THE DISTRIBUTION OF THE VITAMINS OF WHEAT IN COMMERCIAL MILL PRODUCTS

W. K. CALHOUN, F. N. HEPBURN, AND W. B. BRADLEY[1]

ABSTRACT

Nine vitamins were determined in a commercial wheat blend (50% hard red spring and 50% hard red winter) and in the products resulting from its milling. Thiamine was assayed chemically. Riboflavin, niacin, pantothenic acid, folic acid, biotin, *p*-aminobenzoic acid, choline, and inositol were determined by microbiological methods. Choline was found not to be concentrated to any extent in any one product. The other vitamins followed, in general, the well-known distribution pattern of the enrichment vitamins (thiamine, riboflavin, and niacin), with the greatest proportion (50 to 90%) contained in bran and shorts. Comparison of vitamin content of patent flour, first-clear flour, and low-grade flour shows pronounced and progressive increases in all vitamins except choline with decreasing grades of flour.

Studies in this laboratory showed some procedures for liberating vitamins from wheat and wheat products to be inadequate. Methods

[1] Manuscript received May 5, 1960. Presented at the 45th annual meeting, Chicago, Illinois, May 1960. Contribution from the American Institute of Baking, Chicago, Illinois. This research supported by a grant from the Max C. Fleischmann Foundation.

adopted for analysis of wheat, flour, and bread were published by Calhoun *et al.* (5). It was of interest to see if these methods could be applied satisfactorily to the analysis of mill products, and to determine the percentage of the vitamins of whole wheat recovered in the products of commercial milling. As stated in the previous communication (5), the determination of vitamin B_6 in wheat and wheat products presents special problems and is requiring extensive investigation. These studies will be presented separately.

Materials and Methods

Sample Description. The same lots of samples from Mill A described in the preceding paper by Hepburn *et al.* (8) in their studies of amino acids were analyzed for vitamins. Sample descriptions are repeated in Table I for convenience. The samples were stored at −20°F. (−28.9°C.) until needed.

TABLE I
SAMPLE DESCRIPTION OF WHEAT BLEND AND OF THE PRODUCTS COMMERCIALLY MILLED FROM IT
(14% moisture basis)

	PERCENT OF CLEANED WHEAT	PROTEIN (N × 5.7)
		%
Whole wheat[a]	100.0	12.9
Farina	1.51	10.7
Patent flour[b]	60.68	11.7
First-clear flour	11.14	14.8
Low-grade flour	2.42	16.4
Red dog	0.48	16.4
Shorts	9.13	16.3
Bran	14.57	14.5
Germ	0.07	22.7

[a] Blend of hard red spring and hard red winter wheats. Extraction rate 75.75%.
[b] 80.1% patent.

Assay Methods. Niacin, pantothenic acid, folic acid, biotin, *p*-aminobenzoic acid, choline, and inositol were determined by microbiological methods previously described by Calhoun *et al.* (5). Thiamine was determined by the thiochrome procedure as revised by Bechtel and Hollenbeck (4).

The fluorometric method of analysis for riboflavin (2) was unsatisfactory for products which, upon hydrolysis, produced highly colored extracts. Attempts to correct for nonriboflavin fluorescence met with varying success. Results of analysis of such products often varied markedly from day to day. However, the microbiological method using the assay organism *Lactobacillus casei* (1) proved satisfactory for all products.

Repeatedly, tests in this laboratory have failed to demonstrate the superiority of digesting wheat and wheat products (except germ) for pantothenic acid determination with the pigeon liver—alkaline phosphatase extract of Neilands and Strong (13) over the use of Mylase P[2] as suggested by Ives and Strong (11). In fact, approximately 10% higher values were obtained by the use of Mylase P. The pantothenate value obtained for germ was 10.4 γ per g. after digestion with Mylase P (Table II) and 15.8 γ per g. following digestion with the pigeon liver – alkaline phosphatase extract. Tests were also carried out with the pigeon liver extract from which most on the pantothenic acid had been removed by adsorption on activated Dowex 1 resin according to the method of Novelli and Schmetz (14) as modified by Toepfer *et al.* (15). Results obtained with this preparation were not different from those previously found with the untreated extract. Clegg (6) found this two-enzyme system liberated approximately 50% more pantothenic acid from wheat and flour than did treatment with takadiastase plus papain.

Two or more hydrolysates were prepared from each sample and either five or six levels of each hydrolysate were analyzed in duplicate. Values falling beyond ±10% of the mean were discarded.

Results and Discussion

The vitamin levels found in the wheat blend and in the mill products are given in Table II. The mill products are arranged in general order of decreasing content of vitamins. It is doubtful if meaningful detailed comparisons can be made between the values given in Table II and values found in the literature. Wheat blends are usually composed of several varieties, often grown under different environmental conditions. Storage times and conditions may vary widely. Also it is difficult to compare product values from different mills because yield values may not be identical. Hinton and co-workers, employing the technique of hand-dissecting wheat and analyzing the various anatomical regions for vitamins, have contributed much to present knowledge of the distribution of vitamins within the kernel. The results obtained in the present study can be related to their findings only in a general way. Where inferences can be drawn, as in case of thiamine (9), niacin (7), riboflavin, and pantothenic acid (10), good agreement was obtained when based upon available knowledge as to the kinds and relative amounts of tissues present in the products.

The literature is replete with values for thiamine, riboflavin, and niacin in wheat and in mill products. It is sufficient to note that values

[2] Wallerstein Laboratories, New York, N.Y.

TABLE II

VITAMIN CONTENT OF PRODUCTS COMMERCIALLY MILLED FROM A SINGLE WHEAT BLEND
(14% moisture basis)

	Whole Wheat	Bran	Shorts	Red Dog	Germ	Low-Grade Flour	First-Clear Flour	Patent Flour	Farina
	γ/g	γ/g	γ/g	γ/g	γ/g	γ/g	γ/g	γ/g	γ/g
Thiamine[a]	3.93	6.29	13.4	28.0	13.5	10.8	2.45	0.76	0.42
Riboflavin	1.07	3.34	3.47	3.22	4.87	1.24	0.48	0.32	0.28
Niacin	54.5	266.0	160.0	80.1	45.3	38.6	20.9	10.1	8.79
Pantothenic acid[b]	10.9	39.1	26.6	18.2	10.4[c]	9.15	6.75	4.83	4.44
Folic acid	0.50	0.88	1.35	1.20	2.05	0.42	0.18	0.11	0.09
Biotin	0.114	0.440	0.350	0.250	0.174	0.108	0.042	0.014	0.010
p-Aminobenzoic acid	3.83	14.8	12.6	7.81	3.70	2.95	1.26	0.33	0.24
	mg/g	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*
Choline[d]	1.63	1.54	1.76	1.74	2.65	1.48	1.51	1.61	1.63
Inositol	3.15	13.4	10.8	8.08	8.52	3.41	1.13	0.33	0.21

[a] As thiamine hydrochloride.
[b] As calcium pantothenate.
[c] Pigeon liver — alkaline phosphatase digestion yielded 15.8γ per g.
[d] As choline chloride.

TABLE III

PERCENTAGE OF THE TOTAL VITAMIN CONTENT OF WHEAT FOUND IN EACH COMMERCIALLY MILLED PRODUCT

	Bran	Shorts	Patent Flour	First-Clear Flour	Low-Grade Flour	Red Dog	Farina	Germ	Total
	%	%	%	%	%	%	%	%	%
Yield from wheat	14.57	9.13	60.68	11.14	2.42	0.48	1.51	0.07	100.0
Choline	13.8	9.9	59.9	10.3	2.2	0.5	1.5	0.1	98.2
Pantothenic acid	52.3	22.3	26.9	6.9	2.0	0.8	0.6	0.1	111.9
Riboflavin	45.5	29.6	18.2	5.0	2.8	1.4	0.4	0.3	103.2
Thiamine	23.3	33.0	15.1	9.0	8.6	3.4	0.2	0.2	92.8
Folic acid	25.6	24.6	13.4	4.0	2.0	1.2	0.3	0.3	71.4
Niacin	71.1	26.8	11.2	4.3	1.7	0.7	0.2	0.1	116.1
Biotin	56.2	28.0	7.4	4.1	2.3	1.0	0.1	0.1	99.2
Inositol	62.0	31.3	6.4	4.0	2.6	1.2	0.1	0.2	107.8
p-Aminobenzoic acid	56.3	30.0	5.2	3.7	1.9	1.0	0.1	0.1	98.3

found in Table II agree broadly with those summarized from the literature by Bailey (3), and by Kent-Jones and Amos (12). Only fragmentary studies of the pantothenic acid, folic acid, biotin, *p*-aminobenzoic acid, choline, and inositol content of mill products have been reported. Some of these studies have been summarized (3,12). Where values are found, the samples are often inadequately described and differences between laboratories could be ascribed either to samples, methods of determination, or both. The authors failed to find complete studies of the content of these vitamins in the products of milling from a single wheat source.

Comparison of the vitamin content of patent flour, first-clear flour, and low-grade flour shows pronounced and progressive increases in all vitamins except choline. This is in contrast to the findings with amino acids (8), which exhibited little or no difference between flour products.

The percent of the vitamin content of whole wheat that appeared in each mill product (Table III) was calculated by multiplying the yield value (Table I) by the vitamin concentration (Table II) and dividing the resulting product by the vitamin content of whole wheat. The mill products are arranged in Table III in general order of decreasing contribution to the total vitamin contained in whole wheat. Patent flour's contribution of choline provided the major exception, since this vitamin was not concentrated in any one product by milling. For example, patent flour constituted 60.68% of the wheat and contained 59.9% of the total choline of that wheat. This relationship between yield of product and contribution of choline was found for the other products. The vitamins are listed in Table III according to decreasing amounts of whole wheat vitamin retained in patent flour. Thus, 59.9% of choline was retained, but only 5.2% of the *p*-aminobenzoic acid of wheat appeared in patent flour.

Bran and shorts contained, except in the case of choline, approximately 50 to 90% of the total vitamins present in the cleaned wheat. Except for niacin and *p*-aminobenzoic acid, the vitamin content of germ was higher than in whole wheat (Table II). However, when the amount of germ contained in wheat is considered, assuming 2 to 2.5% of the kernel is germ, the amounts of vitamins removed in this product by milling are far less than the total contained in bran or shorts.

The sums of vitamins found in the mill products (Table III) were within about 3% of the amount contained in whole wheat in case of choline, riboflavin, biotin, and *p*-aminobenzoic acid. Ninety-three percent of the thiamine and 108% of the inositol appeared in the products. Less satisfactory, but acceptable recoveries of pantothenic acid (112%) and of niacin (116%) were obtained. Unaccountably, only 71% of the folic acid of wheat was found in the mill products.

It is hoped that the methods adopted for use in these studies will prove useful in future work designed to understand better the extent to which various factors influence the vitamin content of wheat and wheat products.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Mrs. Evelyn Cashman, Miss Clara Deaton, and Miss Pauline Persing.

Literature Cited

1. Association of Official Agricultural Chemists. Official methods of analysis (8th ed.). The Association: Washington, D.C. (1955).
2. Association of Vitamin Chemists, Inc. Methods of vitamin assay (2nd ed.). Interscience: New York (1951).
3. Bailey, C. H. The constituents of wheat and wheat products. Reinhold: New York (1944).
4. Bechtel, W. G., and Hollenbeck, C. M. A revised thiochrome procedure for the determination of thiamine in cereal products. Cereal Chem. **35:** 1–14 (1958).
5. Calhoun, W. K., Bechtel, W. G., and Bradley, W. B. The vitamin content of wheat, flour, and bread. Cereal Chem. **35:** 350–359 (1958).
6. Clegg, K. M. The microbiological determination of pantothenic acid in wheaten flour. J. Sci. Food Agr. **9:** 366–370 (1958).
7. Heathcote, J. G., Hinton, J. J. C., and Shaw, B. The distribution of nicotinic acid in wheat and maize. Proc. Roy. Soc. **B139:** 276–287 (1952).
8. Hepburn, F. N., Calhoun, W. K., and Bradley, W. B. The distribution of the amino acids of wheat in commercial mill products. Cereal Chem. **37:** 749–755 (1960).
9. Hinton, J. J. C. The distribution of vitamin B_1 and nitrogen in the wheat grain. Proc. Roy. Soc. **B134:** 418–429 (1947).
10. Hinton, J. J. C., Peers, F. G., and Shaw, B. The B-vitamins in wheat: The unique aleurone layer. Nature **172:** 993–995 (1953).
11. Ives, Margaret, and Strong, F. M. Preparation of samples for the microbiological assay of pantothenic acid. Arch. Biochem. **9:** 251–258 (1946).
12. Kent-Jones, D. W., and Amos, A. J. Modern cereal chemistry (5th ed.). Northern Publishing Co.: Liverpool (1957).
13. Neilands, J. B., and Strong, F. M. The enzymatic liberation of pantothenic acid. Arch. Biochem. **19:** 287–291 (1948).
14. Novelli, G. D., and Schmetz, F. J., Jr. An improved method for the determination of pantothenic acid in tissues. J. Biol. Chem. **192:** 181–185 (1951).
15. Toepfer, E. W., Zook, E. G., and Richardson, L. R. Microbiological procedure for the assay of pantothenic acid in foods: results compared with those by bioassay. J. Assoc. Offic. Agr. Chemists **37:** 182–190 (1954).