Stability of Vitamin B_6 During Bread Making and Storage of Bread and Flour[1]

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ABSTRACT

Stability of vitamin B_6 was determined during bread making using three flours: whole wheat, white, and white enriched with vitamin B_6 (WB6). Breads made commercially and by two procedures (straight dough and sponge dough) under home conditions were compared. Stability of vitamin B_6 in stored WB6 flour and WB6 bread was also studied. Vitamin B_6 content of dough during different stages of bread making and of bread and flour during storage was determined by a microbiological method employing *Saccharomyces carlsbergensis*. Vitamin B_6 content decreased slightly during fermentation and baking, except that in bread made by the sponge method, the vitamin B_6 content increased during fermentation. Vitamin B_6 losses of 0–15% occurred during baking of the breads. Vitamin B_6 in the WB6 bread was stable when stored in the freezer (−5°C) or refrigerator (4–5°C). Vitamin B_6 content dropped 10% in bread stored at room temperature for three days. The level of vitamin B_6 of the WB6 flour did not change significantly when stored at room temperature or in a cold room for six months.

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Vitamin B_6 in food occurs as a group of closely related compounds. Pyridoxal and pyridoxamine are the predominant forms in foods of animal origin; pyridoxine (PIN) occurs mainly n plant foods. One of these plant foods, wheat, contains a relatively high amount of vitamin B_6 (Orr 1969). However, in the milling process, the content of vitamin B_6 is reduced to about 15–18% of the level in whole wheat (Aykroyd and Doughty 1970).

The importance of wheat in the diets of most people makes the loss of nutrients due to milling and processing of food an important consideration. The Food and Nutrition Board of the National Research Council has given evidence of potential risk of deficiency of certain vitamins, including vitamin B_6, among some population groups (NAS/NRC 1974). Because of this, the Food and Nutrition Board has suggested that vitamin B_6 be added, with several other vitamins and minerals, to milled wheat flour. The level of enrichment recommended for vitamin B_6 is 0.44 mg (as PIN) per 100 g of flour, or 2 mg/lb.

PIN is quite stable in acid solutions but is rapidly destroyed by light and heat at neutral or alkaline pH (Hochberg et al 1944), and pyridoxal and pyridoxamine are relatively less stable than PIN (Cunningham and Snell 1945). The processing of certain foods results in a loss of vitamin B_6. Heating and storage losses of vitamin B_6 in milk (Woodring and Storvick 1960) and loss from processing and preservation of various foods (Schroeder 1971) have been reported. However, Bunting (1965) determined that more than 90% of the vitamin B_6 added to cornmeal and macaroni was retained after storage for one year. Recently Cort et al (1976) and Rubin et al (1977) studied the stability of vitamin B_6 added to cereal products and reported little or no loss of vitamin B_6.

The present study was conducted to determine the stability of flour and bread enriched with vitamin B_6. We studied the effects of two methods of bread making and the effect of storage of vitamin B_6-enriched bread and flour on the retention of vitamin B_6.

MATERIALS AND METHODS

Preparation of Breads

Breads were prepared from whole wheat (WHW), white (W), and white flour enriched with vitamin B_6 (WB6) under commercial and home conditions. Commercial breads were prepared on a large scale at a local bakery. Brominated bakers patent flour was used in the commercially prepared breads WB6 and W, and 100% whole wheat flour was used in the WHW bread (Centennial Mills, Portland, OR 97208). These breads were prepared by formulas generally employed in the bakery. All-purpose flour and 100% WHW flour were used in the preparation of bread under home conditions.

Straight dough breads were prepared using the Finney and Barmore formula (1945), modified by replacing 2% of compressed yeast with 1% of active dry yeast (on flour basis). Sponge dough breads were made using the AACC approved method (1969) but omitting yeast food. The quantities of ingredients were kept at the same level as in the straight dough formula. The major difference between the two methods was the time of fermentation and proofing. The totals were 235 min for the straight dough method and 330 min for the sponge dough method.

Crystalline pyridoxine monohydrochloride (PIN-HCl; Calbiochem-Behring Corp., San Diego, CA 92112) was used as the source of vitamin B_6 in the enrichment of WB6 breads. The level of enrichment recommended by the National Academy of Sciences (1974), 2.00 mg/lb of flour, was used in the WB6 breads prepared under home conditions. In the commercially prepared breads, only 1.12 mg/lb of flour was used to obtain a level of vitamin B_6 in WB6 bread equal to that in WHW bread. This level was chosen because the commercially prepared breads were used in a subsequent study on bioavailability of vitamin B_6 in human subjects,[3] which necessitated a constant level of intake of bread and of vitamin B_6.

Sampling of Dough and Bread

During the bread making, representative samples were taken in triplicate at three stages: after mixing all the ingredients (mix), at the end of proofing (proof), and after baking (bread). These samples were analyzed for vitamin B_6 and for moisture content. Samples of mix and proof taken for the determination of vitamin B_6 were weighed and immediately frozen between two slabs of dry ice to stop fermentation. All samples were held frozen until analyzed.

For the bread, five slices of the same approximate weight were removed from different positions in the loaf and ground (dry) in a Waring Blendor. Triplicate samples were taken for the determination of vitamin B_6 content. The differences in the vitamin content between mix and proof and between proof and bread were tested for significance using Student's t test.

Storage of WB6 Breads

Several randomly selected loaves from the commercial batch of bread were wrapped in 1.5-mil plastic bags and placed under one of three conditions: at room temperature (25–27°C), exposed to indirect daylight only; in a refrigerator (4–5°C); or in a freezer (−5°C). The zero day samples were obtained after the loaves had

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been stored for 3 hr. Sample preparation was similar to that described. The bread stored at room temperature was sampled at three-day intervals until mold growth was seen. Loaves stored in the refrigerator and freezer were sampled at weekly intervals for four and eight weeks, respectively.

Storage of WB6 Flour

Crystalline PIN-HCl was added to all-purpose flour at the level of 2 mg/lb of flour. The vitamin was mixed in a small quantity of flour, and the rest of the flour was gradually added with constant mixing. This WB6 flour was placed in amber-colored closed jars and stored under three conditions: at room temperature (25–27° C), under refrigeration (5–7°C), and in a freezer (−40°C). The last temperature was chosen as a control condition. Triplicate samples were analyzed for vitamin B_6 content after 0, 1, 4, 8, 13, 19, and 26 weeks of storage.

Determination of Vitamin B_6

All operations were performed under subdued light to minimize photodegradation of vitamin B_6. The samples were hydrolyzed by autoclaving in 0.44*N* HCl for 2 hr at 121°C. After the hydrolysate was cooled to room temperature, the pH was adjusted to 4.5. The hydrolysate was made up to a known volume and filtered. This filtrate was used in the determination of vitamin B_6 by the AOAC method (1975). In commercially prepared breads, the three forms of vitamin B_6 (PIN, pyridoxal, and pyridoxamine) were separated by column chromatographs and Dowex 50 W (K^+) resin (100–200 mesh) according to the method of Toepfer and Polansky (1970). These column eluates were assayed for vitamin B_6 components using the AOAC method.

The standard AOAC method was modified by replacing the 100 ml of acid-hydrolyzed casein solution with 10 g of vitamin-free casamino acid (Difco Laboratories, Detroit, MI 48232). A 6 hr culture of *Saccharomyces carlsbergensis* (S. uvarum, ATCC No. 9080) was used in place of a 20-hr culture. The 6-hr culture was incubated at 30° C and gave adequate growth.

RESULTS AND DISCUSSION

Stability of Vitamin B_6 During Bread Making

The total vitamin B_6 content for the mix, proof, and bread prepared using WHW, WB6, and W flour under commercial and home conditions is shown in Fig. 1. Regardless of baking conditions, WHW and W breads showed little change in the vitamin B_6 level during fermentation and a slight decrease during baking. The commercial WB6 bread had a slightly lower level of vitamin B_6 than did the homemade breads. An increase in the level of vitamin B_6 in WB6 bread during fermentation was followed by a decrease during baking. The only significant increase in the vitamin B_6 content during fermentation was for the sponge dough bread $P \leqslant 0.05$). The fermentation time was 95 min longer for this type of bread. The reason for this increase is not clear. Keagy et al (1975) observed an increase in the folacin content during the fermentation of bread and attributed this to synthesis of folacin by yeast.

As expected, PIN was the predominant form of vitamin B_6 in the WHW bread (Fig. 2). Because PIN-HCl was used for fortification, this was also the predominant form in the WB6 bread. The increase in vitamin B_6 content of the WB6 proof in contrast to a decrease or no change in vitamin B_6 in the WHW and W proofs, suggests that if yeast synthesizes vitamin B_6, factors in the WHW and W breads may either be unavailable to the yeast or inhibit the synthesis of vitamin B_6. Using a microbiological assay of total vs free vitamin B_6, Siegel et al (1943) calculated that nearly 80% of the vitamin B_6 in WHW flour is bound, compared with about 55% bound in patent flour.

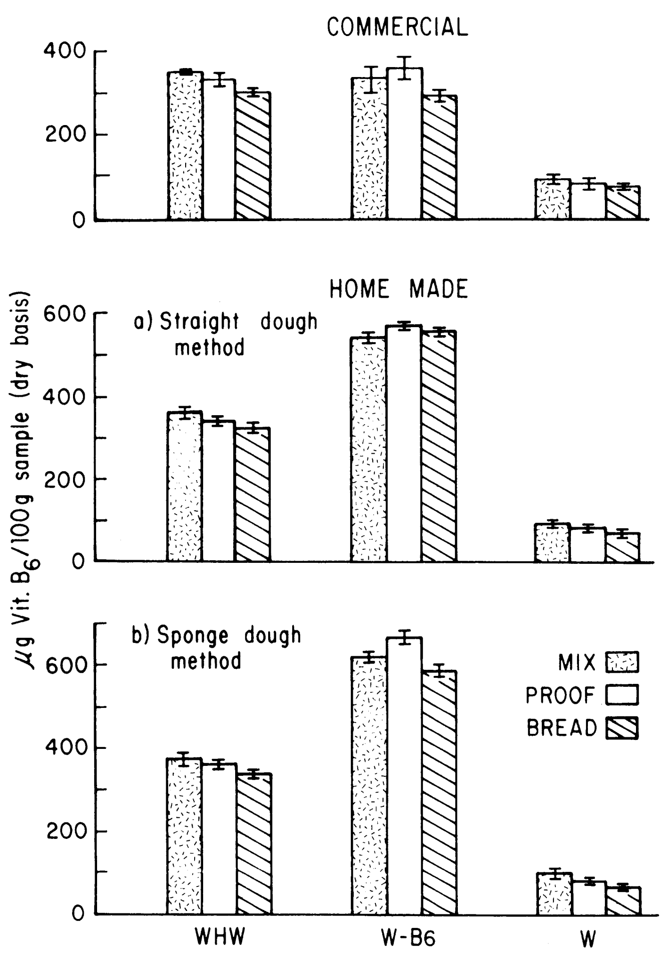

Fig. 1. Vitamin B_6 levels in mix, proof, and bread. For the straight dough method, all ingredients were mixed at the same time (total fermentation time, 235 min). In the sponge dough method, 60% of the flour was mixed with other ingredients and fermented for 4 hr before the remaining flour was added. W = white flour, WB6 = white flour enriched with vitamin B_6, WHW = whole wheat flour.

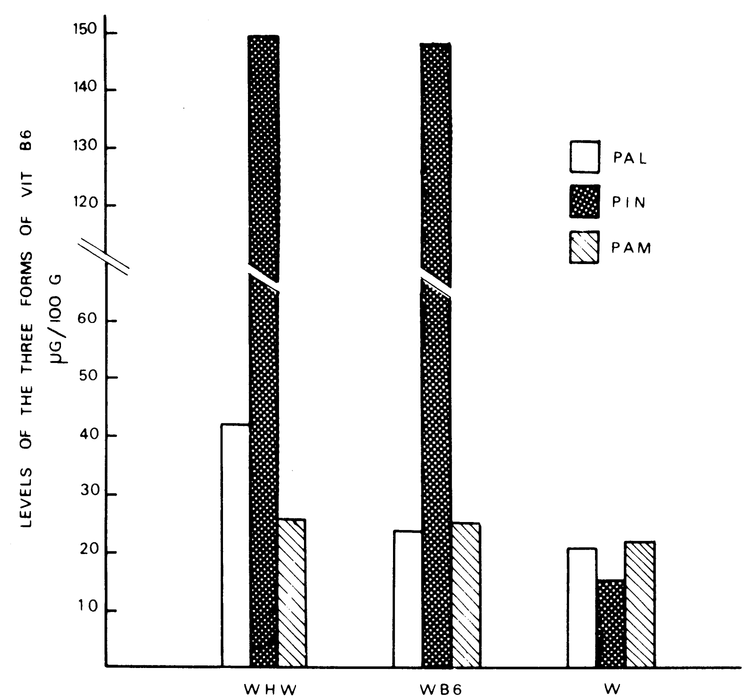

Fig. 2. Levels of the three forms of vitamin B_6 in the three commercial breads. PAL = pyridoxal, PIN = pyridoxine, PAM = pyridoxamine, W = white flour, WB6 = white flour enriched with vitamin B_6, WHW = whole wheat flour.

The vitamin B_6 content of the bread was decreased compared with that of the mix. This decrease was significant in WHW and W breads made commercially ($P \leqslant 0.05$). Significant differences also occurred between the W mix and bread and between the WHW and WB6 mixes and breads made by the sponge dough method ($P \leqslant 0.05$, $P \leqslant 0.05$, and $P \leqslant 0.001$, respectively). These baking losses of vitamin B_6 were 15% or less. Data from studies using somewhat different fortification and sampling procedures indicate a vitamin B_6 stability slightly better than that observed. Rubin et al (1977) reported vitamin B_6 retention of 100–105% in bread prepared by a sponge dough method. In a study in which a school lunch procedure for bread baking was used, vitamin B_6 retention of more than 100% was reported for bread after baking and after storage for five days at room temperature (Cort et al 1976). Differences in methods of baking and in addition of vitamin B_6 to flour may have contributed to the slightly lower retention observed in the present study.

Among the three forms of vitamin B_6, PIN is the most stable (Cunningham and Snell 1945). We determined that PIN was the predominant form of vitamin B_6 in the WHW and WB6 breads (Fig. 2). Our values for total vitamin B_6 and the three forms of vitamin B_6 in bread are similar to those reported by Polansky et al (1964).

TABLE I
Vitamin B_6 Content of WB6[a] Bread Stored Under Different Conditions[b]

Time of Analysis	Storage: Freezer (−5°)	Refrigerator (5°C)	Room Temp (27°C)
Day			
0	204 ± 2	191 ± 12	192 ± 3
3	194 ± 12	181 ± 6	172 ± 2[c]
7	192 ± 2	185 ± 5	
14	186 ± 11	181 ± 3	
21	193 ± 4	192 ± 11	
28	187 ± 3	185 ± 5	
35	194 ± 8		
42	197 ± 9		
49	193 ± 5		

[a] WB6 = White flour enriched with vitamin B_6.
[b] Mean ± standard deviation of three replications, expressed as milligrams of pyridoxine per 100 g of flour. Values are on wet weight.
[c] Student's t test; significantly lower than day 0 sample ($P < 0.01$).

TABLE II
Vitamin B_6 Content of WB6[a] Flour Stored Under Cold and Room Temperatures

Time of Analysis	Storage Temperature: 5°C	27°C
Week		
0	589 ± 23[b] (99.6)[c]	592 ± 48 (100.2)
1	586 ± 14 (100.5)	557 ± 18 (98.3)
4	542 ± 15 (94.4)	515 ± 12 (89.7)
8	593 ± 7 (99.9)	590 ± 14 (99.4)
12	578 ± 13 (110.0)	558 ± 21 (106.3)
19	580 ± 13 (99.4)	591 ± 18 (101.2)
26	574 ± 48 (98.0)	579 ± 23 (98.9)

[a] WB6 = White flour enriched with vitamin B_6.
[b] Mean ± standard deviation of three replications, expressed as milligrams of pyridoxine per 100 g of flour. Moisture content of the flour ranged from 9.8 to 9% for the three storage conditions over the 26-wk period.
[c] Vitamin B_6 content expressed as a percentage of the −45°C control flour analyzed at the same time.

Stability of Added Vitamin B6 During Storage of Bread

Vitamin B_6 content of bread stored for varying lengths of time under three different conditions is presented in Table I. No significant differences were observed in the values of frozen and refrigerated samples, but the vitamin B_6 level in bread stored at room temperature dropped significantly after three days, from 192 to 172 μg/100 g on moist weight basis ($P \leqslant 0.01$). On day 5 of the storage, mold growth was observed. In contrast, the results of Cort et al (1976) indicated no loss of vitamin B_6 even after five-day storage at room temperature. The flour we used contained no preservative, which probably contributed to rapid mold growth. This mold growth (even though unobserved) may have resulted in the low vitamin B_6 level observed at day 3.

Stability of Added Vitamin B_6 During Storage of Flour

Table II presents the vitamin B_6 levels of all purpose flour enriched with PIN-HCl and stored refrigerated (5–7°C) and at room temperature (25–27°C) for six months. No definite trend was observed in the changes of the vitamin B_6 levels of the flour, indicating good stability of the added PIN-HCl in flour. This observation was similar to that for added folacin in flour (Keagy et al 1975). Excellent stability of PIN-HCl enriched flour was also observed by Rubin et al (1977) and Cort et al (1976).

Results of the present study and previous studies (Cort et al 1976, Rubin et al 1977) indicate that fortification of wheat flour with vitamin B_6 is feasible in terms of its stability in flour and bread.

CONCLUSIONS

Changes in vitamin B_6 levels during bread fermentation were not significant except for an increase in WB6 bread dough prepared by the sponge dough method. The retention of vitamin B_6 in WHW, WB6, and W breads was greater than 85%, which indicated good stability of the vitamin. The vitamin B_6 level did not change in WB6 bread stored under frozen and refrigerated conditions, whereas the level in WB6 bread stored at room temperature dropped by 10%. The vitamin B_6 content of the WB6 flour did not change when stored at 5°C and 27°C for 26 weeks. The results indicate fairly good stability of native and added vitamin B_6 in wheat flour during home and commercial bread making and during storage.

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