Folacin Retention and Cookie Diameter in Enriched Cookies: Regression Analysis Using Factorial Design[1]

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Cereal Chem. 58(3):239-244

ABSTRACT

Folacin retention in cookies baked from flour fortified to National Research Council recommendations (0.07 mg of folic acid per 100 g of flour) is predicted by a least squares regression equation using baking time (7.6-12.7 min), baking temperature (334-432°F), unneutralized soda squared (0.3-35 mmol/100 g of flour), and thickness (5.0-10.5 mm) as the independent variables. The proportion of leavening gas from ammonium bicarbonate (50-90%) and the rest time of the dough had no measurable effect. An average of 85% of the dough folate was retained by the control cookies (time, 9.8 min; temperature, 385°F; excess soda, 3.3 mmol; and thickness, 7.0 mm). Folacin retention may also be predicted by cookie color or cookie pH and soda level. Regression equations for product diameter indicate that at an initial dough thickness of 9.5 mm, diameter can be predicted by time × temperature, soda × temperature, and soda. The presence of the time-temperature interaction enables the model to describe samples that are removed from the oven during the initial spreading phase before the dough has set or later when the cookies are drying and shrinking.

In 1974, the Food and Nutrition Board of the National Academy of Sciences (NAS/NRC 1974) recommended that all products based on the major cereal grains be fortified with additional vitamins and minerals. Folacin was included among these at a recommended fortification level of 0.07 mg of folic acid per 100 g of flour. The board also suggested that studies be conducted on cereal grain products to determine stability of the added nutrient and effects of the additives on product quality. Prior studies examining the retention of folacin in bread fortified with 0.5 mg of folic acid per 100 g of flour showed an average loss of 11% during baking (Keagy et al 1975). Using flour fortified with the recommended levels of vitamins and minerals, Cort et al (1976) found that 92-97% folic acid was retained during baking.

This article presents data and regression equations of folacin retention as a function of six processing variables—baking time, temperature, dough thickness, unneutralized soda, leavening ratio, and dough rest time. A fractional factorial experimental design was chosen to estimate the individual effects of the variables (linear and quadratic) and their interaction effects. Cookies were chosen as the test system because extreme as well as conventional baking conditions could be explored in this product. Prediction equations are presented that quantitatively estimate the effects of the six variables on folacin stability and product diameter. These equations should be useful in optimizing vitamin retention and product quality. Equations predicting thiamin retention, product color, and pH have been described previously (Keagy et al 1979).

MATERIALS AND METHODS

The selection and measurement of variables, experimental design, cookie formulation, baking procedures, and sample preparation have been described previously (Keagy et al 1979), except as noted below.

Vitamin Addition

Folic acid, Vitamin A palmitate, and pyridoxine HCl were blended into increasingly larger amounts of enriched, unbleached commercial cake and pastry flour to form a premix, then blended with the majority of the flour in a "V" blender to reach final concentrations of: folic acid, 0.3 mg/lb; Vitamin A palmitate (retinol equivalent), 2.2 mg/lb; and pyridoxine HCl, 2.0 mg/lb.

[1]Presented in part at the AACC Milling and Baking Division Spring Technical Conference, Manhattan, KS, 1978.

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Folacin Extraction and Assay

Two grams of fat-free samples were mixed 5 min with 45 ml of 0.1 *M* phosphate buffer, pH 6.1, containing 5 mg of ascorbic acid per milliliter to protect folacin from oxidative destruction, then steamed 30 min to extract folacin into the solution and precipitate proteins. Samples were made up to a total weight of 50 g, frozen overnight, and thawed to break the starch gel. They were filtered and the first few milliliters of filtrate discarded to avoid errors due to adsorption of vitamin on the filter paper. Aliquots of the filtrate were diluted to an appropriate concentration for assay with 0.05 *M* phosphate buffer, pH 6.1, containing 1.5 mg of ascorbic acid per milliliter. No conjugase treatment was performed during the extraction because 98% of the total folacin present was in the free form. Eighty percent of natural flour folacin (Butterfield and Calloway 1972) and all added pteroylglutamic acid was available to the test bacteria.

The microbiological assay of folacin was performed as described by Tamura et al (1972), using *Lactobacillus casei* (ATCC 7469) and 1.5 mg of ascorbic acid per milliliter in the buffer. Calculations used the parallel curve assay of Schatzki and Keagy (1975). Up to five replicates were determined on each sample.

Lighting Conditions

Folacin extraction and assay were performed using lighting designed to minimize photodegradation of folacin. Approximately 4 fc of working light were supplied by General Electric red fluorescent lights supplemented by daylight through two 1½ × 1-ft windows covered with amber film.

Measurements of Diameter

Cookie diameter was determined on four to six cookies, according to AACC method 10-50B (1976).

RESULTS AND DISCUSSION

The values of the six independent baking variables and resulting folacin retention, color, pH, and diameter for each sample are given in Table I. Transformations used to linearize and center the data for least squares fitting of the prediction equations are given in Table II, which also gives the values of the theoretical −2, −1, +1, and +2 levels of each variable. The cookies are shown in Fig. 1.

Folacin Retention

Folacin retention ranged from 93% in a very underbaked cookie (Sample 1) to 50% in one appearing almost burned (Sample 16). The center point cookies (Samples 17-20 and 34) retained an average of 85% of the dough folate. Table III lists the regression coefficients and mean squares of the baking variables that are significant at $P = 0.05$ or better in the regression equation predicting folacin retention. Less significant terms have been

combined with the error in the residual.

Baking time, baking temperature, and soda squared are significant at $P = 0.01$ and thickness at $P = 0.05$. Changing the amount of ammonium bicarbonate or dough rest time had no measurable effect within the range of this experiment. No interactions were found significant for folacin retention. Predicted values of folacin may be calculated by substituting the regression coefficients from Table III and the transformed, coded values (Table II) of the independent variables into the model:

$$\text{lnln}\,(C_0/C) = -1.89 + 0.16\,X_1 + 0.47\,X_2 + 0.18\,X_3 + 0.15\,X_4^2$$

where C_0 is the initial vitamin concentration (dough value) and C is the final vitamin concentration (sample value). We assumed that the kinetics of the thermal destruction of folacin under normal baking conditions of excess oxygen would follow pseudo first order reaction kinetics; thus, the retention of folacin is described in terms of lnln (C_0/C). A detailed derivation of the transform is described in Keagy et al (1979).

The positive coefficients indicate that as the design levels of the variables increase (ie, bake time, bake temperature, and excess soda squared increase and thickness decreases), lnln (C_0/C) increases and percent retention of folacin decreases.

The coefficient of determination (R^2) gives the proportion of the variation accounted for by the model, here 65%. This relatively low R^2 results from the variations of the folacin assay and baking conditions relative to the small range of folacin losses. No lack of fit is indicated ($F = 1.33$).

Time and Temperature Effects

If thickness and soda are held constant at the control levels, folacin retention becomes a function of time and temperature, as seen in Fig. 2. The data has been plotted in degrees Fahrenheit, minutes, and percent folacin retention for ready interpretation.

TABLE I
Values of Untransformed Variables

Sample Number	X_1 Thickness (mm)	X_2 Time (min)	X_3 Temperature (°F)	X_4 Excess Soda (mmole)	X_5 NH_4HCO_3 (mmole)	X_6 Rest Time (min)	Y_1 Folacin Percent Retained (100 C/C_0)	Y_2 Color (Absorbance)	Y_3 pH	Y_4 Diameter (cm)
1	9.50	7.6	359	1.0	12.8	43	93.3	0.2409	7.5	8.84
2	5.56	8.0	388	1.0	7.10	19	86.5	0.2771	8.0	7.92
3	9.50	12.5	360	1.0	7.10	19	84.9	0.3658	7.6	9.28
4	5.56	11.9	364	1.0	12.8	43	80.1	0.5330	6.7	7.82
5	9.50	8.3	404	1.0	7.10	43	83.8	0.3785	7.7	8.93
6	5.56	8.1	410	1.0	12.8	19	73.8	0.4543	7.0	7.91
7	9.50	12.5	414	1.0	12.8	19	75.1	0.6804	6.6	8.60
8	5.56	12.7	408	1.0	7.10	43	54.4	0.9069	6.5	7.68
9	9.50	7.9	361	10.7	12.8	19	90.8	0.2741	9.1	8.51
10	5.56	8.3	398	10.7	7.10	43	91.9	0.3180	8.7	7.92
11	9.50	12.6	360	10.7	7.10	43	80.3	0.4665	8.0	8.72
12	5.56	12.1	374	10.7	12.8	19	76.0	0.5839	7.4	7.58
13	9.50	7.9	413	10.7	7.10	19	90.2	0.3689	8.4	8.94
14	5.56	8.1	396	10.7	12.8	43	82.0	0.4987	8.0	7.92
15	9.50	12.5	413	10.7	12.8	43	75.9	0.6942	7.4	8.75
16	5.56	12.3	415	10.7	7.10	19	49.7	0.8608	6.7	7.61
17	6.97	9.8	399	3.27	9.94	31	86.8	0.4186	7.6	8.11
18	6.97	9.5	393	3.27	9.94	31	85.0	0.3977	7.7	8.24
19	6.97	10.2	377	3.27	9.94	31	85.9	0.4593	7.5	8.14
20[a]	6.97	...	...	3.27	9.94	31	77.4	0.4547	7.8	8.07
21	5.01	9.6	384	3.27	9.94	31	87.3	0.5227	7.4	7.25
22	10.66	10.0	387	3.27	9.94	31	87.7	0.3433	8.0	11.46
23	6.97	9.8	379	0.30	9.94	31	89.3	0.4923	7.0	8.22
24	6.97	9.2	369	35.2	9.94	31	68.8	0.5870	7.9	7.35
25	6.97	9.8	373	3.27	9.94	65	80.3	0.4791	7.5	8.38
26	6.97	10.0	369	3.27	9.94	12	90.8	0.4031	7.6	8.09
27	6.97	10.0	385	3.27	9.94	65	88.8	0.4455	7.5	8.16
28	6.97	8.3	378	3.27	9.94	31	91.9	0.3304	7.9	8.45
29	6.97	7.9	359	3.27	9.94	31	89.7	0.3556	7.5	8.40
30	6.97	12.2	383	3.27	9.94	31	69.6	0.6073	7.0	8.33
31	6.97	9.8	334	3.27	9.94	31	90.8	0.3070	7.9	8.06
32	6.97	9.9	409	3.27	9.94	31	78.8	0.5907	7.1	8.58
33	6.97	9.4	432	3.27	9.94	31	85.3	0.6291	7.1	8.72
34	6.97	9.5	372	3.27	9.94	31	90.9	0.3678	7.8	8.38
35	6.97	8.1	386	35.2	9.94	31	79.5	0.4182	10.0	7.54

[a] Sample 20 was only used to calculate the variability of replicate bakes due to missing time and temperature.

TABLE II
Transformations and Levels of Independent Variables

Symbol	Variable	Transformation	Levels				
			−2	−1	0	+1	+2
X_1	Thickness, mm	−8 lnln mm + 5.36	12.3	9.2	7	5.6	4.6
X_2	Baking time, min	4.34 ln min − 9.88	...	7.7	9.7	12.2	...
X_3	Temperature, °F	$-2.08 \times 10^4\ °K^{-1} + 44.4$	348	366	385	404	425
X_4	Soda excess, mmole	0.84 ln mmoles −1	0.30	1	3.29	10.8	35.5
X_5	Ammonium bicarbonate, mmole	−0.352 mmoles + 3.50	...	12.78	9.94	7.10	...
X_6	Rest time, min	−0.0833 min + 2.58	55	43	31	19	7
X_7	Color (absorbance)	6.37A − 3.01					
X_8	pH	0.83 pH − 6.33					

Ninety percent of the dough folate is retained by baking 8 min at 389° F, 10 min at 343° F, or 12 min at 308° F. From the 8-min 90% retention point, an additional 10% decrease in retention results if the cookie is baked 3.7 min longer or at a 90°-higher temperature.

The control samples averaged 15% folacin loss during processing. The flour used in this study contains 13% naturally occurring free folacin as measured by *L. casei*. Under normal baking conditions, losses may be primarily the result of destruction of the thermally labile natural forms. The principal folates naturally occurring in plants are 5-CH_3-H_4 folic acid and its derivatives (Scott and Weir 1976). Paine-Wilson and Chen (1979) showed that destruction of 5-CH_3-H_4 folic acid at 212° F occurs very rapidly and follows first order kinetics at all pH levels, losing 50% activity in about 10 min at pH 7 and 8 and losing more rapidly at most other pH levels. Folic acid (the form of the vitamin used for enrichment) was shown to be relatively stable at 212° F over the pH range 5–12. As the sample was subjected to extremes of heating, losses of even the stable folic acid appeared to occur. In the two samples (8 and 16) that were baked at such times and temperatures to cause burned and blackened cookies, losses averaged 48%.

Paine-Wilson and Chen (1979) and Dick (1948) showed that destruction of folic acid followed first order reaction kinetics. Garrett (1956) found it to be zero order, then first order. A coefficient of 1 for the ln time term, X_2, is consistent with first order kinetics. However, an experimental value of 2.04 (0.47 × 4.34) was obtained. Because the temperature was not constant with time, as assumed by the first order reaction equation, the latter is not truly applicable and a higher power for time is to be expected.

As explained by Keagy et al (1979), as the oven temperature setting is increased, the oven temperature profile increases more than the cookie temperature profile; therefore the regression should predict a lower observed "activation energy" (E_a) than that derived from the Arrhenius equation. Garrett (1956) calculated E_a for folic acid at pH 3.2 to be 16.8 kcal/mole. Chen and Cooper (1979) calculated the E_a for 5-CH_3-H_4 folic acid to be 9.5 kcal/mole. The regression coefficient for 1/T corresponds to an observed E_a of 7 kcal/mole.

Direct comparison of this coefficient with the previously studied activation energies is invalid because of the continual change of cookie temperature with time during baking and the measurement of oven temperature rather than cookie temperature. However, both the form and order of magnitude of the temperature relationship in this nonideal system are consistent with previous kinetic studies under more ideal conditions.

Effect of Excess Soda

Folic acid has good thermal stability over the pH range 5–12 (Paine-Wilson and Chen 1979); thus one would expect folacin retention to be little affected by excess soda. This is verified by the regression equation. Excess soda (linear) does not come into the regression equation at all. Excess soda squared is significant at $P=0.05$. However, this term arises solely from the +2 level of soda in the design, in which 27 ml of 1 *N* NaOH was added to 100 g of flour to achieve the design level. Even a quadratic equation is unable to fit the data from the −2 to the +2 levels of excess soda. When the range of fit is restricted to that from the −1 to the +2 levels of excess soda, the R^2 increases to 0.75, indicating that the model fits the data better. The regression equation becomes:

$$\ln\ln (C_0/C) = -1.93 + 0.17\ X_1 + 0.48\ X_2 + 0.17\ X_3 + 0.25\ X_4.$$

If one calculates percent retention as a function of soda squared, holding the other variables in the regression equation at the control level, the maximum retention, 86%, is at the 0 level of soda (3.29 mmoles of soda, 0.28 g of excess soda). At the +1 level, percent retention decreases 3.5% from the control level and at the +2 level, it decreases 19% from the control level. In contrast, 30% more thiamin is lost as the excess soda is raised from the 0 to the +1 level and 65% at the +2 level.

TABLE III
Folacin Retention [lnln (C_0/C)] Predicted by Processing Variables: Analysis of Variance for Regression

Source of Variation	Coefficient	Mean Square	Degrees of Freedom
Regression		1.67	4
Constant	−1.89		
Bake time, X_2	0.47	3.80[a]	1
Bake temperature, X_3	0.18	1.34[a]	1
Soda × soda, X_4^2	0.15	0.95[a]	1
Thickness, X_1	0.16	0.60[b]	1
Residual		0.126	29
Lack of fit		0.133	23
Error		0.099	6
Corrected total		0.313	33
$R^2 = 0.65$			

[a] $P = 0.01$.
[b] $P = 0.05$.

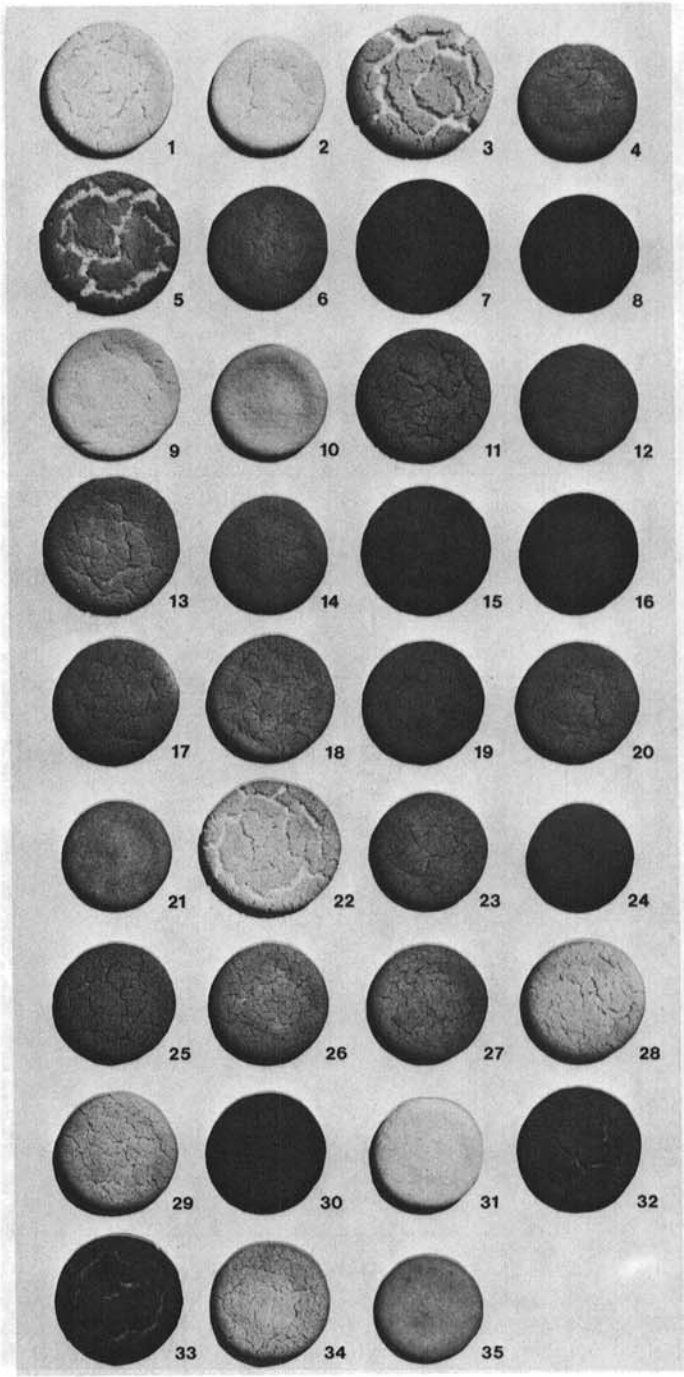

Fig. 1. Cookie samples described in Table I.

Folacin Retention Predicted by Color

Cookie color (absorbance) is a positive function of the transformed bake time, temperature, thickness, time × temperature, and soda squared ($P = 0.01$; $R^2 = 0.87$), as shown by Keagy et al (1979). If transformed color is included as a variable in the regression predicting folacin, it replaces all the other terms and improves predictive value, as indicated by the larger R^2 (Table IV). Thus, at $P < 0.01$, $\ln\ln (C_0/C) = -1.76 + 0.048\ X_7$ (color absorbance, transformed). The positive coefficient indicates that as color (absorbance) increases (that is, more browning occurs), $\ln\ln (C_0/C)$ increases and percent retention of folacin decreases. Figure 3 shows a plot of percent retention vs color (absorbance). The regression line has been fitted through the actual sample values showing the distribution about the regression line. Of the variables considered, cookie color is the best indicator of folacin losses.

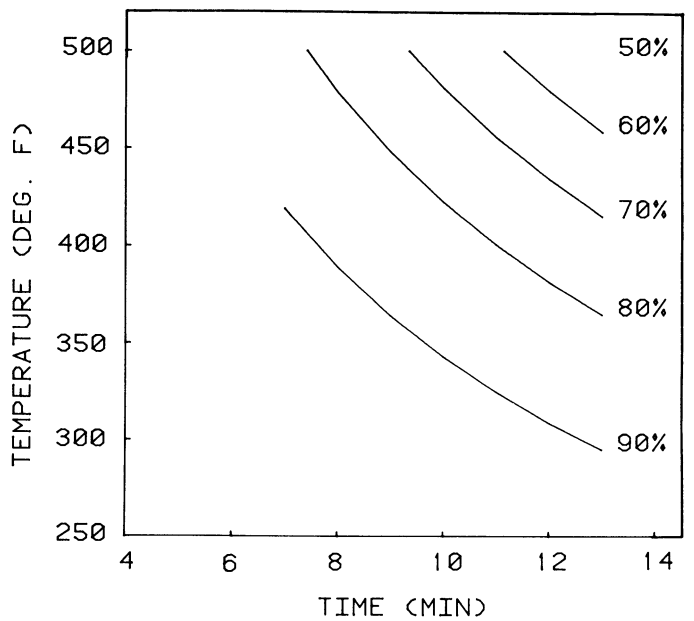

Fig. 2. Prediction curves of percent folacin retention as a function of time and temperature, with other variables at control levels.

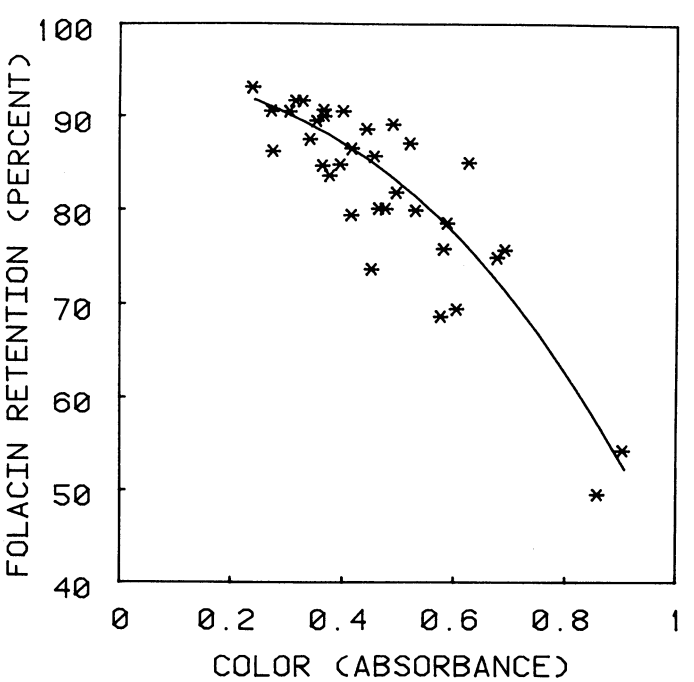

Fig. 3. Prediction curve of percent folacin retention as a function of color (absorbance). Experimental values show the distribution about the regression line.

Increased color also limits acceptability and hence should limit the impact of potential folacin losses.

Folacin Retention as a Function of Product pH

Cookie pH is predicted by the heat-related terms of time, temperature, and thickness in addition to excess soda (Keagy et al 1979). When pH is introduced into the regression equation predicting folacin retention (Table V), pH replaces the temperature-dependent terms, leaving pH (linear and squared terms) and excess soda in the equation. The R^2 is 0.66. This means that at a constant soda level, product pH predicts folacin retention as well as the processing variables studied do.

Product Diameter

Automated packaging equipment and embossed designs on cookies require manufacturers to control cookie spread. This is most frequently accomplished by using high levels of soda in the formulation. Alternative means of controlling spread might permit a reduction in soda content and an increase in thiamin retention. Product diameter was investigated as the component of cookie spread most affected by the variables investigated.

Samples 20 and 21 (extremely thick and thin cookies, respectively) were not included in the data set because they contributed large residuals even when thickness squared was included in the regression. Fitting the remaining 32 data sets resulted in a regression equation containing thickness, soda, soda squared, and time × temperature terms significant at $P < 0.01$ and temperature × soda and temperature terms significant at $P < 0.05$. Examination of the residuals indicated that the thick cookies (9.5 mm rolled thickness) were primarily responsible for the significant terms and that the thin (5.01 mm rolled thickness) cookies had very little influence. Separate regressions were then computed for the thick and thin cookies because a single regression did not adequately describe the response surface over the entire thickness range. The eight thick and thin cookies each form a complete 2^3 factorial design in time, temperature, and soda if the leavening and

TABLE IV
Folacin Retention [$\ln\ln (C_0/C)$] Predicted by Color: Analysis of Variance for Regression

Source of Variation	Coefficient	Mean Square	Degrees of Freedom
Regression		7.55	1
Constant	−1.76		
Color, X_7	0.048	7.55[a]	1
Residual		0.087	32
Lack of fit		0.084	26
Error		0.099	6
Corrected total $R^2 = 0.73$		0.313	33

[a] $P < 0.01$.

TABLE V
Folacin Retention [$\ln\ln (C_0/C)$] Predicted by pH: Analysis of Variance for Regression

Source of Variation	Coefficient	Mean Square	Degrees of Freedom
Regression		2.28	3
Constant	−1.90		
pH, X_8	−1.07	6.52[a]	1
Excess soda, X_4	0.36	2.38[a]	1
pH × pH, X_8^2	0.44	2.28[a]	1
Residual		0.117	30
Lack of fit		0.121	24
Error		0.099	6
Corrected total $R^2 = 0.66$		0.313	33

[a] $P = 0.01$.

rest variables are unimportant.

The reduced regression model follows:

$$Y = B_0 + B_2X_2 + B_3X_3 + B_4X_4 + B_{2,3}X_2X_3 + B_{2,4}X_2X_4 + B_{3,4}X_3X_4 + B_{2,3,4}X_2X_3X_4$$

where Y is the cookie diameter and X_2, X_3, and X_4 are baking time, temperature, and soda excess, respectively.

A new confounding pattern is observed if a constant thickness value of ±1 is substituted for X_1 in the interaction terms of the original model. Splitting the original design confounds the time × temperature interaction with the effect of leavening, the soda × temperature term with leavening × rest, and the soda term with time × rest. The regression results for the thick cookies are presented in Table VI.

Rest and its interactions did not appear significant when available data from the second bake from each batch were included in the data or when all 32 data sets were fit together. Therefore, we assume that the leavening × rest and time × rest terms do not affect the soda × temperature and soda terms. Increased amounts of ammonium bicarbonate have been shown to increase cookie spread (Finney et al 1950). The time × temperature constant is negative despite its being confounded with the leavening constant, which would be expected to be positive. In addition, the leavening term did not appear significant when the combined data was fit. Therefore, the leavening effect must be small relative to the time × temperature effect and has been assumed negligible.

The response surface of cookie diameter as a function of time and temperature at three soda levels is shown in Fig. 4. The surfaces are curved because these are the actual baking times and temperatures rather than the transformed linearized times and temperatures. Figure 4B shows the surface at the zero soda level. The surface appears as a saddle with the seat in the center of the time and temperature ranges. Baking a long time at a low temperature probably delays setting the dough structure and allows more time for the dough to flow. A high temperature, short time bake also increases cookie diameter. The high temperature probably decreases the dough viscosity and allows the dough to spread more rapidly before the dough sets. A short time, low temperature bake reduces cookie diameter but also produces an underbaked cookie, which has probably not stopped flowing. A long time, high temperature bake yields a cookie with smaller diameter, overdone, and bordering on unacceptable. The reduced diameter appears to be due to shrinking when all free water is lost and browning increases rapidly. The effect of soda is illustrated in Fig. 4A and C. Figure 4C shows the response surface at higher soda level (+1), comparable to that of commercial cookies. The average cookie diameter is smaller and the seat of the saddle is displaced with respect to time. Thus, a long time, low temperature bake increases the diameter very little, whereas a high temperature, short bake still allows the diameter to expand. Figure 4A shows the response surface with very little soda (−1 level) in the formulation. The average diameter is larger and the seat of the saddle is moved toward shorter bake times.

When the effect of time, temperature, and soda was examined in the thin cookies, time was the only variable significant at $P<0.01$. Its correlation with cookie diameter was −0.89. The thinner cookies baked more rapidly at a given temperature and all reached their maximum diameter. The time factor indicates shrinking as the cookies became overbaked.

Careful examination of Fig. 4A–C reveals that adjustment of time and temperature cannot entirely compensate for changes in the soda level of the formulation and still produce an optimally baked product. Although folacin retention is little affected by soda, the retention of other nutrients, especially thiamin, is profoundly affected by soda level. Reducing the excess soda level from the +1 level, which yields 35% thiamin retention (Keagy et al 1979; predictions used time = 0, temp = 0, thickness = +1), to the −1 level increases thiamin retention to 72% but increases the cookie diameter by only 2.5%.

SUMMARY

Folacin retention in cookies baked with fortified flour is predicted by bake time, temperature, unneutralized soda squared, and dough thickness. If the color is introduced into the regression equation, it replaces all the independent variables and improves prediction. Introduction of product pH (linear and square terms) into the equation results in replacement of all but unneutralized excess soda. Regression equations are presented showing the effect of bake time, temperature, and excess soda on product diameter.

TABLE VI
Diameter of Thick Cookies Predicted by Processing Variables: Analysis of Variance for Regression

Source of Variation	Coefficient	Mean Square	Degrees of Freedom
Regression		0.127	3
Constant	8.83		
Time × Temperature, $X_2 \times X_3$	−0.11	0.165[a]	1
Soda × Temperature, $X_4 \times X_3$	0.10	0.133[a]	1
Soda, X_4	−0.11	0.086[a]	1
Residual		4.5×10^{-3}	4
Corrected total $R^2 = 0.955$		0.0568	7

[a] $P = 0.05$ using $S^2 = 0.0136$, df = 7, estimated from center points (Samples 17–20 and 34) and three sets of duplicates (Samples 25, 27; 28, 29; and 32, 33).

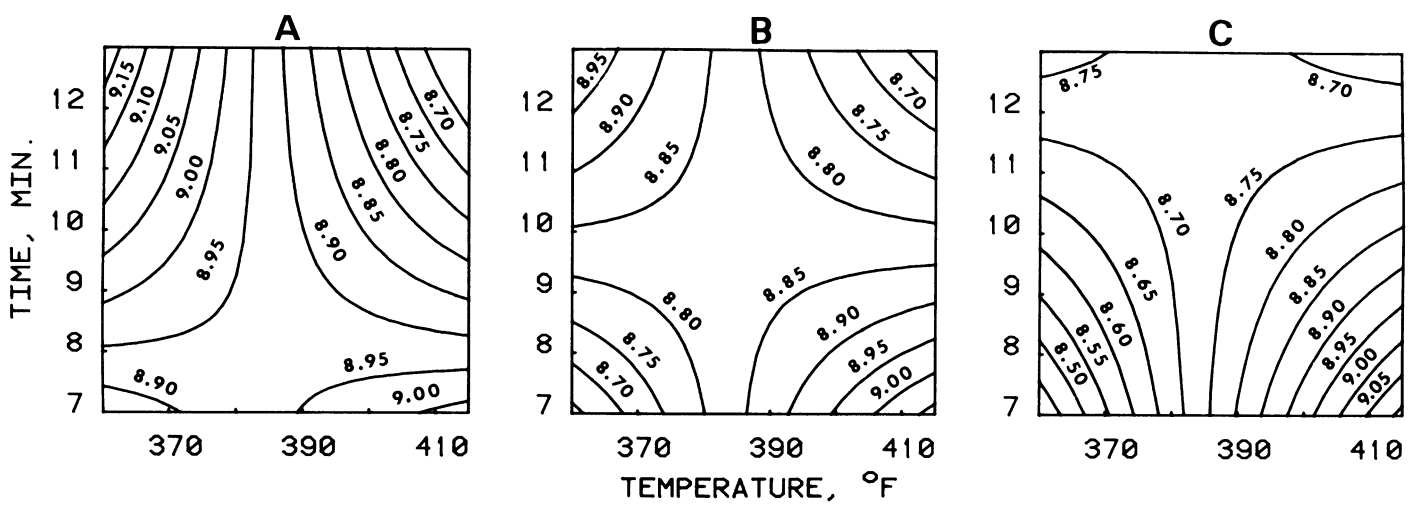

Fig. 4. Predicted diameter (cm) of thick cookies (−1 level) as a function of time and temperature with three levels of excess soda: **A**, −1; **B**, 0; **C**, +1.

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[Received July 23, 1980. Accepted November 21, 1981]