Effect of Bleaching, Maturing, and Oxidizing Agents on Vitamins Added to Wheat Flour[1]

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ABSTRACT

Bread and cake flours fortified with vitamins B_1, B_2, B_6, A, niacin, and folacin to the National Research Council's proposed levels and with pantothenic acid to the optional Canadian enrichment standards were subjected to a number of commonly used flour treatments (azodicarbonamide, benzoyl peroxide, ascorbic acid, potassium bromate, chlorine, and chlorine dioxide) alone and in typical combinations from normal up to 16 times normal treatment rates. The three vitamins included in current enrichment programs (B_1, B_2, and niacin) were unaffected by any of the applied treatments. Chlorination was the only flour treatment found to have any detrimental effect on the other vitamins, significantly ($P < 0.05$) reducing the level of vitamin B_6 an average of 16% and having a lesser, but not statistically significant, effect on vitamin A, pantothenic acid, and folacin. Unlike the natural vitamin E in wheat flour, added *d*-α-tocopheryl acetate was found to be resistant to destruction by flour bleaching. The mill application of bleaching, maturing, and oxidizing agents is therefore quite compatible with vitamin fortification.

The enrichment of wheat flour with thiamin, riboflavin, and niacin has been a common practice in the United States, Canada, and a number of other countries for many years. The Agriculture Stabilization and Conservation Service of the U.S. Department of Agriculture requires that vitamin A also be added to wheat flour and soy-fortified flour used in the Food For Peace program. An expanded fortification policy proposed for cereal-grain products by the Food and Nutrition Board of the National Research Council (NAS/NRC 1974) calls for the addition of vitamin A, pyridoxine, and folacin. Canada has expanded its flour enrichment program to allow the optional addition of pyridoxine, folacin, and pantothenic acid (Canada Department of National Health and Welfare 1978).

A number of studies have reported on the stability of vitamins added to cereals during storage and baking. One area that has received little formal investigation is the effect of typical flour treatments on added vitamins. Wheat flour is generally treated at the mill with bleaching, maturing, and other oxidizing agents to improve its color and baking quality. Bleaching is known to destroy much of the indigenous vitamin E in flour (Frazier and Lines 1967), but whether flour treatments such as bleaching have a detrimental effect on added vitamins, including vitamin E, has not been documented. The objective of this study was to determine what effect, if any, typical flour treatments have on vitamins which are now, or might be, added to wheat flour.

MATERIALS AND METHODS

Sources of Added Vitamins

The sources of the vitamins used were those recommended by Ranum (1977b) for the American Bakers Association Inter-Industry Committee on Cereal Fortification, as used in other studies to investigate the feasibility of fortifying flour and bread with additional nutrients (Emodi and Scialpi 1980, Ponte 1979). Thiamin mononitrate was used as the source of vitamin B_1 because this has been shown to be the more stable form of thiamin in flour and baking (Hollenbeck and Obermeyer 1952). Niacinamide was used as the source of niacin because it does not have the vasodilation effect of niacin (nicotinic acid), which could be irritating to experimenters. Both forms are nutritionally equivalent. Vitamin A palmitate [Hoffmann-La Roche code 250-SD containing 250,000 international units (IU) vitamin A activity per gram] was used as the source of vitamin A. This product, which is spray dried and contains an antioxidant to improve stability, has been shown to be well suited for flour fortification (Anderson and Pfeifer 1970, Borenstein 1969). Vitamin B_6 and pantothenic acid were added in the form of pyridoxine hydrochloride and d-calcium pantothenate, respectively. Iron was also added in the form of electrolytically reduced iron powder, but its inclusion is only incidental to the purpose of this study. Vitamin E was added in the form of dry *d*-α-tocopherol acetate (50%-SD, Hoffmann-La Roche Co., containing 500 IU of vitamin E activity per gram).

Flour Fortification

Vitamins were added to flour by means of the fortification premix shown in Table I. The formulation of this premix was based on the recommended levels of nutrients to add for meeting the National Research Council's (NRC) proposed fortification levels, as given by Ranum (1980), when used at a rate of 10 g per 100 lb of flour. The added levels take into account the naturally occurring vitamin content of the flour and provide a 10% overage to serve as a safety margin. The premix itself was made with a 2% overage to ensure meeting the stated levels. Pantothenic acid and vitamin E, which are not included in the NRC standards, were added separately.

The vitamin fortification standards given in Table I represent the vitamin reference form in which any assay results are expressed.

TABLE I
Composition of Flour Fortification Premix[a]

Ingredient/Assay Referenced Standard	Level in Premix[b] Minimum (%)	Assay (%)	Fortification[c] Standard (mg/lb)
Thiamin mononitrate/	2.57	...	...
thiamin HCl	2.65	2.78	2.9
Riboflavin	1.80	1.94	1.8
Niacinamide/	21.0	...	...
niacin	...	23.4	24.0
Pyridoxine HCl/	2.0	...	...
pyridoxine	(1.65)[d]	...	2.0
Folic acid/	0.26	...	...
folacin	...	0.31	0.3
Vitamin A palmitate[e]/	20.0	...	...
vitamin A (IU/100 mg)	5,000	5,100	5,000
Reduced iron/	11.46	...	...
iron	11.0	12.3	13–16.5

[a] Carrier: 3% tricalcium phosphate (free-flowing agent) with starch as the remainder.
[b] Premix was designed to be added to flour at the rate of 10 g per 100 lb. This would make the level added (in milligrams per pound) equal to the percent level in the premix. A 2% manufacturing overage was allowed for each nutrient to insure meeting the minimum standard.
[c] NAS/NRC (1974).
[d] Corrected value.
[e] Stabilized form containing 250,000 international units (IU) of vitamin A per gram.

[1] Presented in part at the 63rd AACC Annual Meeting (World Bread Congress) in Winnipeg, September 1978.
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Only riboflavin and folacin actually used the reference form. Where the molecular weight of the reference form differs from that of the form actually used (such being the case for thiamin, pyridoxine, and pantothenic acid), a molecular weight adjustment must be made in the amount added. This correction was made for thiamin mononitrate and calcium pantothenate but not for pyridoxine hydrochloride. The level of vitamin B_6 actually added was thus lower (1.65 mg/lb) than that called for under the NRC proposed standards (2.0 mg/lb).

Two types of flour were fortified: a hard wheat baker's flour (Bay State Milling Co.), having 12.2% protein and 0.50% ash contents at a 10.9% moisture level, and a soft wheat cake flour (Peavey Co.), with 8.8% protein and 0.34% ash contents at a 10.3% moisture level. For the baker's flour, 6.0 g of the premix and 0.30 g of calcium pantothenate were mixed with 5 lb of flour in a Hobart mixer. This preblend was then mixed with 55 lb of flour in a large rotating drum. The addition was similarly performed for the cake flour.

Flour Treatments

The fortified flours shown in Table II were treated with azodicarbonamide (Maturox®, Pennwalt Corp.), potassium bromate (Bromolux 2300®, Pennwalt Corp.), ascorbic acid (Fine powder, Hoffmann-La Roche, Inc.), and benzoyl peroxide (LA Novadelox®, Pennwalt Corp.) by mixing with flour for 7 min in a Hobart mixer. The two gases, chlorine (Cl_2) and chlorine dioxide (ClO_2), were applied by a previously described lab apparatus (Parker and Fortmann 1949).

Except for chlorine, the three treatment rates used were the normal rate used in the milling industry, four times normal, and 16 times normal. The highest level of chlorine that could be applied without gas escaping from the flour was 12,500 ppm or eight times normal.

A number of the treatments shown in Table II were combinations of two or more agents. This was done to better duplicate actual mill practices and to see if any synergistic effects might occur.

Fortification with Vitamins A and E

The stability of vitamin A was studied separately, using a commercial fortification premix (type 6 N-Richment-A®, Pennwalt Corp.), which contained 66,000 IU of vitamin A per gram in addition to thiamin, riboflavin, niacin, and reduced iron. An untreated baker's flour was fortified with this premix at a rate of 0.25 oz/cwt, adding 4,700 IU of vitamin A per pound of flour, and subjected to the various treatments shown in Table III. This flour was baked into white pan bread, chappaties, and wheat tortillas, using standard baking methods.

For the vitamin E results given in Table III, an untreated baker's flour was supplemented with 6.8 g/cwt of *d*-α-tocopheryl acetate containing 500 IU of vitamin E per gram and subjected to the various flour treatments shown.

Vitamin Assays

Thiamin, riboflavin, and niacin were determined by automated methods, using a common extraction procedure described by Ranum (1977a). Riboflavin was assayed by the AACC automated procedure, and thiamin was run by an automated version of the AACC thiochrome method. Niacin was determined by a modification of the AACC automated method, in which the bound niacin contained in the common extract was released by incubating the sample stream at a high pH (using NaOH) for 1 min, followed by buffering back to pH 7 before dialysis. A niacinamide standard was used, with the results corrected on the basis of the unfortified flour containing niacin. The three vitamins were run in triplicate one month after treatment on separate days using separate extracts.

Vitamins A, E, and folacin were assayed according to standard AOAC (1975) procedures. Pyridoxine and pantothenic acid were assayed by the microbiological methods of Atkin et al (1943, 1944).

TABLE II
Niacin, Thiamin, and Riboflavin Contents[a] of Treated Flours

Flour Treatments[b] (ppm)						Vitamin Contents: Niacin (mg/lb)		Thiamin (mg/lb)		Riboflavin (mg/lb)		Vitamin B_6 (mg/lb)		Vitamin A (IU/lb)		Pantothenic Acid (mg/lb)	Folic Acid (mg/lb)
ADA	$KBrO_3$	AA	BPO	ClO_2	Cl_2	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Hard wheat, bread flour																	
Unfortified, untreated						5.0	0.3	0.85	0.03	0.17	0.01	···	···	···	···	···	···
Fortified, untreated						25.1	0.6	3.52	0.06	2.15	0.01	1.71	0.07	5,350	420	7.3	0.32
···	···	···	···	5.5	···	24.7	0.4	3.70	0.07	2.17	0.01	2.17	···	5,290	···	5.9	···
···	···	···	···	22.0	···	25.7	0.7	3.60	0.03	2.17	0.01	1.88	···	5,250	···	···	0.39
5.5	···	···	···	···	···	24.4	0.4	3.62	0.05	2.16	0.01	···	···	···	···	···	···
22.0	···	···	···	···	···	24.7	1.3	3.59	0.09	2.17	0.02	···	···	···	···	···	···
···	15	···	···	···	···	25.6	0.4	3.70	0.15	2.18	0.03	···	···	···	···	···	···
···	60	···	···	···	···	25.8	1.4	3.55	0.07	2.18	0.02	1.64	···	···	···	···	···
5.5	15	···	···	···	···	24.4	1.4	3.63	0.06	2.17	0.03	1.74	0.10	5,220	90	4.9	···
22.0	60	···	···	···	···	26.3	0.4	3.66	0.14	2.25	0.04	1.83	···	5,170	···	6.9	···
88.0	240	···	···	···	···	25.0	0.6	3.65	0.07	2.18	0.00	2.00	···	5,120	···	5.8	0.39
···	···	50	···	···	···	25.1	0.7	3.64	0.06	2.21	0.00	1.74	0.28	5,330	···	4.2	···
···	···	200	···	···	···	24.9	1.3	3.58	0.09	2.19	0.02	1.69	···	5,590	···	4.9	0.36
5.5	15	···	50	···	···	24.8	0.2	3.72	0.06	2.20	0.02	1.49	···	···	···	···	···
22.0	60	···	200	···	···	24.8	0.9	3.58	0.09	2.16	0.03	1.59	···	···	···	···	···
···	15	50	50	···	···	25.2	0.4	3.61	0.14	2.22	0.05	1.86	···	···	···	···	···
Soft wheat cake flour																	
Unfortified, untreated						4.9	0.1	0.38	···	0.14	···	···	···	···	···	···	···
Fortified, untreated						23.6	2.6	3.17	0.22	2.04	0.23	1.81	0.09	5,130	230	4.0	0.41
			···	···	1,560	27.8	1.7	3.57	0.11	2.38	0.02	1.51	···	···	···	···	···
			···	···	6,250	27.9	1.6	3.47	0.15	2.26	0.03	1.59	···	···	···	···	···
			···	···	12,500	27.7	1.2	3.15	0.09	2.13	0.03	1.46	···	4,810	···	3.3	···
			50	···	···	26.6	1.4	3.33	0.09	2.17	0.02	1.64	···	···	···	···	···
			200	···	···	25.3	0.7	3.30	0.11	2.11	0.03	1.79	···	···	···	···	···
			800	···	···	26.2	2.4	3.27	0.16	2.13	0.03	1.63	···	5,220	···	5.3	···
			50	···	1,560	24.5	0.9	3.04	0.17	1.98	0.02	1.46	0.12	4,740	50	5.0	···
			200	···	6,250	23.4	1.7	3.03	0.33	1.91	0.04	1.46	···	4,680	···	5.0	0.25

[a] Vitamin contents shown are on 14% moisture basis.
[b] ADA = azodicarbonamide, $KBrO_3$ = potassium bromate, AA = ascorbic acid, BPO = benzoyl peroxide, ClO_2 = chlorine dioxide, Cl_2 = chlorine.

RESULTS AND DISCUSSION

Niacin, Thiamin, and Riboflavin

The assay means and standard deviations for these three vitamins are shown in Table II. The values are expressed at a 14% moisture basis in milligrams per pound. The theoretical levels of niacin, thiamin, and riboflavin contained in the fortified untreated flours are 27.6, 3.53, and 2.04 mg/lb for the bread flours and 27.3, 3.06, and 2.00 mg/lb for the cake flours, respectively. These figures are calculated from the assayed levels in the premix (Table I) corrected to a 14% flour moisture basis and the natural levels in the unfortified flour (Table II). The experimentally observed levels in the fortified flours compare favorably with the theoretical values, although the levels of recovered niacin ran slightly low and those for riboflavin ran slightly high.

A Student's t test showed that no significant reduction in the level of these three vitamins resulted from any of the applied flour treatments. Thiamin and riboflavin levels were lowered slightly in cake flour bleached with a combination of benzoyl peroxide and chlorine but not enough to cause the assayed level to drop below the minimum standard.

Niacin, thiamin, and riboflavin, the three vitamins generally included in flour enrichment programs, were essentially unaffected by flour treatments, even when the rate of those treatments greatly exceeded normal application rates.

Pyrodoxine, Folacin, and Pantothenic Acid

The results for these three vitamins, given in Table II, represent single determinations except where a standard deviation is shown, in which case they were run in triplicate. A Student's t test for significance can still be applied by pooling the assay results for individual treatment agents.

Chlorination of cake flour resulted in a significant ($P < 0.05$) reduction of pyridoxine (vitamin B_6), averaging a 16% loss from the level in the untreated control. None of the other agents caused a significant reduction in pyridoxine nor was any trend toward lower levels with higher treatment rates noticeable.

The mean assay levels of pyridoxine (1.84 ± 0.19 and 1.65 ± 0.13 mg/lb, "as is" moisture basis, for bread and cake flour, respectively) are below the 2.0 mg/lb fortification standard. This was because a molecular weight correction for the hydrochloride salt was not applied to the amount added; flour treatment effects were not responsible for the apparently low values.

Wide variation was found in the levels of pantothenic acid found in the flour samples. The overall average, on an "as is" moisture basis, was 5.9 ± 1.2 mg/lb for bread flour and 4.7 ± 0.9 mg/lb for cake flour. Three of the 12 flour samples tested failed to meet the 4.5 mg/lb minimum standard, including even the untreated cake flour. Although the highest level of chlorine treatment gave the lowest pantothenic acid level (3.3 mg/lb), adequate levels of this vitamin in the other two chlorinated samples suggest that chlorine is not a serious problem at normal treatment rates.

The only flour sample that failed to meet the 0.3 mg/lb folacin standard was the cake flour bleached with both chlorine and benzoyl peroxide. As in the work of Keagy et al (1975), none of the bread flour treatments exhibited any deleterious effects on this vitamin. An insufficient number of pantothenic acid and folacin assays were performed to statistically determine if any of the flour treatments resulted in a significant reduction in these two vitamins.

TABLE III
Vitamin A and Vitamin E Contents of Fortified and Unfortified Flours

Flour Treatment		Vitamin A,	Vitamin E	
Agent	Amount (ppm)	Fortified[a] (IU/lb)	Unfortified (IU/lb)	Fortified[b] (IU/lb)
Untreated		4,700	6.4	43
Azodicarbonamide	6.6	4,900	2.7	43
Benzoyl peroxide	50	4,800	1.3	42
Chlorine dioxide	4.4	···	0.9	···
	8.8	4,800	···	41
Chlorine	1,250	4,600	0.6	40
+ benzoyl peroxide	50	···	0.4	40
Acetone peroxide	20	4,700	2.1	43

[a]4,700 IU of vitamin A palmitate added per pound of flour.
[b]34 IU of d-α-tocopheryl acetate added per pound of flour containing 7.7 IU per pound.

Vitamin A

None of the bread flour treatments resulted in a significant loss of vitamin A (Table II). Chlorinated cake flours showed a slight reduction, averaging around 10%, in vitamin A content. This reduction was not statistically significant and was unrelated to increasing chlorine application rates. In the second study (Table III), none of the treatments resulted in a serious loss of vitamin A.

A more severe test was made by combining equal amounts of mixtures containing 32% benzoyl peroxide and 50% vitamin A palmitate. No reduction in vitamin A activity was found after 10 min. At the end of three weeks, a 10% loss of vitamin A was observed. This would indicate that the form of vitamin A palmitate used in these studies is extremely resistant to oxidative destruction by benzoyl peroxide, the most commonly used flour bleaching agent.

In a separate study, flour fortified with vitamin A palmitate showed a 90% retention of vitamin A in white pan bread just after baking. Five days later the bread still showed 89% of the initial vitamin A activity added. Chappaties and tortillas showed 85 and 96% retention of vitamin A, respectively. This confirms the study by Anderson and Pfeifer (1970), which showed similar resistance of vitamin A palmitate to destruction during baking.

Vitamin E

The results on unfortified treated flours shown in Table III confirm what a number of other investigators (Engel 1942, Menger 1957, Mason and Jones 1958, Moran et al 1954) have reported, ie, that bleaching is very destructive to the natural vitamin E content in flour.

Vitamin E was considered, but not included, in the proposed fortification policy (NAS/NRC 1974) for cereal-grain products because, as stated in the report, it "may not be stable when subjected to current bleaching practices in the flour industry." However, the results in Table III show that added d-α-tocopheryl acetate was quite stable in flour subjected to the typical bleaching treatments shown. Flour bleaching is destructive to the natural forms of vitamin E in wheat but not to d-α-tocopheryl acetate, the form in which it would be added, because the reactive hydroxyl group in the natural forms is replaced by a stabilizing acetate group that protects it from oxidative destruction.

Chlorination of Fiour

Of the flour treatments tested in this study, chlorination was the only one found to cause any problems in vitamin fortification. It lowered pyridoxine levels and was possibly detrimental to added vitamin A, pantothenic acid, and folacin. None of the other agents exhibited a similar effect on added vitamins.

This is understandable because chlorine is a highly reactive oxidizing agent, immediately acting on contact with dry flour, and is generally applied at rates higher than those used for the other flour treatment agents. It also lowers the flour's pH by forming hydrogen chloride and other acids that may be destructive to some vitamins.

Despite these factors, the amount of vitamins lost because of chlorination was relatively small, the highest being pyridoxine at 16%, and may be even lower under actual mill practice. By chlorinating fortified flour, as was done in this study, we allowed the chlorine gas to come into actual contact with the added vitamins, enchancing its destructive effect. This differs from the normal mill practice of adding vitamins to flour that has been previously chlorinated so that the chlorine gas does not come into direct contact with the added vitamins. Although the effect of adding vitamins to chlorinated flour was not investigated in this study, that procedure would be expected to be no more, and probably less, harmful to added vitamins.

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