Natural Levels of Nutrients in Commercially Milled Wheat Flours. II. Vitamin Analysis[1]

P. M. KEAGY,[2] B. BORENSTEIN,[3] P. RANUM,[4] M. A. CONNOR,[2] K. LORENZ,[5] W. E. HOBBS,[6] G. HILL,[7] A. L. BACHMAN,[8] W. A. BOYD,[9] and K. KULP[10]

ABSTRACT

Mean vitamin levels and variances of 63 unenriched, commercially milled and treated U.S. and Canadian flours are reported according to wheat type (hard or soft) and flour end use. In general, milling variables greatly influenced the vitamin content of the flour, as shown by correlations with flour ash content. Pyridoxine gave the highest correlation (0.90), followed by thiamin, folacin, and niacin (0.74–0.53), but riboflavin and pantothenic acid showed little or no correlation (0.31 to −0.10). Multiple regression equations predict thiamin, niacin, pyridoxine, and folacin content of flours as a function of flour ash and protein. Hearth flours had the highest vitamin content, followed by baker's bread, cookie, family, and cake flours. Parent wheats were analyzed for three vitamins. Vitamin content of flours as a percent of whole wheat levels were: pyridoxine 15%, thiamin 32%, and riboflavin 35–42%. Wheat kernel protein was significantly correlated with wheat kernel thiamin and riboflavin but not with pyridoxine. Two laboratories determined flour thiamin, riboflavin, niacin, and pyridoxine. Thiamin and niacin results showed excellent agreement. Riboflavin and pyridoxine results were significantly different due to laboratories or methods.

Cereal Chem. 57(1):59–65

Flour and cereal products provide 19% of the calories, 17% of the protein, and 35% of the carbohydrate available for civilian consumption in the U.S. diet. Significant amounts of total available thiamine (41%), riboflavin (21%), and niacin (27%) are also contributed, but only small amounts of pyridoxine (8.7%) and vitamin A (0.4%) are supplied by these sources (Marston and Friend 1976). The high proportions of thiamin, riboflavin, and niacin are due to the current cereal enrichment program. The National Research Council has proposed adding pyridoxine, folacin, and vitamin A to the list of enrichment vitamins for the United States (NAS/NRC 1974). Recent Canadian regulations have included pyridoxine, folacin, and pantothenic acid enrichment of wheat flours (Kulp et al 1980). Amounts of vitamins to be added must be based on knowledge of their initial concentration in flours. Several extensive studies have reported vitamin contents of wheat and flours (Calhoun et al 1958, Toepfer et al 1972, Waggle et al 1967), but none contains a sample statistically adequate to estimate variation on a national basis. In addition, new wheat varieties, changes in milling procedures, and advances in analytical methodology may result in different values.

Eight laboratories analyzed 63 wheat and flour samples for thiamin, riboflavin, niacin, pyridoxine, folacin, and pantothenic acid. This article reports mean results and variances according to wheat and flour types, plus correlations of vitamin levels with ash and protein content. Others (Kulp et al 1980, Lorenz et al 1980) present proximate composition and mineral analyses of these samples.

[1]Presented at the AACC 61st Annual Meeting, New Orleans, October 1976. Joint cooperative study of the American Bakers' Association Inter-Industry Committee on Cereal Fortification and the Technical and Nutrition Committee of the Bakery Council of Canada.

[2]Western Regional Research Center, USDA-SEA/AR Berkeley, CA 94710.

Use of a company or product name by the USDA does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

[3]Hoffmann-LaRoche Inc., Nutley, NJ. Present Address: CPC International Inc., Englewood Cliffs, NJ 07632.
[4]Pennwalt Corp., Broadview, IL 60153.
[5]Colorado State University, Fort Collins, CO 80523.
[6]General Mills Inc., Minneapolis, MN 55427.
[7]ITT Continental Baking Co., Inc., Rye, NY 10580.
[8]W. E. Long Co., Chicago, IL 60606.
[9]Merck & Co., Inc., Rahway, NJ 07065. Present address: Stauffer Chemical Co., Westport, CT 06880.
[10]American Institute of Baking, Manhattan, KS 66502.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Association of Cereal Chemists, Inc., 1980.

MATERIALS AND METHODS

Samples

The samples used are described by Kulp et al (1980). At the mill, 36 flours received normal treatments, which may or may not affect vitamin content. Details regarding these treatments are given by Kulp et al (1980). Because the treatments were used in 25 different combinations, the effect of the treatments on vitamin levels in the flours is difficult to evaluate.

Methods

All laboratories were selected on the basis of expertise and availability. The analytical procedures applied for determination of the individual nutrients were those routinely used by the respective collaborators, and no effort was made to select one method for each nutrient. Consequently, variations in testing methods were encountered.

Thiamin. Two laboratories analyzed the flours for thiamin. Both used the thiachrome procedure with slight modifications.

Laboratory C extracted 10-g samples at 40°C in 100 ml of a solution of 2% acetic acid and 25% KCl (Hoffer et al 1943, 1945) and filtered through Whatman No. 2 filter paper. The oxidation step and thiachrome fluorescence determination were done both manually by means of AACC method 86-80 and by a Technicon autoanalyzer.

Laboratory D analyzed the flours according to AOAC method 43.024-43.030, using 0.1*N* H_2SO_4 instead of 0.1*N* HCl in the extraction.

Riboflavin. This vitamin was estimated chemically in two laboratories by four procedures.

Laboratory C analyzed the flours by three methods. One was AACC method 86-73, which calls for extraction of samples by autoclaving 30 min in 0.1*N* HCl followed by automated permanganate oxidation of impurities and fluorometric determination of riboflavin. Laboratory C also assayed for riboflavin in the extract prepared for thiamin determination and measured fluorescence both with and without the permanganate oxidation step (Hoffer et al 1944, 1945).

Laboratory D used the enzyme-treated flour extract prepared for the thiamin assay and determined riboflavin fluorescence according to AOAC method 43.039-43.043, omitting permanganate oxidation.

Niacin. Three laboratories determined niacin.

Laboratory D used AOAC microbiological method 43.126-43.127.

Laboratory E used AOAC method 43.A01-43.A05 (Gross 1975, Anonymous 1975) which extracts niacin with $Ca(OH)_2$ for 2 hr at 15 psi, cleaves pyridine groups with CNBr, and reacts with sulfanillic acid to develop a yellow complex read at an absorbance

of 470nm.

Laboratory F analyzed niacin by the AOAC microbiological method (43.126-43.129) using *Lactobacillus plantarum* ATCC 8014. Difco niacin assay medium, and Difco microinnoculum agar. This method extracts 30 min at 121° C with $1N$ H_2SO_4.

Pyridoxine. Laboratory K used AOAC microbiological method 43.159-43.164 with reference to Atkin et al (1943) and the Association of Vitamin Chemists (1966). Briefly, the samples were autoclaved 2 hr at 121°C in $0.44N$ HCl. Aliquots were diluted and assayed microbiologically using *Saccharomyces uvarum* (formerly *carlsbergensis*) and pyridoxine HCl standard.

Laboratory L extracted 2 g of flour with 180 ml of $0.055N$ H_2SO_4 for 16 hr at 127°C. The sample was adjusted to pH 5.0, brought to 250 ml, filtered through Whatman No. 42 paper, and assayed by the method of Atkin et al (1943) using *S. uvarum* and pyridoxine HCl. Minor modifications of the original method included the use of wort molasses agar to maintain the organism, sodium citrate in place of potassium citrate in the buffer, and 25 μg per tube of niacin added to the medium.

Folacin. Laboratory M extracted triplicate 2.5-g samples (30 min at 100°C) with 30 ml of $0.1M$ sodium phosphate buffer (pH 6.1) containing ascorbic acid (5 mg/ml) and 1 ml of 5% takadiastase solution that had been treated 1 hr in an ice bath with 2.5% Dowex 1-X8 chloride resin to remove folate. Samples were cooled and incubated 4 hr at 37°C with 3 ml of hog kidney conjugase (Bird et al 1965), 1 ml of Dowex-treated takadiastase, and 0.75 ml of $2.5M$ sodium acetate buffer (pH 4.5). Extracts were filtered, diluted with $0.05M$ phosphate buffer (pH 6.1) containing ascorbic acid (1.5 mg/ml), and assayed microbiologically (Schatzki and Keagy 1975, Tamura et al 1972) with *Lactobacillus casei* ATCC 7469. Important points of this method are the use of ascorbic acid to protect reduced folate forms, conjugase to liberate polyglutamate folate for the microorganism, and *L. casei,* which utilizes methyl folate not available to *Streptococcus faecalis* ATCC 8043 (formerly the most common assay organism).

Free Pantothenic Acid. Laboratory H extracted and analyzed free pantothenic acid as described by Michela and Lorenz (1976). Briefly, 1-g samples were extracted with papain and takadiastase at pH 4.5 for 24 hr at 39°C. The samples were steamed, centrifuged, and refrigerated until assay with *L. plantarum* ATCC 8014 as

TABLE I
Mean Nutrient Content and Variances of Commercial Flours, mg/100 g, Dry Weight Basis

Nutrient	Lab./Method[a]	All Flours	Wheat Type[b]		Flour End Use[c]					
			Hard	Soft	Hearth	Bread	Cookie	Family	Cake	$\overline{s^2_p}$[d]
Thiamin[e]	C/M	0.146	0.144	0.150	0.168 ab	0.144 b	0.203 a	0.132 bc	0.089 c	
	C/T	0.164[f]	0.162[f]	0.168	0.191 ab	0.160[f] b	0.228 a	0.147 bc	0.102 c	
	D	0.158	0.154	0.166	0.174 ab	0.155 bc	0.210 a	0.140 bc	0.114 c	
	s^2_p[g] $\times 10^2$	0.3562	0.2380	0.6183	0.2095	0.2576	0.4266	0.2535	0.1078	0.260
Riboflavin[e]	C/M1	0.040	0.041	0.038	0.045 a	0.037 a	0.037 a	0.045 a	0.036 a	
	C/TA	0.046[h]	0.047[h]	0.043	0.048[d] a	0.047[f] a	0.045 a	0.050 a	0.034 a	
	C/M2	0.050[f]	0.052[f]	0.046	0.058 a	0.049[f] a	0.048 a	0.054 a	0.041 a	
	$s^2_p \times 10^2$	0.0452	0.0470	0.0428	0.0223	0.0829	0.0238	0.0206	0.0538	0.0463
	D	0.067	0.059	0.083	0.063	0.055	0.072	0.068	0.089	
	$s^2 \times 10^2$	0.1100	0.426	0.2176	0.0206	0.0740	0.1868	0.0399	0.2755	
Niacin[e]	D	1.42	1.48	1.31	1.61 a	1.42 ab	1.42 ab	1.46 ab	1.17 b	
	E	1.31	1.44	1.02	1.80 a	1.35 b	1.17 b	1.34 b	0.87 c	
	F	1.38	1.48	1.16	1.68 a	1.42 ab	1.32 ab	1.44 b	0.97 c	
	s^2_p	0.1313	0.1274	0.0738	0.1279	0.1442	0.0835	0.0845	0.0234	0.1015
B_1, B_2, niacin	N	58	39	19	7	19	10	14	8	
Pyridoxine	K	0.046[i]	0.052[i]	0.034	0.067 a	0.052 b	0.048 b	0.038[i] c	0.020 d	
	L	0.069	0.075	0.054	0.088 a	0.076 b	0.066 c	0.064 c	0.041 d	
	$s^2_p \times 10^2$	0.0285	0.0167	0.0280	0.0182	0.0076	0.0130	0.0224	0.0070	0.0127
Folacin	M	0.019	0.021	0.016	0.024 a	0.020 b	0.019 b	0.019 b	0.014 c	
	$s^2 \times 10^2$	0.0020	0.0015	0.0021	0.0011	0.0011	0.0021	0.0021	0.0002	0.0014
Pantothenic acid	H	0.37	0.39	0.34	0.42 a	0.39 ab	0.37 ab	0.36 ab	0.31 b	
	$s^2 \times 10^2$	0.982	1.206	0.4028	1.068	1.516	0.403	0.734	0.236	0.9481
Protein %	A	12.6	13.8	9.9	15.8	14.0	10.4	12.2	9.6	
	s^2	4.870	1.982	0.3467	0.9659	0.5911	0.2382	2.2101	0.1121	
Ash %	A	0.518	0.540	0.469	0.608 a	0.545 b	0.517 bc	0.484 c	0.419 d	
	$s^2 \times 10^2$	0.4709	0.3383	0.4225	0.4188	0.1411	0.2252	0.2832	0.1210	0.2138
B_6, FA, Pan Pro, Ash	N	63	43	20	7	23	11	14	8	

[a] Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, TA = AACC Method 86-73, M2 = thiamin M extract without permanganate step.
[b] Flours from blends of soft and hard wheats were classed with the wheat type contributing the majority of the blend.
[c] Duncan's multiple range test. Flour end use means within a method are not significantly different at $P \leq 0.05$ if followed by the same letter.
[d] Flour end use s^2_p values were tested for homogeneity using Bartlett's test. If not different at $P \leq 0.05$, they were pooled into $\overline{s^2_p}$ and used for Duncan's multiple range test (Steel and Torrie 1960).
[e] Five flour samples were enriched with thiamin, riboflavin, niacin, and iron before collection. They were excluded from the data for these nutrients.
[f] Mean contains n−1 observations.
[g] Variances (s^2) for each laboratory and method were pooled (s^2_p) after testing for homogeneity (Bartlett's test) at $P \leq 0.01$ (Steel and Torrie 1960).
[h] Mean contains n−3 observations.
[i] Mean contains n−2 observations.

described by Difco (1953). Like official AOAC and AACC methods, this method does not measure the maximum amount of pantothenic acid obtainable from wheat products (Clegg 1958, Nielands and Strong 1948). However, estimates of relative vitamin concentrations and variability should be valid.

Statistical Methods

Table I presents means and pooled variances. Variances were presented instead of standard deviations or standard errors to avoid rounding errors and to allow computing whichever statistic is appropriate for the reader's comparisons. In order to provide the best variance estimate and facilitate multiple comparisons, variances for each laboratory and method were tested for homogeneity (Bartlett's test) and pooled (s^2_p) if not significantly different (Steel and Torrie 1960). Pooled flour type variances (s^2_p) were again tested for homogeneity and pooled ($\bar{s}^2_p$) for use in

TABLE II
Multiple Regression Equation Values[a] of Vitamins with Flour Ash[b] and Protein[c]

Vitamin (mg/100 g)	Lab./Method[d]	B_0[e] ± SE	B_1[f] ± SE	B_2[g] ± SE	B_{12}[h] ± SE	MSE[i]	r^2[j]
Thiamin	C/M	−0.099 ± 0.041	0.845 ± 0.112	−0.015 ± 0.004		0.18×10^{-2}	0.52
	C/T	−0.115 ± 0.047	0.937 ± 0.127	−0.016 ± 0.004		0.23×10^{-2}	0.51
	D	−0.032 ± 0.038	0.714 ± 0.104	−0.014 ± 0.003		0.15×10^{-2}	0.47
Riboflavin	C/M1	No significant regression					
	C/TA	−0.006 ± 0.022	0.101 ± 0.042			0.48×10^{-3}	0.10
	C/M2	0.006 ± 0.021	0.086 ± 0.041			0.47×10^{-3}	0.07
	D	No significant regression					
Niacin	D	0.207 ± 0.262	2.36 ± 0.50			0.71×10^{-1}	0.28
	E	−0.817 ± 0.249	2.98 ± 0.68	0.048 ± 0.022		0.65×10^{-1}	0.58
	F	−0.191 ± 0.314	3.04 ± 0.60			0.10	0.31
Pyridoxine	K	−0.071 ± 0.007	0.226 ± 0.014			0.55×10^{-4}	0.82
	L	−0.132 ± 0.032	0.350 ± 0.064	0.009 ± 0.002	−0.013 ± 0.005	0.39×10^{-4}	0.87
Folacin	M	−0.0025 ± 0.0033	0.0205 ± 0.0087	0.0009 ± 0.0003		0.11×10^{-4}	0.47
Pantothenic acid	H	No significant regression					

[a] All coefficients are significant at $P \leq 0.05$.
[b] Ash data ranges from 0.382 to 0.710 g/100 g of flour, moisture free basis.
[c] Protein data ranges from 8.9 to 17.1 g/100 g of flour, moisture free basis.
[d] Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, TA = AACC Method 86-73, M2 = thiamin M extract without permanganate step.
[e] B_0 = Constant.
[f] B_1 = Regression coefficient for flour ash (g/100 g).
[g] B_2 = Regression coefficient for flour protein (g/100 g).
[h] B_{12} = Regression coefficient for flour ash × flour protein.
[i] MSE = Mean squared error.
[j] r^2 = Coefficient of determination.

TABLE III
Correlations of Flour Vitamins with Ash and Protein

Nutrient	Lab./Method[a]	Flour Ash			Flour Protein		
		All	Hard Wheats	Soft Wheats	All	Hard Wheats	Soft Wheats
Thiamin	C/M	0.60[b]	0.67[b]	0.82[b]	0.14	0.27	0.72[b]
	C/T	0.61[b]	0.69[b]	0.81[b]	0.16	0.32	0.74[b]
	D	0.53[b]	0.61[b]	0.83[b]	0.08	0.26	0.72[b]
Riboflavin	C/M1	0.21	0.31	0.06	0.16	0.19	0.39
	C/TA	0.31[c]	0.31	0.28	0.22	0.20	0.63[b]
	C/M2	0.27[c]	0.28	0.18	0.24	0.23	0.49[c]
	D	−0.10	0.28	−0.07	−0.24	0.13	0.08
Niacin	D	0.53[b]	0.46[b]	0.58[b]	0.43[b]	0.38[c]	0.55[c]
	E	0.74[b]	0.64[b]	0.66[b]	0.66[b]	0.50[b]	0.49[c]
	F	0.56[b]	0.37[c]	0.68[b]	0.45[b]	0.19	0.55[c]
Pyridoxine	K	0.90[b]	0.84[b]	0.92[b]	0.62[b]	0.44[b]	0.66[b]
	L	0.91[b]	0.88[b]	0.90[b]	0.74[b]	0.60[b]	0.65[b]
Folacin	M	0.61[b]	0.39[b]	0.69[b]	0.65[b]	0.61[b]	0.58[b]
Pantothenic acid	H	0.23	0.10	0.35	0.23	0.07	0.53[c]
Protein	A	0.70[b]	0.66[b]	0.57[b]	1.00[b]	1.00[b]	1.00[b]

[a] Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, TA = AACC Method 86-73, M2 = thiamin M extract without permanganate step.
[b] Significant at $P < 0.01$.
[c] Significant at $P < 0.05$.

Duncan's new multiple range test comparing flour type means.

Regression equations in Table II were computed by least squares stepwise multiple regression. Coefficients reported are significant at $P \leq 0.05$. The complete model is as follows:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{12}X_1X_2$$

where Y is the flour vitamin concentration (mg/100 g); B_0 is a constant; B_1 is the regression coefficient for flour ash, X_1 (g/100g); B_2 is the regression coefficient for flour protein, X_2 (g/100 g); and B_{12} is the regression coefficient for the interaction term X_1X_2. All data is on a moisture free basis.

This model assumes that flour protein indicates the nutrient potential of the parent wheat and is determined by wheat protein (r = 0.96). Flour ash is determined in the milling process (flour ash vs. wheat grain ash, r = 0.04) and indicates the effect of milling. Other significant variables probably exist and could refine this model. Examination of the regression coefficients will indicate the magnitude of flour vitamin changes expected if higher protein wheats are used or if higher ash flours are produced. For illustrative purposes, expected changes in the vitamin content of flours have been calculated for an increase of 0.07% ash or 2.2% protein. These levels were selected because they represent one standard deviation in the range of ash or protein values respectively.

RESULTS AND DISCUSSION

Table I presents the mean nutrient content of all flours combined and of subgroups according to wheat class, flour end use, and analytical method. Table II presents regression equations of flour vitamins with ash and protein. Table III presents correlations of flour vitamins with flour ash and protein. Table IV presents correlations between laboratories for thiamin, riboflavin, niacin, and pyridoxine.

Thiamin

The mean thiamin content for all flours (excluding enriched samples) and all methods was 0.156 mg/100 g of flour. Means were similar for hard (0.153 mg/100 g) and soft (0.161 mg/100 g) wheat flours although soft wheat flours included cake and cookie flours with the lowest and highest thiamin contents, respectively. Thiamin content varied with flour end use. Cookie-cracker flour thiamin content (0.214 mg/100 g) was highest, followed by hearth (0.178 mg/100 g), baker's bread (0.153 mg/100 g), family (0.140 mg/100 g), and cake (0.102 mg/100 g) flours in descending order. This trend was probably due to milling differences as indicated by flour ash (Kulp et al 1980). The distribution of vitamins, ash, and protein in the wheat kernel was reviewed by MacMasters et al (1971). Endosperm, which is known to comprise 80–85% of the wheat kernel, contains small portions of the total ash and thiamin. As nonendosperm material increases in the flour, raising the ash content, thiamin would also be expected to increase. This trend was confirmed by significant correlations of flour ash and thiamin. Multiple regression of thiamin with ash and protein indicated that protein was also a significant factor in predicting the thiamin content of the flours. This means that as breeders produce higher protein wheats, the potential for higher thiamin flours increases. However, millers, through their control of flour ash, have much greater impact; a 0.07% increase in flour ash will have approximately 1.7 times the effect of a 2.2% increase in protein.

Thiamin values reported here are generally higher (20–100%) than previously reported (Calhoun et al 1958, Toepfer et al 1972, Waggle et al 1967, Watt and Merrill 1963). This probably reflects an increase in extraction rate for commercial flours during recent years. Results of different laboratories correlated well (Table IV), and differences between laboratories of the all flour means were not significant ($P > 0.10$). The acetic acid-KCl extract used by laboratory C would be expected to give values 8% lower than the standard method using takadiastase (Hoffer et al 1943). However, results with this extract were numerically both higher and lower than the AOAC procedure with no significance observed.

Riboflavin

The mean riboflavin content of all flours ranged from 0.040 to 0.067 mg/100 g depending on laboratory and method. No significant differences between hard and soft wheat flours or flour types were determined by laboratory C ($P > 0.10$). Wheat endosperm contains a large proportion of the kernel riboflavin (MacMasters 1971), so increased extraction rates indicated by higher ash content have little effect on flour riboflavin. This was borne out by the very low correlation (−0.10–0.31) of riboflavin with flour ash (Table III). Flour protein and ash did not predict riboflavin well, as indicated by the low r^2 (nonsignificant to 0.10, Table II).

Values reported here are approximately the same as those given by Toepfer (1972) and Waggle (1967) but considerably lower then those in USDA Handbook 8 (Watt and Merrill 1963) for the hard wheat categories.

Results of the three methods from laboratory C agreed quite well (r = 0.86–0.95, Table IV). Agreement between laboratories was lower (r = 0.58–0.72), with laboratory D reporting higher values than C. The discrepancy between laboratories was greatest for soft wheat flours.

Niacin

The mean niacin content of all flours varied from 1.31 to 1.42 mg/100 g of flour depending on laboratory and method. Niacin levels varied significantly between hard and soft wheat flours ($P < 0.001$) and among flour end uses. This variation followed a trend similar to that of flour ash. The niacin correlation with flour ash (Table III) was significant and analogous to that of thiamin. In addition, niacin gave significant correlations with flour protein but thiamin did not. Multiple regression with data from laboratory E indicated that both protein and ash were significant variables in predicting flour niacin. A 2.2% increase in flour protein indicates a rise in niacin of 0.11 mg/100 g of flour, and a 0.07% increase in flour ash indicates a rise in niacin of 0.20 mg/100 g of flour. Regressions using niacin data from laboratories D and F did not fit as well, as indicated by the smaller r^2. In these regressions, protein was not significant, possibly because the mean squared errors were larger.

Niacin values reported here are within 20% of the Handbook 8 values and compare favorably with other studies (Calhoun et al 1958, Hepburn 1971, Waggle et al 1967). With the exception of cake flours, mean values did not differ significantly between laboratories. Table IV shows that correlation between laboratory E (chemical method) and laboratory F (microbiological method) was

TABLE IV
Correlations Between Laboratories for All Flours[a]

Nutrient	Lab./ Method[b]	C/M	C/T	D	
Thiamin	C/M	1.00			
	C/T	0.99	1.00		
	D	0.96	0.95	1.00	
		C/M1	C/TA	C/M2	D
Riboflavin	C/M1	1.00			
	C/TA	0.86	1.00		
	C/M2	0.95	0.92	1.00	
	D	0.72	0.58	0.66	1.00
		D	E	F	
Niacin	D	1.00			
	E	0.66	1.00		
	F	0.53	0.85	1.00	
		K	L		
Pyridoxine	K	1.00			
	L	0.88	1.00		

[a] All correlations significant at $P < 0.01$.
[b] Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, TA = AACC Method 86-73, M2 = thiamin M extract without permanganate step.

TABLE V
Mean Values and Variances[a] for Selected Nutrients of Wheat Grain, mg/100 g Dry Basis

Nutrient	Lab./ Method[b]	All Wheats	Wheat Type[c]		Flour End Use[d]				
			Hard	Soft	Hearth	Bread	Cookie	Family	Cake
Thiamin	C/M	0.466	0.475	0.445	0.496 a	0.472 b	0.443 c	0.468 b	0.446 c
	C/T	0.514	0.524	0.492	0.561 a	0.519 b	0.486 d	0.510 bc	0.496 c
	$s^2_p \times 10^2$	0.1099	0.1132	0.0394	0.0691	0.0733	0.0275	0.1521	0.0557
Riboflavin	C/M1	0.113	0.117	0.105	0.123 a	0.118 a	0.099 c	0.114 a	0.107 b
	C/M2	0.118	0.121	0.109	0.130 a	0.121 ab	0.107 c	0.117 b	0.110 c
	$s^2_p \times 10^2$	0.0171	0.0170	0.0071	0.0242	0.0161	0.0045	0.0123	0.0025
Pyridoxine	K	0.315[e]	0.317[f]	0.309[f]	0.326 a	0.324[g] a	0.272[g] a	0.310[h] a	0.325[h] a
	$s^2 \times 10^2$	0.3472	0.4256	0.1524	0.4457	0.4844	0.1647	0.2770	0.1040
Ash	A	1.86[h]	1.84[h]	1.87	1.90 a	1.85[h] a	1.85 a	1.83 a	1.88 a
	$s^2 \times 10^2$	1.964	2.107	1.670	1.417	2.011	1.596	2.563	2.444
Protein	A	14.0	14.9	11.9	16.6 a	14.9 b	11.8 d	13.9 c	12.0 d
	s^2	3.021	1.493	0.1480	0.5697	0.4530	0.1220	1.812	0.1991
	N	61	42	19	7	23	10	13	8

[a] Variances (s^2) for each method were pooled (s^2_p) after differences were found to be nonsignificant by F at $P \leq 0.01$.
[b] Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, M2 = thiamin M extract without permanganate step.
[c] Blends of soft and hard wheats were classed with the wheat type contributing the majority of the blend.
[d] Student's t test of unpaired observations. Flour end use means within a method are not significantly different at $P \leq 0.05$ if followed by the same letter (Steel and Torrie 1960).
[e] Mean contains n−10 observations.
[f] Mean contains n−5 observations.
[g] Mean contains n−4 observations.
[h] Mean contains n−1 observations.

good (r = 0.85), but correlations of laboratories E and F with laboratory D were lower (r = 0.66 and 0.53, respectively.)

Pyridoxine

Mean pyridoxine content for all flours was 0.046 and 0.069 mg/100 g of flour for laboratories K and L, respectively. Differences were significant between hard and soft wheat flours ($P < 0.001$) and among flour types. The relative values followed those of ash, and pyridoxine correlated highly with ash (r = 0.90, 0.91). Correlation of pyridoxine with protein was somewhat lower (r = 0.62, 0.74). Regression analysis of laboratory L pyridoxine values indicated that ash and protein were both significant variables, as was an ash × protein interaction. As with thiamin and niacin, increasing flour ash has greatest effect on flour pyridoxine values. A 0.07% increase in the ash content of a 10% protein flour raises flour pyridoxine 0.015 mg/100 g whereas an increase of 2.2% protein in a 0.5% ash flour raises pyridoxine 0.005 mg/100 g.

Optimum extraction methods for pyridoxine in wheat have been controversial for some time. Differences between laboratories were statistically significant, with laboratory L results 50% higher than those of laboratory K. Laboratory L included comparison data on seven of the flour samples analyzed by two methods. In addition to the method described for laboratory L, these flours were extracted 2 hr at 127°C, with 100 ml of 0.44N H_2SO_4 (similar to the laboratory K method). Correlation between the two methods performed by laboratory L was good (r = 0.96), but overnight autoclaving with more dilute acid gave results 38% higher for these seven samples.

Values from laboratory K, using the AOAC method, tend to agree with data by Polansky and Toepfer (1969, Polansky et al 1964, Toepfer et al 1972) using the same method, whereas the higher laboratory L values correspond more with Orr's tabular data (1969). Despite the different mean values between laboratories, correlation was good (r = 0.88).

Folacin

One laboratory measured folacin. Mean folacin content for all flours was 0.019 mg/100 g. Folacin level varied significantly with hard (0.021 mg/100 g) and soft (0.016 mg/100 g) wheat flours

TABLE VI
Correlations of Vitamins with Wheat Protein in All Wheat Grains

Nutrient	Lab./Method[a]	Wheat Protein
Thiamin	C/M	0.62[b]
	C/T	0.67[b]
Riboflavin	C/M1	0.61[b]
	C/M2	0.73[b]
Pyridoxine	K	0.01

[a] Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, M2 = thiamin M extract without permanganate step.
[b] Significant at $P < 0.01$.

($P < 0.001$) and with flour type. Flour type means decreased with decreasing ash, as might be expected with a folacin-ash correlation of r = 0.61. Like niacin and pyridoxine, folacin correlated with protein (r = 0.65). Regression analysis of folacin data indicates that both protein and ash are significant variables indicating the folacin content of flours. Flour protein indicates more in this case than it does with the other vitamins; a 0.07% ash increase (0.001 mg of folacin per 100/g of flour) has less effect than a 2.2% protein increase (0.002 mg of folacin per 100 g of flour). Values reported here are within the range of single samples reported using the same method (Butterfield and Calloway 1972, Keagy et al 1975) and are 50% higher than those reported previously (Calhoun et al 1958, Waggle et al 1967) using a method and organism that did not respond to all folacin forms.

Pantothenic Acid

One laboratory measured pantothenic acid. The mean in all flours was 0.37 mg/100 g of flour. Content varied slightly with flour type and followed the same pattern as that of flour ash. However, pantothenic acid had no overall significant correlations or regressions with flour ash or protein. Values reported here are about 25% lower than those of Calhoun et al (1958), Orr (1969), and Waggle et al (1967). This could be due to the absence of avian liver enzyme in the extraction technique (Clegg 1958, Neilands and Strong 1948).

TABLE VII

Mean Flour Nutrient Content as Percent of Wheat Grain Nutrient

Nutrient	Lab./Method[a]	All Flours	Wheat Type		Flour End Use				
			Hard	Soft	Hearth	Bread	Cookie	Family	Cake
Thiamin	C/M	31	30	34	34	31	46	28	20
	C/T	32	31	34	34	31	47	29	21
Riboflavin	C/M1	35	35	36	37	31	37	39	34
	C/M2	42	43	42	45	40	45	46	37
Pyridoxine	K	15	16	11	21	16	18	12	6
Ash	A	28	29	25	32	29	28	26	22
Protein	A	90	93	83	95	94	88	88	80

[a]Methods: M = AACC Method 86-80, T = Technicon autoanalyzer, M1 = thiamin M extract with permanganate step, M2 = thiamin M extract without permanganate step.

Effect of Flour Type

The effect of flour type on different vitamins may be compared by expressing the flour type mean (from Table I) as a percent of the all-flour mean. Pyridoxine has the widest range of vitamin concentration varying from 46–28% higher in hearth flours to 41–57% lower in cake flours. Thiamin, niacin, and folacin have narrower ranges of concentrations, with the highest flour (cookie or hearth) 26–39% above the combined mean, and cake flours 18–39% below. Riboflavin and pantothenic acid are more evenly distributed throughout the kernel and therefore less affected by variations in milling different flour types. Hearth flours are 4–16% above and cake flours 16–26% below the all-flour mean (excluding laboratory D riboflavin).

Wheat Vitamins

Parent wheats were analyzed for thiamin, riboflavin, and pyridoxine (Table V). In all cases the vitamin content of hard wheats was higher than that of soft wheats, although the difference for pyridoxine was not statistically significant ($P > 0.15$). Wheat protein correlated significantly (Table VI) with riboflavin ($r = 0.61$ and 0.73 for the two methods used) and thiamin ($r = 0.62, 0.67$) but not with pyridoxine ($r = 0.01$). Pyridoxine showed a weak relationship to wheat ash ($r = 0.34$, $P < 0.05$), and thiamin and riboflavin showed none.

Comparison of these results with those in the literature shows similar levels of thiamin (Calhoun et al 1958, Waggle et al 1967) and pyridoxine (Polansky and Toepfer 1969). Riboflavin levels reported here are somewhat lower than those reported by Calhoun et al (1958), Waggle et al (1967), and Watt and Merrill (1963). An interesting sidelight is that the riboflavin enrichment standard for flour is based on the level of riboflavin in wheat. After the original standards were adopted, the riboflavin standard was found to be much too high because the assay methods used on wheat gave erroneously high values (Anonymous 1966).

Milling Losses

Table VII expresses the mean flour nutrient content as a percent of wheat nutrient content. This indicates the loss of individual nutrients due to conversion of wheat to flour by milling. Pyridoxine losses were greatest, with the flour retaining 6–21% of the original wheat pyridoxine. Thiamin retention ranged from 20 to 47%, depending on flour type, and closely matched ash retention. Cookie flours seem to be the major exception; they retain more thiamin than would be expected from the ash values. Riboflavin, which is more evenly distributed throughout the kernel, showed better retention, ranging from 31 to 46% depending on flour type and analytical method. These results are simlar to Toepfer's (1972) for riboflavin and pyridoxine but somewhat higher for thiamin.

SUMMARY

This article presents means and variances of six vitamins in 63 commercially milled flours. The mean vitamin concentrations have been used to calculate supplemental amounts of vitamins needed to meet proposed enrichment standards (Ranum 1980). Information important for the administration of such a program is also included, such as the extent of variation among flours and the effects of different analytical methods or laboratories. The relationships of the vitamins with flour ash and protein have been explored by means of correlations and multiple regression equations. These relationships indicate the potential for increasing the natural vitamin content of flours through the selection of higher protein wheats and/or production of higher ash flours.

ACKNOWLEDGMENT

We are grateful for the statistical guidance of Linda Whitehand and Bruce Mackey.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. The Association: St. Paul, MN.

ANONYMOUS. 1966. Enrichment: Remembrance of things past. Cereal Sci. Today 11:258.

ANONYMOUS. 1975. Niacin and niacinamide automated method—Official first action. J. AOAC 58:407.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Methods of Analysis. (12th ed.). The Association: Washington, DC.

ASSOCIATION OF VITAMIN CHEMISTS, INC. 1966. Methods of Vitamin Assay, 3rd ed. pp. 37-62, 209-221. Interscience: New York.

ATKIN, L., SCHULTZ, A. S., WILLIAMS, W. L., and FREY, C. N. 1943. Yeast microbiological methods for determination of vitamins. Pyridoxine. Ind. Eng. Chem. Anal. Ed. 15:141.

BIRD, O. D., McGLOHON, V. M., and VAITKUS, J. W. 1965. Naturally occurring folates in the blood and liver of the rat. Anal. Biochem. 12:18.

BUTTERFIELD, S., and CALLOWAY, D. H. 1972. Folacin in wheat and selected foods. J. Am. Diet. Assoc. 60:310.

CALHOUN, W. K., BECHTEL, W. G., and BRADLEY, W. B. 1958. The vitamin content of wheat, flour, and bread. Cereal Chem. 35:350.

CLEGG, K. M. 1958. The microbiological determination of pantothenic acid in wheaten flour. J. Sci. Food Agric. 9:366.

DIFCO LABORATORIES. 1953. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures, 9th ed. Difco Laboratories: Detroit, MI.

GROSS, A. F. 1975. Automated method for the determination of niacin and niacinamide in cereal products: Collaborative study. J. AOAC 58:799.

HEPBURN, F. N. 1971. Nutrient composition of selected wheats and wheat products. VII. Total and free niacin. Cereal Chem. 48:369.

HOFFER, A., ALCOCK, A. W., and GEDDES, W. F. 1943. A rapid method for the determination of thiamine in wheat and flour. Cereal Chem. 20:717.

HOFFER, A., ALCOCK, A. W., and GEDDES, W. F. 1944. A rapid method for the determination of riboflavin in wheat and wheat products. Cereal Chem. 21:524.

HOFFER, A., ALCOCK, A. W., and GEDDES, W. F. 1945. A rapid method for the combined determination of thiamine and riboflavin in cereal products. Cereal Chem. 22:271.

KEAGY, P. M., STOKSTAD, E. L. R., and FELLERS, D. A. 1975.

Folacin stability during bread processing and family flour storage. Cereal Chem. 52:348.
KULP, K., RANUM, P. M., WILLIAMS, P. C., and YAMAZAKI, W. T. 1980. Natural levels of nutrients in commercially milled wheat flours. I. Description of samples and proximate analysis. Cereal Chem. 57:54.
LORENZ, K., LOEWE, R., WEADON, D., and WOLF, W. 1980. Natural levels of nutrients in commercially milled wheat flours. III. Mineral analysis. Cereal Chem. 57:65.
MacMASTERS, M. M., HINTON, J. J., and BRADBURY, D. 1971. Microscopic structure and composition of the wheat kernel. In: POMERANZ, Y. (ed.). Wheat Chemistry and Technology. Am. Assoc. Cereal Chem.: St. Paul, MN.
MARSTON, R., and FRIEND, B. 1976. Nutritional Review. Natl. Food Situation 158:25.
MICHELA, P., and LORENZ, K. 1976. The vitamins of triticale, wheat, and rye. Cereal Chem. 53:853.
NAS/NRC. 1974. Proposed Fortification Policy for Cereal Grain Products. National Academy of Sciences, National Research Council: Washington, DC.
NEILANDS, J. B., and STRONG, F. M. 1948. The enzymatic liberation of pantothenic acid. Arch. Biochem. 19:287.
ORR, M. L. 1969. Pantothenic acid, vitamin B-6, and vitamin B-12 in foods. Home Economics Research Report No. 36, USDA.
POLANSKY, M. M., MURPHY, E. W., and TOEPFER, E. W. 1964. Components of vitamin B-6 in grains and cereal products. J. Assoc. Off. Agric. Chem. 47:750.
POLANSKY, M. M., and TOEPFER, E. W. 1969. Nutrient composition of selected wheats and wheat products. IV. Vitamin B-6 components. Cereal Chem. 46:664.
RANUM, P. M. 1980. Note on levels of nutrients to add under expanded wheat flour fortification programs. Cereal Chem. 57:70.
SCHATZKI, T. F., and KEAGY, P. M. 1975. Analysis of nonlinear response in microbiological assay for folacin. Anal. Biochem. 65:204.
STEEL, R., TORRIE, J. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw Hill: New York.
TAMURA, T., SHIN, Y. S., WILLIAMS, M. A., and STOKSTAD, E. L. R. 1972. *Lactobacillus casei* response to pteroylpolyglutamates. Anal. Biochem. 49:517.
TOEPFER, E. W., POLANSKY, M. M., EHEART, J. F., SLOVER, H. T., MORRIS, E. R., HEPBURN, F. N., QUACKENBUSH, F. W. 1972. Nutrient composition of selected wheats and wheat products. XI. Summary. Cereal Chem. 49:173.
WAGGLE, D. H., LAMBERT, M. A., MILLER, G. D., FARRELL, E. P., DEYOE, C. W. 1967. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. Cereal Chem. 44:48.
WATT, B. K., and MERRILL, A. L. 1963. Composition of Foods, Raw, Processed, Prepared. USDA Handbook No. 8.

[Received May 7, 1979. Accepted August 7, 1979]