FOLACIN STABILITY DURING BREAD PROCESSING AND FAMILY FLOUR STORAGE[1]

P. M. KEAGY, E. L. R. STOKSTAD[2], and D. A. FELLERS, Western Regional Research Center[3], Berkeley, California 94710

ABSTRACT

Synthetic pteroylglutamic acid (PteGlu) added at levels of 1 or 5 μg per g of all-purpose wheat flour showed small losses after 1 year at 120°F. Potassium bromate, ascorbic acid, azodicarbonamide, and benzoyl peroxide added at maximum levels allowed by Federal Bread Standards had no effect on the stability of added PteGlu in sponge and dough, straight dough, or mechanically developed breads. An average 11% loss was found in baking the doughs. Native folacin activity determined using *L. casei,* ascorbate, and hog kidney conjugase, decreased in all-purpose flour (0.12 μg/g) with increasing storage time and temperature. Total folacin in bread dough (65% yeast origin) increased with fermentation time but decreased an average 31% during baking. Thus, fermentation time determined whether final bread folacin was above or below concentrations expected from ingredient composition. Oxidizing-maturing agents had little or no effect on natural folacin activities in breads from baker's patent flour.

Folacin deficiency is probably the most prevalent vitamin deficiency in man (1,2,3). In the United States and Great Britain low serum folacin levels and megaloblastic anemia have been reported in association with conditions of nutritional stress (pregnancy, infancy, alcoholism, and disease states) (4,5), low family income (3,6), and poor dietary habits (7,8,9). Cooking procedures may result in varying losses of food folacin (10,11,12). Consequently, folacin enrichment of cereal products has been recommended by the National Research Council Committee on Food Standards and Fortification Policy at 0.07 mg/100 g of cereal grain products (13). Folacin enrichment of staple foods has been suggested in other countries as well (14). Before any enrichment program is adopted, the nutrient added should be shown to be stable under customary conditions of storage and use (15,16). Modern baking techniques often use oxidizing-maturing agents which could be detrimental to folacin stability. Light, oxidants, extremes of pH, and molecular oxygen at elevated temperatures are known to degrade folacin (17).

Many investigators (12, 18–24) have determined the amount of folacin in bread and flour with differing results which may be due in part to improvements and changes in the assay method. Early studies used *S. faecalis,* which does not respond to methyl folate derivatives (used by man) and does respond to pteroates (not used by man). The response of *Lactobacillus casei* more closely parallels man's response (4). In addition, ascorbic acid was not used in early studies to preserve labile folacin forms.

This investigation was carried out to determine the stability of folacin in a bread and flour enrichment program. Two subjects were studied: 1) The effect of bread-baking methods and oxidizing-maturing agents on folacin retention in bread. 2) The effect of storage of family flour on folacin retention.

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[2]Professor, Department of Nutritional Sciences, University of California, Berkeley.

[3]Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

TOPIC I: BREAD PROCESSING

MATERIALS AND METHODS

Flours and Breads

Breads were baked using an untreated, commercial baker's patent flour [12.4% moisture, 11.7% protein (N × 5.7), 0.47% ash, 0.12 μg folacin/g]. Straight-dough breads were baked using the Finney and Barmore (25) formula, 2% compressed yeast (flour basis), 3-hr fermentation, 55-min proof, and 25-min bake at 425° F. Sponge-dough breads were baked using AACC method 10-11 (26) modified to use a 70% sponge omitting yeast food. Total fermentation and proof time was 5 hr and 50 min. Mechanically developed breads, based on the English Chorleywood process, were mixed from the following formula.

Flour (14% moisture)	1000 g
Water	600 g
Malted wheat flour	2 g
Salt	20 g
Hydrogenated shortening	10 g
Compressed yeast	20 g

Each batch was mixed at atmospheric pressure to 5.2 W hr/lb dough in a Tweedy 10 laboratory mixer, scaled, moulded after 10-min floor time, proofed 65 min at 105° F, and baked 25 min at 425° F.

The control and conditioner breads were baked without additives, the conditioner being the first loaf baked. Ascorbic acid (200 ppm flour basis) and potassium bromate (75 ppm) were added in solution, and azodicarbonamide (Maturox®, 450 ppm) was added dry at the mix or sponge stages. Benzoyl peroxide[4] (Novadel®, 0.25%) was added to the folacin-containing flour in a closed system 24 hr prior to baking. Bread and dough moisture contents were calculated from ingredients and weight changes; calculated and representative analytical values showed good agreement.

Sampling

Dough samples were weighed, frozen between Dry Ice slabs, and held at −10° F until extraction. Bread samples were 10-g center slices with the proportion of crust and crumb held constant for each set of breads (straight-dough breads, 61% crumb; mechanical-dough, 75% crumb; sponge-dough native folacin, 61% crumb; added folacin 67%).

Extracts

All operations were performed without artificial light to minimize photodegradation of folacin. Approximately 12 ft-candles of working light was supplied by a south window. Samples were blended with pH 6.1, 0.1 *M* phosphate buffer containing 5 mg/ml ascorbic acid for 1 min in 4-oz Osterizer

[4]Oxidizing-maturing agents were added at the highest levels allowed by federal standards. Benzoyl peroxide is allowed "in a quantity not more than sufficient for bleaching, etc." (27). The amount used in this study is ten times commercial practice.

blend-and-store jars, autoclaved 10 min at 121°C, reblended, frozen overnight to break the starch gel, and filtered through glass wool. Aliquots were stored at −10°F until used. For release of total folacin the samples were treated with hog kidney conjugase by the method of Bird *et al.* (28) with fresh ascorbate (5 mg/ml) added to the buffer.

Microbiological Assay

The microbiological assay of folacin was performed as described by Tamura *et al.* (29) using *L. casei* and 1.5 mg/ml ascorbate in the buffer. Calculations were based on Finney's parallel line assay (30) and have been fully described by Schatzki and Keagy (31).

Statistics

Each sample value is the mean of two or more replicate extractions and analyses. Standard errors given in Tables I and II apply to these means. Significant differences from the control were calculated using Student's t test at the $\alpha = 0.1$ as well as the more conventional 0.05 and 0.01 probability levels. Small true changes in folacin content may not appear significant at $\alpha = 0.05$ due to the variabilities of some samples. Therefore, significant differences at $\alpha = 0.1$ have been included to indicate apparent but less certain differences. Variances within treatments are pooled for each processing stage. Replicate batches to determine bread processing variability were not taken. However, conditioner and control treatments approximate replicate batches.

RESULTS AND DISCUSSION

Stability of Native Folacin during Bread Processing

Table I, part A gives the native total folacin content of doughs and breads baked according to the straight-dough method. The first two batches baked were controls with no improving agent. However, the first batch baked on any day is considered least reliable and designated the conditioner; all test comparisons were made to the control (second batch baked). The calculated value is derived from analysis of the ingredients. Among the individual treatments, doughs made with benzoyl peroxide treated flour gave the lowest folacin levels at the mix and proof stages. However, the final bread folacin was similar to the levels given by other treatments. Flour treated with benzoyl peroxide analyzed before (0.133μg folacin/g) and 24 hr after treatment (0.130μg folacin/g) showed no effect of the bleaching treatment on folacin content. No other additive showed significant effects.

Other interesting phenomena were observed. The folacin content of the dough was much greater than that of the flour due to the contribution by yeast and milk. Yeast contributed approximately 65% of the initial folacin. The average folacin content increased from 0.44 μg/g dough (dry basis) at the mix to 0.76 μg after proofing. This is probably due to folacin synthesis accompanying yeast growth during fermentation. Baking losses averaged 0.30 μg, leaving a final concentration near the initial concentration.

Table I, part B shows similar trends for native folacin using the sponge and dough method. The calculated folacin value decreased at the proof stage as low-folacin-content flour, sugar, salt, and shortening were added in composing the

TABLE I
Stability of Natural Folacin during Bread Processing
0.12 μg Total Folacin/g Flour

Treatment	Mean μg Folacin/g Sample, Dry Basis								
	A. Straight Dough Method			B. Sponge Dough Method			C. Mechanical Dough Method		
	Mix[a]	Proof[b]	Bread	Mix[a]	Proof[c]	Bread	Mix[a]	Proof[d]	Bread
Control	0.45	0.85	0.39	0.53	0.58	0.48	0.39	0.42	0.27
Conditioner[e]	0.44	0.76	0.59[f]	0.46	0.64	0.38			0.26
Ascorbic acid	0.44	0.77	0.53	0.49	0.66	0.58			
Potassium bromate	0.46	0.83	0.36	0.51	0.63	0.49	0.37	0.44	0.27
Benzoyl peroxide	0.36[f]	0.57[g]	0.37	0.38	0.44[h]	0.44			
Azodicarbonamide	0.47	0.80	0.53	0.57	0.62	0.43			
Average	0.44	0.76	0.46	0.49	0.59	0.47	0.38	0.43	0.27
Calculated	0.40	0.40	0.40	0.47	0.30	0.30	0.40	0.40	0.40
Standard error treatments	0.028	0.046	0.062	0.042	0.044	0.049	0.009	0.016	0.016

[a]Freshly mixed dough.
[b]Total fermentation and proof time = 235 min.
[c]Total fermentation and proof time = 350 min.
[d]Total fermentation and proof time = 75 min.
[e]Second control.
[f]Student's t test—significantly different from control at $\alpha = 0.10$.
[g]Significantly different from control at $\alpha = 0.01$.
[h]Significantly different from control at $\alpha = 0.05$.

TABLE II
Stability of Added Folacin During Bread Processing
5 μg PteGlu Added/g Flour

Treatment	Mean μg Folacin/g Sample, Dry Basis								
	A. Straight Dough Method			B. Sponge Dough Method			C. Mechanical Dough Method		
	Mix[a]	Proof[b]	Bread	Mix[a]	Proof[c]	Bread	Mix[a]	Proof[d]	Bread
Control	4.9	5.4	4.6	6.8	5.4	4.5	5.0	5.1	4.6
Conditioner[e]	5.6	5.6	5.2	6.8	5.2	4.7			
Ascorbic acid	4.7	5.0	4.4	6.2	5.3	4.2			
Potassium bromate	4.9	4.1	4.1	4.8[f]	4.4[f]	4.2	5.8[h]	5.2	4.3
Benzoyl peroxide	4.0	4.2	3.9	5.2[f]	4.9[g]	4.0			
Azodicarbonamide	4.8	4.7	4.4	5.4[f]	4.9[g]	4.1			
Average	4.8	4.8	4.4	5.9	5.0	4.3	5.4	5.1	4.5
Calculated	5.0	5.0	5.0	5.5	5.0	5.0	4.8	4.8	4.8
Standard error, treatments	0.36	0.42	0.39	0.31	0.20	0.26	0.17	0.20	0.24

[a]Freshly mixed dough.
[b]Total fermentation and proof time = 235 min.
[c]Total fermentation and proof time = 350 min.
[d]Total fermentation and proof time = 75 min.
[e]Second control.
[f]Significantly different from control at $\alpha = 0.01$.
[g]Student's t test—significantly different from control at $\alpha = 0.10$.
[h]Significantly different from control at $\alpha = 0.05$.

dough, diluting the folacin content. However, the measured folacin was double the expected value due to the folacin synthesis by the yeast. An average of 0.12 μg/g was lost in baking leaving final levels 50% higher than expected from ingredient composition.

Using the mechanical dough method, (Table I, part C) only potassium bromate was tested. Although the standard errors were small for this set of breads, there were no significant differences due to treatment. During the short fermentation period, the folacin content of proof samples increased by only 0.05 μg/g from the mix and an average loss of 0.16 μg/g on baking led to contents in the bread significantly below the expected value.

These results indicate the most important determinant of native folacin in bread is the yeast content achieved by initial formulation and fermentation growth. Most losses occur during baking.

Stability of Added Folacin during Bread Processing

Crystalline PteGlu was blended into the flour in a pilot plant "V" blender for several hours. The 5 μg/g level gave more than 1000 particles per g of flour based on microscopic estimation of the size of the largest particles. Flour enrichment levels were confirmed by microbiological assay. These analytical results were used to determine the calculated values in Table II. (Exception: PteGlu was added to the straight-dough breads in solution at the mix.) Because the concentration of commercial folacin was high compared with free native folacin (less than 0.1 μg/g flour), conjugase treatment was omitted and these results reflect stability of added commercial folacin.

Table II, part A gives the folacin content of doughs and breads baked by the straight-dough procedure with 5 μg PteGlu added per g flour. There were no statistically significant differences due to additives. However, dough and bread in which benzoyl peroxide was used tended to have low values at all stages. Flour with 5 μg of PteGlu added per g showed no significant difference before (5.47 μg/g) and 24 hr after (5.52 μg/g) benzoyl peroxide treatment.

Comparison of values at the mix and proof stages showed no apparent increase with fermentation. These samples were not conjugase treated and, therefore, yeast folacin, which is 95% conjugated, was not measured in this assay. Baking resulted in 8% loss from the proof, indicating good stability of added PteGlu.

Table II, part B shows the effect of the sponge and dough method on stability of added folate. Several differences appear to be significant during the sponge stage but are not apparent in the final bread. The calculated folacin drops at the proof stage because additional solids are used in composing the dough. Baking losses averaged 14%, a value not significantly different from the 8% value observed with straight-dough breads.

Table II, part C shows baking losses averaging 12% for the mechanical dough method.

Of the many folacin analogs the oxidized, unconjugated, unsubstituted pteroylglutamic acid was the first to be isolated in pure form and appears most stable (32). This property accounts for the much greater stability of added folacin in bread compared to native folacin.

TOPIC II: ALL-PURPOSE FLOUR STORAGE

Of flours used in the United States, all-purpose or family flour is expected to

have the longest storage history, including time at the mill, wholesaler's warehouse, retail store, and consumer's shelf. Storage may vary from a week to months under the wide range of temperature and humidity conditions encountered throughout the U.S. In contrast, most bread flour is purchased directly from the mills by large bakeries and used in a relatively short time.

The storage study was conducted on a commercial all-purpose flour [12.5% moisture, 10.9% protein (N × 5.7), 0.49% ash, 0.12 μg folacin/g] treated with chlorine (15 g/100 lb) benzoyl peroxide (4 g Novadel/100 lb), barley malt (100 g/100 lb), and enriched to federal standards. The flour was stored in the dark, in 1-qt mason jars at: −10°, 84°, 100°, and 120° F during one year and analyzed for folacin after various periods. Accelerated storage tests at 120° F are frequently part of U.S. purchase specifications. Previous experience with flour has suggested this temperature may not be representative of changes occurring at lower temperatures over longer times. Therefore, 100° F was included as an alternative accelerated test. Storage at summer temperatures is represented by 84° F and −10° F serves as a zero time control and standard.

RESULTS AND DISCUSSION

Stability of Native Folacin during Flour Storage

Figure 1 presents these results expressed as per cent of the −10° F control analyzed at the same time. These data seem to follow an exponential decay of the form

$$R = A + (I-A)e^{-DT}$$

where R is the response expressed as per cent of the −10° F control, A is the asymptote at infinite time, I is the intercept at zero time, D is the decay exponent

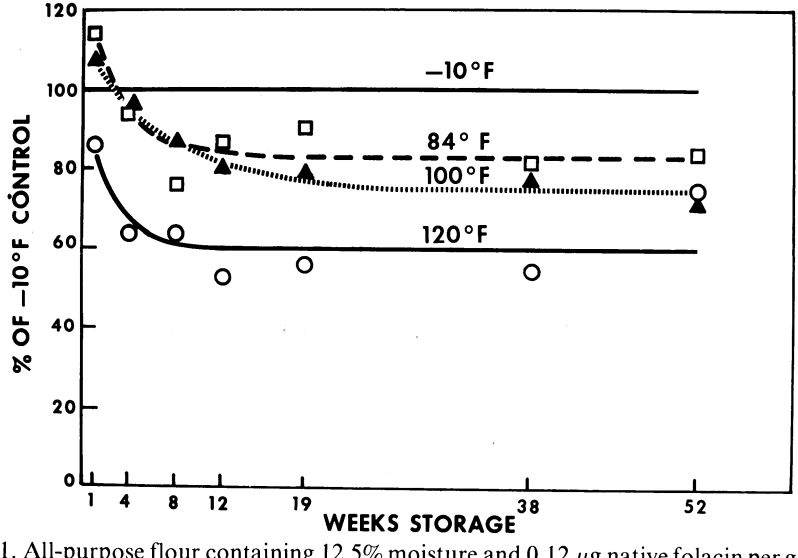

Fig. 1. All-purpose flour containing 12.5% moisture and 0.12 μg native folacin per g flour (dry basis)was stored in glass jars at the temperatures indicated. Folacin was determined at the times indicated and expressed at per cent of the −10° F control flour analyzed at the same time.

per week, and T is time in weeks. Curves of best fit have been drawn through the data using the above model and the constants given in Table III. Within the temperature range observed, the final folacin retention (A) after storage is an indirect function of temperature. The zero storage intercept (I) and decay exponent (D) are greatly determined by the first two time periods and do not follow any trend with temperature. However, the folacin increase of the 84° and 100° F flours at 1 week may be real as bacterial growth or inhibitor destruction could result in these observations. The labile folacin (I-A) appears to have half-life of 2 to 4 weeks based on the range of D values ($T_{1/2} = 0.69/D$). These results are specific for this type of flour at this moisture level. Flours with different water activities would probably give different decay rates and asymptotes.

Stability of Added Folacin during Flour Storage

Commercial folacin (PteGlu) was blended with the all-purpose flour at levels at 1 and 5 μg per g flour. Because the concentration of commercial folacin was high compared with free native folacin (less than 0.1 μg/g flour), conjugase treatment was omitted and these results reflect stability of added commercial folacin. After 52 weeks storage (Table IV) the flours at 84° and 100° F had lost little or no folacin activity when compared to the −10° F control flour. At 120° F the flours supplemented with 1 and 5μg PteGlu/g had lost 17 and 8% of their folacin activity, respectively, indicating great stability for PteGlu in flour.

TABLE III
Curve Parameters for Native Folacin Loss in Flour Stored at Various Temperatures

Temperature °F	84°	100°	120°
A − Asymptote per cent	83	75	56
I − Intercept per cent	126	114	100
D − Decay exponent wk^{-1}	0.32	0.16	0.43
Standard deviation about fitted line per cent	4.8	1.9	7.5

TABLE IV
Effect of 52 Weeks Storage on PteGlu Added to Flour

PteGlu Added μg/g	μg Folacin/g Flour, Dry Basis			
	−10° F	84° F	100° F	120° F
1	1.2	1.1[a]	1.1[a]	1.0[b]
5	5.3	5.4	5.5	4.9[c]

[a]Significantly different from control at $\alpha = 0.05$.
[b]Significantly different from control at $\alpha = 0.01$.
[c]Significantly different from −10° F control by Student's t $\alpha = 0.10$.

CONCLUSIONS

PteGlu appears very stable throughout bread processing (11% average baking loss) compared to native folacin (31% average baking loss). However, folacin synthesis by the yeast during fermentation compensates for the greater baking loss of native folacin, making fermentation time a major factor in determining the final native folacin content of bread. Ascorbic acid, potassium bromate, benzoyl peroxide, or azodicarbonamide showed no significant effect on PteGlu retention or native folacin content in bread.

Native flour folacin decreased early during storage and stabilized at a level dependent on temperature. Added PteGlu showed small losses at high temperature. These data suggest flour and bread are practical vehicles for folacin enrichment of the American diet.

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