±20	273±66	205	N/A
White corn meal & enr wheat flour	10	30	40	76±9	254±29	190	N/A

[a] Abbreviations: DV, daily value for folate, 400 μg; serv, serving size; regul, amount specified in regulation, 154–220 μg/100 g product.
[b] Analyzed values are means±S.D. of 3 or more independent determinations by the tri-enzyme procedure.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label) × 100]. Analyzed values falling within the limits defined in the regulations are ''in range'. Analyzed values falling above the maximum value or below the minimum value specified in the regulation are expressed as ''% of max' or ''% of min', as appropriate.
[d] N/A, not applicable.

ingredient were also analyzed. While it is not possible to predict the folic acid content of such products without knowledge of the composition, we observed that the range of concentrations was large (i.e. 80–217 μg/100 g). Among three unenriched flours analyzed, whole grain soy flour contained the highest level of folate.

3.2.2.2. Enriched corn grits and corn meals (Table 4). Mean analyzed values for folate in 3 of 13 products examined fell within the range of 154–220 μg folic acid/100 g specified in the regulations. Three values were below the minimum of the range, and 7 were above the maximum. With one exception, statements of % DV on

Table 5
Total folate in enriched farina[a]

Enriched farinas	Label information Folate			Analyzed values[b] Folate		Folate	
	% DV	Serv g	μg/serv	μg/serv	μg/100 g	% of label value[c]	Regulation % of max or min
Wheat cereal-farina	20	35	80	159±1	453±4	198	236% of max
Wheat cereal-farina	10	33	40	99±13	300±39	247	156% of max
Wheat farina	10	33	40	75±10	226±30	186	118% of max
Wheat farina	10	33	40	13	39	33	25% of min

[a] Abbreviations: DV, daily value for folate, 400 μg; serv, serving size; regul, amount specified in regulation, 154–192 μg/100 g product.
[b] Analyzed values for the first three products are means ±S.D. of 3 or more independent determinations by the tri-enzyme procedure. Analyzed value for the fourth product is the result of a single determination.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label) × 100]. Analyzed values falling within the limits defined in the regulations are "in range'. Analyzed values falling above the maximum value or below the minimum value specified in the regulation are expressed as "% of max' or "% of min', as appropriate.

Table 6
Total folate in enriched and unenriched rice[a]

Enriched rice	Label information Folate			Analyzed values[b] Folate		Folate	
	% DV	Serv g	μg/serv	μg/serv	μg/100 g	% of label value[c]	Regulation % of max or min
Enriched rice							
10-minute rice	20	54	80	359±10	665±18	449	216% of max
10-minute rice	20	54	80	107±6	198±12	248	In range
Converted rice	20	49	80	96±5	196±10	120	In range
Instant white rice	15	44	60	184±26	418±60	307	135% of max
Precooked rice	15	44	60	118±6	267±14	197	In range
Long grain rice	15	44	60	101±10	230±23	168	In range
Precooked rice	15	44	60	94±16	213±37	156	In range
Precooked rice	15	44	60	95±9	215±20	156	In range
Rice	15	45	60	87±14	192±31	145	In range
Rice	15	45	60	84±21	186±45	140	In range
Parboiled rice	15	47	60	61±2	130±4	102	84% of min
Rice,long grn	15	45	60	59±6	131±13	98	85% of min
Yellow rice	–	56	N/A[d]	44±3	78±5	N/A	51% of min
Rice	15	45	60	21±4	46±9	35	30% of min
Yellow rice	–	28	N/A	8±1	30±4	N/A	19% of min
Chick&wild rice	20	57	80	106±42	184±70	133	–
Beans&rice	–	33	N/A	27±9	86±31	N/A	–
Folic acid-fortified rice products not labelled as "enriched'							
Rice pilaf	20	70	80	163±27	233±39	205	–
Spanish rice	20	56	80	110±19	197±34	138	–
Lng grn & wld rice	15	56	60	96±8	171±14	159	–
Unenriched (unfortified) rice products							
Brown rice	–	39	N/A	22±3	56±9	N/A	N/A
Brown rice	–	39	N/A	14±1	36±3	N/A	N/A

[a] Abbreviations: DV, daily value for folate, 400 μg; serv, serving size; regul, amount specified in regulation, 154–308 μg/100 g product.
[b] Analyzed values are means ± S.D. of 3 or more independent determinations by the tri-enzyme procedure.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label) × 100]. Analyzed values falling within the limits defined in the regulations are "in range'. Analyzed values falling above the maximum value or below the minimum value specified in the regulation are expressed as "% of max' or "% of min', as appropriate.
[d] N/A, not applicable.

the product labels were exceeded, often by significant amounts. Two additional products consisting of blends of enriched white corn meal and enriched wheat flour were analyzed. Label declarations for these products stated that each contained 10% DV folate per serving (i.e. 40 μg folate per serving). Analyzed values were 190 and 205% of the label statements.

3.2.2.3. Enriched farinas (Table 5). Analyzed values in three of four products exceeded the upper end of the range specified in the regulations. As a result, analyzed values exceeded label statements of % DV.

3.2.2.4. Enriched rice (Table 6). Federal regulations require 154–308 μg folic acid/100 g in enriched rice.

Table 7
Total folate in enriched macaroni products[a]

Macaroni products	Label information Folate			Analyzed values[b] Folate		Folate	
	% DV	Serv g	μg/serv	μg/serv	μg/100 g	% of label value[c]	Regulation % of max or min
Enriched macaroni products							
Macaroni	35	56	140	255±18	455±32	182	172% of max
Campanelle	30	56	120	275±45	492±80	229	186% of max
Spaghetti	30	56	120	265±19	473±34	221	179% of max
Gemelli	30	56	120	246±23	439±41	205	166% of max
Thin spaghetti	30	56	120	198±32	354±57	165	134% of max
Spaghetti	30	56	120	197±8	351±14	164	133% of max
Rigatoni	30	56	120	187±7	334±13	156	126% of max
Jumbo shells	25	56	100	238±16	424±28	238	161% of max
Elbow macaroni	25	56	100	184±9	328±16	184	124% of max
Thin spaghetti	25	56	100	157±18	280±32	157	106% of max
Rotini	25	56	100	156±11	278±20	156	105% of max
Folic acid-fortified pasta products not labelled as "enriched"							
Pasta	25	70	100	175±5	250±7	174	–
Pasta	20	56	80	188±15	337±27	235	–
Angel hair pasta	20	56	80	147±23	262±46	184	–
Enriched macaroni-containing food							
Macaroni & cheese dinner	–	70	–	174±19	249±27	–	In range

[a] Abbreviations: DV, daily value for folate, 400 μg; serv, serving size; regul, amount specified in regulation, 198–264 μg/100 g product.
[b] Analyzed values are means ±S.D. of 3 or more independent determinations by the tri-enzyme procedure.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label)×100]. Analyzed values falling within the limits defined in the regulations are "in range'. Analyzed values falling above the maximum value or below the minimum value specified in the regulation are expressed as "% of max' or "% of min', as appropriate.

Table 8
Total folate in enriched noodle products[a]

Enriched noodle products	Label information Folate			Analyzed values[b] Folate		Folate	
	% DV	Serv g	μg/serv	μg serv	μg/100 g	% of label[c] value	Regulation % of max or min
Egg noodles	30	56	120	173±24	308±43	144	117% of max
Egg noodles	30	55	120	148±24	269±44	123	In range
Egg noodles	–	55	–	147±0.1	266±0.2	–	In range
Chol-free noodles	30	56	120	148±4	268±7	107	In range
Fine egg noodles	30	56	120	136±3	243±5	113	In range
Noodles	30	56	120	135±5	240±9	113	In range
Hrty egg noodles	30	56	120	126±5	225±10	105	In range
Chol-free noodles	30	56	120	80±14	143±24	67	72% of min
Egg noodles	30	56	12	46±4	83±8	39	42% of min
Fettucine	25	56	100	194±22	347±40	194	132% of max
Egg noodles	25	56	100	182±24	325±42	182	123% of max
Fettucine	25	70	100	153±99	219±13	153	In range

[a] Abbreviations: DV, daily value for folate, 400 μg; serv, serving size; regul, amount specified in regulation, 198–264 μg/100 g product.
[b] Analyzed values are means ±S.D. of 3 or more independent determinations by the tri-enzyme procedure.
[c] Calculations: % of label value = [(analyzed amount/amount declared on label)×100]. Analyzed values falling within the limits defined in the regulations are "in range'. Analyzed values falling above the maximum value or below the minimum value specified in the regulation are expressed as "% of max' or "% of min', as appropriate.

Table 9
Total folate in ready-to-eat cereals[a]

Cereals	Label information Folate			Analyzed values[b] Folate	Folate
	% DV	Serv g	μg/serv	μg/serv	% of label value
Multigrain cereal	100	30	400	546±20	137
Multigrain, rice, oats	100	50	400	508±58	127
Corn cereal	100	30	400	505±75	126
Raisin bran cereal	100	55	400	499±104	125
Corn flakes	100	30	400	466±74	117
Multigrain cereal	100	30	400	422±4	106
Whole grain oats	50	30	200	248±30	124
Natural wheat bran	25	29	100	320±48	320
Multi-bran cereal	25	58	100	292±59	292
Bran flakes cereal	25	30	100	250±37	250
Corn cereal	25	30	100	207±17	207
Corn cereal	25	30	100	204±17	204
Wheat & barley cereal	25	48	100	202±13	202
Corn cereal	25	30	100	197±14	197
Corn cereal	25	30	100	186±27	186
Whole grain oat cereal	25	32	100	183±42	183
Oat cereal	25	28	100	168±34	168
Wheat cereal	25	51	100	161±30	161
Corn & oat cereal	25	27	100	144±0.6	144
Corn & rice cereal	25	29	100	139±21	139
Multigrain cereal	25	55	100	135±15	135
Multigrain cereal	25	30	100	132±25	132
Corn cereal	25	30	100	114±16	114
Rice cereal	25	33	100	98±10	98
Instant oatmeal	20	28	80	122±17	153
Oat bran flake cereal	10	28	40	86±6	216
Shredded wheat	4	46	16	28±4	178
Granola (unfortified)	–	64	–	44±11	N/A

[a] Abbreviations: DV, daily value for folate, 400 μg.
[b] Analyzed values are means±S.D. of 4 or more independent determinations by the tri-enzyme procedure; % of label value = [(analyzed amount/amount declared on label)×100].

Among 15 products studied, mean analyzed values for 8 fell within the range specified. Two exceeded the maximum and 5 fell below the minimum. With respect to agreement between label statements and amounts found on analysis, the analyzed values exceeded labelled amounts by as much as 300% or more. Three additional products were analyzed. These carried label declarations of 15 or 20% DV for folate but were not labelled as ''enriched'. Folate content was found to be 159–205% of the label values. Two unenriched brown rice products contained low levels of folate.

3.2.2.5. Enriched macaroni products (Table 7). Federal regulations require 198–264 μg folic acid/100 g in enriched macaroni products. Mean analyzed values in all 11 products examined contained more folic acid than specified for the maximum value. The amounts found on analysis, expressed as % DV or as μg/serving, exceeded amounts stated on the product labels by 153–238%. Three folic acid-fortified pasta products that were not labelled as ''enriched' contained 184–235% of the amounts declared on product labels.

3.2.2.6. Enriched noodle products (Table 8). Federal regulations require 198–264 μg folic acid/100 g in enriched noodle products. Levels of folic acid fell within the specified range in 7 of 12 products examined. Mean analyzed values in 3 products exceeded the maximum value specified in the regulation and failed to meet the minimum in two other products. Label values also differed significantly from analyzed values for several of the enriched noodle products.

3.2.2.7. Ready-to-eat breakfast cereals (Table 9). A total of 28 ready-to eat breakfast cereals was also analyzed. These cereals contribute significantly to total dietary folate intake in the USA (IOM report, 1998, Tables 8–10). Ready-to-eat cereals may contain up to 400 μg folate/serving (i.e. 100% DV/serving) (FDA, 1996c). Label statements indicated that these products contained between 4 and 100% of the DV per serving (i.e. 16–400 μg/100 g), with the majority stating that they contained folate at the level of 25% DV/serving. For many products, mean analyzed values were significantly higher than label statements. Bran-containing

cereals contained the highest levels relative to statements on product labels.

3.2.2.8. Cereal bars and toaster pastries (Table 10). Seventeen cereal bars and toaster pastries were also analyzed. Most of these products contain an enriched cereal-grain component, usually in the form of enriched flour. In addition, many contain additional folic acid in amounts sufficient to raise the folate level to 10–30% DV (i.e. 40–120 μg/serving). Other products in this group contain an enriched cereal-grain component but are not additionally fortified. For most products, mean analyzed values were close to or below values stated on product labels. Labels of 4 products did not declare a % DV for folate. These products contained 14–29 μg folate/serving (i.e. 4–7% DV of the vitamin).

We noted in our earlier study (Rader et al., 1998) 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 25–35% of the DV. These products make up a significant proportion (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 in our earlier study (Rader et al.) that the analyzed values for folate were 100–189% of the label declarations for a sampling of such products. While we did not repeat the analyses of the total range of such foods in this survey, the earlier results show that a wider range of food products is currently fortified with folic acid than is shown in the present work.

3.2.2.9. Miscellaneous products containing enriched cereal-grain ingredients (Table 11). A number of other products that are fortified with folic acid, that contain an enriched cereal-grain ingredient, or that contain naturally occurring folates were also analyzed. In those cases in which it could be determined, analyzed values were 105–221% of label declarations for folate content. A variety of other enriched cereal-grain-containing products were found to contain approximately 5% to more than 15% of the DV for folate.

3.2.2.10. Summary table (Table 12). For the major classes of enriched cereal-grain products that are the subjects of the new regulations, we calculated the means ±S.D. of analyzed values for all products examined and compared them with the applicable regulation (Table 12). In some groups, several products appeared to be under- or un-fortified. In order to prevent the inclusion of such values from skewing the distribution of values, we also calculated the means ±S.D. of values including only those products that contained at least 85% of the

Table 10
Total folate in cereal bars and toaster pastries[a]

Cereal bars and toaster pastries	Label information Folate			Analyzed values[b] Folate	Folate
	% DV	Serv g	μg/serv	μg/serv	% of label value
Cereal bar	30	37	120	152±6	127
Cereal bar	30	37	120	139±17	116
Cereal bar	25	37	100	124±15	124
Cereal bar	25	37	100	97±5	97
Cereal bar	25	37	100	94±14	94
Cereal bar	25	37	100	91±4	91
Cereal bar	25	37	100	87±15	87
Toaster pastry	20	52	80	72±20	90
Toaster pastry	10	52	40	94±4	235
Cereal bar	10	37	40	63±7	158
Cereal bar	10	37	40	60±9	150
Toaster pastry	10	54	40	55±3	138
Twists	10	37	40	30±9	75
Cereal bar	–	37	N/A	29±9	N/A
Cereal bar	–	37	N/A	16±4	N/A
Cereal bar	–	37	N/A	16±6	N/A
Cereal bar	–	37	N/A	14±0	N/A

[a] Abbreviations: DV (daily value) for folate, 400 μg.
[b] Analyzed values are means ± S.D. of 4 or more independent determinations by the tri-enzyme procedure. % of label value = [(Analyzed amount/amount declared on label)×100].

Table 11
Total folate in miscellaneous products containing enriched cereal-grain ingredients[a]

Miscellaneous products	Label information Folate			Analyzed values Folate[e]	Folate
	% DV	Serv g	μg/serv	μg/serv	% of label value
Breakfast powder	20	37	80	130±5	163
Breakfast powder	20	37	80	112±14	140
Toasted wheat germ	10	13	40	57±11	143
Toasted wheat germ	10	13	40	51±0.4	128
Graham crackers[b]	10	22	40	42±4	105
Blueberry muffin mix[b]	6	40	24	53±7	221
White cake mix[d]	6	43	24	39±3	161
Wheat snack crackers[d]	4	30	16	35±8	219
Corn muffin mix[c]	–	36	–	74±10	–
Hot muffin mix[b]	–	37	–	75±10	–
Pepper crackers[c]	–	15	–	42±1	–
Baked snack crackers[b]	–	31	–	47±2	–
Yellow cake mix[b]	–	45	–	47±16	–
Corn muffin mix[b]	–	38	–	40±5	–
Raisn oatml cookies	–	33	–	24±7	–

[a] Abbreviations, DV (daily value) for folate, 400 μg.
[b] First-listed ingredient, enriched flour.
[c] First-listed ingredient, enriched corn meal.
[d] Second-listed ingredient, enriched flour.
[e] Analyzed values are means ±S.D. of 4 or more independent determinations by the tri-enzyme procedure. % of label value = [(Analyzed amount/amount declared on label)×100].

Table 12
Summary: consistency between fortification levels and fortification regulations in enriched cereal-grain products[a]

Enriched cereal-grain product	Regulation folic acid, μg/100 g	Products	(*N*)	Microbiological assay total folate	
				Mean±S.D.[b] μg/100 g	Range μg/100 g
Part 136 Bakery Products					
Enr bread, rolls, buns	95	All	(11)	162±22	131–191
Part 137 Cereal Flours & Related Products					
Enr flour	154	All	(8)	145±88	33–229
		Fortified	(6)	209±22	174–229
Enr corn grits	154–220	All	(6)	208±107	32–361
		Fortified	(5)	243±71	173–361
Enr corn meals	154–220	All	(7)	233±104	83–387
		Fortified	(6)	258±88	139–387
Enr farina	154–192	All	(4)	255±173	39–453
		Fortified	(3)	326±116	226–453
Enr rice	154–308	All	(15)	213±157	30–665
		Fortified	(12)	253±149	131–665
Part 139 Macaroni and Noodle Products					
Enr macaroni products	198–264	All	(11)	383±77	278–492
Enr noodle products	198–264	All	(12)	243±74	83–347
		Fortified	(10)	271±43	219–347

[a] Specifications for standardized enriched cereal-grain products are found in 21 CFR Parts 136, 137 and 139. Values are expressed above as amounts of folic acid (μg/100 g) that are required to be added to the enriched products.

[b] Means±S.D. for all standardized products analyzed in each group were determined (''all', above). In some groups, several products appeared to be under- or un-fortified. We recalculated the means ±S.D. of values for all products that contained at least 85% of the minimum specified for the

minimum value specified for those products. The greatest differences between the amounts required by the regulations and amounts found on analysis were found for enriched bakery products, enriched farinas, and enriched macaroni products. In general, enriched products appeared to be fortified at or above the maximum amount specified in the regulations.

4. Discussion

4.1. Microbiological assay with tri-enzyme digestion

The need to establish a credible database for folate in foods containing naturally occurring folates and for foods that are now fortified with folic acid has focused attention on the importance of reliable folate analyses. In 1998, we reported a modification of AOAC official method 992.05 (AOAC, 1995a) that utilized a tri-enzyme extraction and described a number of parameters of the assay including modifications of the extraction steps, pH during extraction, pH of the assay, response of the assay to calibrants such as 5-methyltetrahydrofolate and 5-formyltetrahydrofolate, analysis of Standard Reference Material 1846 (Sharpless et al., 1997) and AACC check samples, and recoveries of folic acid added to food samples (Rader et al., 1998). The method was then used to measure folate in enriched cereal-grain samples collected before the full compliance date for the U.S.'s folate fortification regulations (Rader et al., 1998). We used this assay in the present study to analyze total folate in more than 150 fortified cereal-grain products collected during the first 16 months of the US fortification program.

During this survey of folic acid-fortified foods, we found that for many enriched cereal-grain products, analyzed values for folate were significantly higher than levels required by Federal regulations. In part, because of this, there were significant differences between amounts of folate found on analysis and amounts stated on product labels. It is not known whether some of the high values represent excesses added by the manufacturers or whether the tri-enzyme assay is measuring endogenous folates that are present at higher- than-expected levels. This latter possibility may be applicable in the case of bran-containing cereals, which contained among the highest levels of folate relative to their label statements.

Consideration must also be given to the possibility that the microbiological assay has a bias resulting from overestimation of basal levels of folate. For example, there may be growth-stimulating components in the media or in sample extracts to which *L. rhamnosus* may respond. In addition, there may be differences in response of the microorganism to endogenous folate cofactor forms relative to response to the folic acid calibrant (i.e. the assay micro-organism may over-estimate endogenous folate forms). The micro-organism responds only to naturally occurring folates and folic acid, as we observe no growth in inoculated ''blanks'

containing folate-free medium. With respect to differences in response to different calibrants, we obtained comparable quantitative results for total folate in a shredded wheat product when 5-methyl-tetrahydrofolate was used in place of folic acid as the calibrant (Rader et al., 1998).

The most applicable data regarding the question of an overestimation bias come from studies that compare results obtained by the microbiological assay to those obtained by HPLC methods that can resolve many folate forms. Two studies have provided data that directly compare results obtained with the microbiological assay to those obtained from an HPLC assay for the same samples. Selhub's group (Selhub, 1989; Selhub, Darcy-Vrillon & Fell, 1988; Seyoum & Selhub, 1993) reported close agreement (within about 10%) between values for total folates obtained by an affinity chromatography/HPLC method and the *L. casei* assay for 10 unfortified foods including baker's yeast, bovine liver, egg yolk, lima beans, soybeans, wheat germ, cabbage, lettuce, orange juice and bananas. Similarly, Pfeiffer et al. (1997) described an optimized adaptation of the components of several procedures including a modified tri-enzyme extraction, affinity chromatography and reversed-phase HPLC for quantifying the most abundant folate forms in fortified and unfortified cereal-grain products. For five fortified products including white breads, white rice, pasta and a breakfast cereal, results of their HPLC analysis were 99–109% of the values found by microbiological assay. These findings support the accuracy of the microbiological assay when proper sample preparation is used (Pfeiffer et al., 1997). Based on these findings, we do not think that there is an overestimation bias in our microbiological analyses.

Some of the high values observed in our study may have resulted from the addition of the folic acid fortificant without consideration of the level of naturally occurring folates present in certain products. It is likely that both manufacturers' overages and unrecognized levels of endogenous folates contributed to the results observed.

4.2. Total folate vs free folic acid

Label declarations for folate are statements of total folate in the products. Methods other than a microbiological one are needed to quantify free folic acid in fortified foods. Chromatographic methods are available that can be used for the separation and quantitation of specific chemical forms of folate. There have also been significant developments in the fields of competitive immunoassays and affinity-based biosensor technology that may soon be successfully validated and prove to be useful in distinguishing between naturally-occurring folate and free folic acid (Rader, Weaver & Angyal, 1999).

4.3. Stability of folic acid added to cereal-grain products

Hoffpauer and Bonnette (1998) discussed manufacturing concerns with respect to folic acid fortification. In considering riboflavin and folic acid, the authors noted that the stability of these two vitamins continues to be a problem in an environment unprotected from heat and light. They noted that stability studies may need to be undertaken in the future to evaluate the effects of the instability, and that before such studies are performed, overages in these nutrients may be necessary to achieve proper levels after application. However, there is a large body of evidence regarding the stability of folic acid added to cereal-grain products. Numerous reports have described the stability of folic acid in fortified breads, vitamin-minerals premixes, fortified flour and grains and during baking applications (Gregory, 1989). The high degree of stability of folic acid in foods of high moisture content has also been demonstrated (Colman, 1982; Day & Gregory, 1983. Thus, to date, there appear to be no data supporting the concept that overages are needed because folic acid is unstable when added to cereal-grain products.

4.4. Food composition databases

With implementation of the new regulations, food composition tables are no longer 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 or from label statements of folate content, our results from the first year of fortification show that this is much more difficult in practice. In comparing "old' and "new' folate data, 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. Reliance on older databases or on compositional information that has a weak analytical underpinning will lead to unsound estimates of folate intake, and hence, of the potential impact of the new fortification program.

4.5. Use of available data to estimate the impact of folic acid fortification

The data reported here indicate that there are certain limitations in current attempts to measure the potential impact of the fortification program. Measurement of changes in indices of folate status following short-term consumption of fortified foods is one method that has

been used to estimate potential effects of fortification. In a study of 75 individuals with coronary heart disease, Malinow et al. (1998) reported that plasma folate increased and plasma homocysteine decreased proportionally with folic acid content when subjects consumed breakfast cereals fortified with 127, 499 or 665 μg folate/30 g for 5 weeks (i.e. 32, 120 and 166% DV, respectively).

The study of Malinow et al. (1998), while providing short-term information on effects of consuming a single fortified food on indicators of folate status, does not address the effects of consuming a wide range of fortified foods for long periods of time. Thus, such short-term studies tend to underestimate potential effects of a broad-based fortification program.

Another approach to assessing the effects of fortification is to measure indices of folate status in individuals consuming currently fortified foods and to compare the data with those obtained from the same individuals several years previously (i.e. prior to fortification). Jacques et al. (1999) measured plasma folate and total homocysteine concentrations using blood samples from the fifth examination (January 1991 to December 1994) of the Framingham Offspring Study cohort for baseline values and samples from the sixth examination (January 1995 to August 1998) for follow-up values. On the basis of observations of significant increases in mean plasma folate from baseline visit to the follow-up visits, and significant decreases in mean total plasma homocysteine concentrations among non-users of vitamin supplements during this same time, the authors concluded that the fortification of enriched grain products with folic acid was associated with a substantial improvement in folate status in a population-based sample of middle-aged and older adults (Jacques et al.).

In considering the apparent rapidity of changes in plasma folate and plasma homocysteine in response to fortification, Jacques et al., 1999 noted that consideration must be given to the possibility that enriched grain products were being fortified at levels above the minimum required by the FDA. Afman, Bagley and Selhub (1999) determined folic acid per se by the affinity/HPLC method cited above (Seyoum & Selhub, 1993). Their analyses of common national brands of enriched flour, pasta, and rice that were available in the Framingham area revealed that folic acid concentrations were 125 and 136 μg/100 g in two brands of flour, 180 and 205 μg/100 g in two ''pasta' products, and 66, 108, and to 176 μg/100 g in three rice products (Afman et al.). On the basis of these findings, Jacques et al. (1999) concluded that overfortification did not appear to be responsible for the rapidity of the changes in plasma folate and homocysteine levels that they observed.

The data of Afman et al. (1999) are noteworthy because these authors report the amount of folic acid per se in their samples. We note that on the basis of the values reported by Afman et al., the products these authors analyzed appeared to be underfortified or fortified at the lower end of specified ranges which are 154 μg/100 g for flour, 198–264 μg/100 g for enriched macaroni and enriched noodle products [''pasta'] and 154–308 μg/100 g for rice. It would have been interesting to know the ''total folate' value for the products analyzed by Afman et al. in order to compare such values with the total folate values we observed. It would also have been interesting to have information from the product's labels regarding folate content. Our observations that many products contain more total folate than required by the regulations or have higher levels than expected from label declarations (i.e. that some overfortification is occurring) are consistent with Jacques et al.'s (1999) observations of rapid changes in plasma folate and plasma homocysteine in their study population. The new data reported here suggest that some cereal-grain products are fortified at higher-than-minimum levels. Frequent consumption of such foods would be expected to lead to more rapid than anticipated changes in indices of folate status.

Lewis et al. (1999) described another approach to estimating the potential impact of fortification. These authors updated two national food consumption surveys to reflect folate intakes as a result of the food fortification program and to correct folate intakes for the apparently higher bioavailability of synthetic folic acid (i.e. the form of folate added to foods or obtained from dietary supplements) compared with that of naturally occurring folate. The findings of Lewis et al. suggested that 67–95% of the population exceeded the new estimated average folate requirement, depending upon the gender and age group and survey used.

In updating their estimates of folic acid intakes from the newly fortified food supply, Lewis et al. (1999) used the amounts of folic acid stated in Federal regulations for enriched bakery products and for enriched flours, and used the midpoint of the range for those products or ingredients for which the Federal regulations provide a range of fortification levels. Our results suggest that use of the upper limit of the ranges would have provided better estimates than use of the mid-points of the ranges. In addition, our observations of significant overages in bakery products suggest that use of the regulation-derived value 95 μg folic acid/100 g for these products would have resulted in underestimations of their actual contribution to folate intake.

5. Conclusions

Issues regarding the US folate fortification program will continue to draw wide interest and attention. Implementation of a fortification program for the reduction in risk of neural tube birth defects is under

debate in other countries, with safety concerns on the one hand and concern that fortification should be at a level high enough to offer significant protection against neural tube defects on the other (Cuskelly, McNulty & Scott, 1999).

5.1. Potential for excesses

FDA's final regulations regarding fortification were published 5 March 1996 with a "full compliance' date of 1 January, 1998. Manufacturers had approximately 22 months before the full compliance date to exhaust supplies of in-stock labels, fortify their products, and prepare new labels. We report here what appear to be significant excesses in some groups of recently-fortified products. We found no trend toward lower folate values in products purchased in the latter months of the study. There is the general concern that lack of precision when using folate assay procedures that have not been adequately validated or for which sample extractions may be inadequate (e.g. single enzyme extractions) may be compensated for by addition of excess amounts of folic acid. Use of analytical procedures with poor accuracy and precision adds to fortification costs and may result in lack of compliance with respect to Federal regulations and with label statements.

Folic acid added to foods must be present in an amount at least equal to the value declared on the label. Reasonable excesses over label claims are acceptable within current good manufacturing practices (21 CFR§101.9 (g)). For example, if stability data warrant, an excess may be needed to ensure that the product contains at least the labelled amount throughout its shelf life. The regulations prescribing conditions under which food additive substances, including folic acid, may be safely used in foods predicate usage under conditions of good manufacturing practice. Good manufacturing practice is defined to include the restriction that the quantity of the substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritive or other technical effect in food (21 CFR§172.5).

As noted above, nutrients added to fortified or fabricated foods must be present in amounts at least equal to the values declared on product labels. With respect to fortified foods, there is no specific overage or deficiency that is viewed by FDA as being consistent with good manufacturing practice. Whether or not an overage or deficiency is consistent with good manufacturing practice is determined on a case-by-case basis. For example, in a recent survey of vitamin content of fortified milks, FDA found a general trend toward a lower level of vitamins A and D in the products than the amounts declared on their labels (Tanner et al., 1988). In discussing compliance with labeling regulations, FDA noted that compliance procedures in 21 CFR§101.9 were applicable and that "any added nutrient declared on the label must be present at the level declared within the limits of the analytical method' (Tanner et al.).

With respect to folic acid, in considering the appropriateness of overages, the variability generally recognized for the analytical method used must be considered as well as factors relating to the stability of the additive. As noted above, available data show that folic acid in cereal-grain products is quite stable. There appear to be no data to suggest that overages should exceed the variability known to be associated with the analytical method.

5.2. Areas requiring additional work

More attention needs to be focused on careful fortification of foods with particular attention to avoidance of overages beyond those consistent with Good Manufacturing Practices. The potential for excesses across a wide range of folic acid-fortified enriched cereal-grain products is of concern because folic acid is the form of the vitamin for which a tolerable upper intake level has been recommended (IOM, 1998). In addition, while short-term studies have addressed the relative effectiveness of increased intakes of foods naturally rich in folate, folic acid supplements and foods fortified with folic acid, in improving folate status, the effect of habitual (life-time) consumption of folic acid-fortified foods on folate status has not been studied and remains unknown (Lewis et al., 1999).

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 identify and validate methods that can accurately quantify folic acid and naturally-occurring folates separately (Rader et al., 1999). There is also a need for careful analysis of the increasing number and types of folic acid-fortified foods.

Questions of how to handle apparent differences in bioavailability among folate forms remain largely unresolved, as are questions of how best to use current food folate data in the context of an evolving data base. A meaningful evaluation of the effectiveness of the folate fortification program cannot be made until current and much more extensive data on the total folate content of foods are available.

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