Regression Analysis of Thiamin and Color Changes in Enriched Cookies Using Factorial Design[1]

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ABSTRACT

Thiamin losses (12–94%) in cookies baked from commercially enriched flour are predicted by a least squares regression equation using baking temperature (334–432°F), baking time (7.6–12.7 min), unneutralized soda (0.3–35 mmol/100 g of flour), and thickness (5.0–10.5 mm) as the major independent variables. Rest time of the dough (12–65 min) and the proportion of leavening gas from ammonium bicarbonate (50–90%) had no measurable effect. Cookie color and pH are also predicted by soda, time, temperature, and thickness. At a constant soda level, cookie color may be used as a quality control guide to thiamin loss. An augmented 2^{6-2} fractional factorial experimental design of the Box-Wilson type was used to determine the combination of variables. Standard vitamin extraction methods were modified slightly to allow microbiological assay of niacin and of thiamin on aliquots of one extract. Average losses in the control cookies (temperature: 385°F; time: 9.8 min; excess soda: 3.3 mmol; and thickness: 7.0 mm) were 30% thiamin and 4.5% niacin.

In 1973, the FDA increased the levels of thiamin, riboflavin, and niacin required in enriched flour and bread (Federal Register 1973). In addition, some states have adopted universal enrichment laws mandating enriched flour in all products containing more than 25% flour (California Health and Safety Code 1971). This has expanded the range of enriched cereal products in the marketplace. The philosophy and history underlying these actions has been reviewed (Miller 1977, NAS/NRC 1974).

For enrichment to be successful, the added nutrient must be stable under proper conditions of storage and use (NAS/NRC 1974). Data on vitamin stability in baked goods other than bread and, in particular in chemically leavened products such as cookies and crackers, is sparse.

This article presents data and regression equations relating vitamin retention in cookies to baking time, temperature, product thickness, leavening system, dough pH, and dough rest time. Cookies were selected as a test system representing extreme temperature and pH conditions in a food product. We hope this information will form a bridge between knowledge of nutrient behavior in model systems and empirical results from a variety of baked products. The results should be especially useful in product development to optimize product quality and nutrient content.

MATERIALS AND METHODS

Experimental Design

The selection of the variables was based on the fact that temperature and pH affect thiamin stability. Temperature response during baking and cooling is governed by oven temperature, baking time, and dough thickness. In preliminary experiments, temperature profiles were slightly higher during baking of aged cookie dough than during baking of fresh dough; rest time was therefore included as a variable that might affect cookie temperature.

Dough ingredients determine dough pH; in our cookie formula, theory suggested that ammonium bicarbonate and excess soda from sodium bicarbonate, components of the leavening system, would have the greatest influence on dough pH. These ingredients were varied according to design levels, but total leavening gas available from these sources was kept constant (see Appendix). The values of the six independent variables in each sample are shown in Table I.

A 2^{6-2} fractional factorial experimental design (Cochran and Cox 1957) of the Box-Wilson type was used to estimate the coefficients of the six variables and their interactions (Table I, samples 1–16), augmented by four center points to estimate variance and test nonlinearity (samples 17–20), and additional samples to estimate quadratic terms after nonlinearity was revealed (samples 21–35). Variables were transformed (Table II) to minimize nonlinear and first order interaction terms and to center the data for least squares fitting of the model.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_5X_5 + B_6X_6$$
$$+ C_1\frac{(X_1X_2 + X_3X_5 + X_4X_6)}{3} + C_2\frac{(X_1X_3 + X_2X_5)}{2}$$
$$+ C_3\frac{(X_1X_4 + X_2X_6)}{2} + C_4\frac{(X_1X_5 + X_2X_3)}{2}$$
$$+ C_5\frac{(X_1X_6 + X_2X_4)}{2} + C_6\frac{(X_3X_4 + X_5X_6)}{2}$$
$$+ C_7\frac{(X_3X_6 + X_4X_5)}{2} + B_{11}X_1^2 + B_{22}X_2^2$$
$$+ B_{33}X_3^2 + B_{44}X_4^2 + B_{66}X_6^2$$

where Y is the value of the dependent variable, X_i is the value of the independent variable, and B_i, C_i, and B_{ii} are the regression coefficients. The theoretical bases of the transformations are given in Appendix I. Because the model is a 2^{6-2} factorial, some two-way (X_iX_j) and higher order interactions are confounded; ie, terms such as $C_1 = (B_{12} + B_{35} + B_{46})$ appear. If C_i is significant, this will result in uncertainty as to which interactions are responsible. The variable assignments were made such that all but one of the confounding interactions could be neglected, based on theoretical considerations; additionally, small cross terms were expected when corresponding linear terms were small. The method of least squares was used to obtain the best estimate of the dependent variables. All terms not significant at the 0.05 probability level were combined with the residual. Random error was estimated from replicate bakes (five centerpoints, samples 17–20 and 34; and three sets of duplicates, samples 25 and 27, 28 and 29, and 32 and 33). Lack of fit was calculated as the difference between the residual sum of squares and the pure error sum of squares. Marginal lack of fit was tolerated where omitted variables were not significant at $P \leq 0.05$.

Cookie Formulation and Baking

The cookies were baked using enriched, unbleached commercial cake and pastry flour (12.2% moisture, 9.8% protein [N × 5.7], 0.58% ash, 1.8 mg/lb of riboflavin, 3.0 mg/lb of thiamin mononitrate, and 27.6 mg/lb of niacin). Additional vitamins were blended into the flour before baking: 0.3 mg/lb of folic acid, 2.2 mg/lb of vitamin A palmitate (retinol equivalent), and 2.0 mg/lb of pyridoxine HCl.

[1] Presented in part at the AACC 61st Annual Meeting, New Orleans, October 1976.
[2] Reference to a company or product name is for purposes of information only and does not imply approval or recommendation by the USDA to the exclusion of others that may also be suitable.

The cookies were baked from the modified commercial sugar cookie formula shown in Table III. Soda, acid salts, and ammonium bicarbonate were adjusted as necessary for the experimental design. Total potential leavening gas (CO_2 plus NH_3) was kept constant at 28.4 mmol/100 g of flour. The amounts of NH_4HCO_3 were adjusted so that 50, 70, and 90% of the potential leavening gas originated from NH_4HCO_3. The mixing procedure was adapted from Micro Method II (Finney et al 1950) as follows: the sugar, salt, nonfat dry milk, and eggs were sifted together eight times. The shortening, invert sugar syrup, and ethyl vanillin were added and the mixture stirred 2 min each at low, medium, and high speeds with a Hobart C-100 mixer. Appropriate amounts of ammonium bicarbonate and sodium bicarbonate were dissolved in separate portions of water, added to a portion of cream mass, and mixed at medium speed for 1 min with a Hobart N-50 mixer. The acid salts were stirred into the flour, and this mixture was added to the cream mass and mixed 2 min at low speed. Rolling, cutting, and baking were according to AACC method 10-50D (1976). Thermocouples were placed in three cookies during each bake, and two batches of cookies were baked from each dough.

Representative cookie and oven temperature curves are shown by the solid curves in Fig. 1. The "set" temperature was 400°F. Oven temperatures were determined as the time-temperature average of the period between the initial and ending temperature drops. Bake times were determined from the initial cookie temperature rise to the initial temperature drop. Thickness was determined by milled steel rolling gauges. Rest time was defined from the end of mixing to the beginning of baking. Final cookie pH was determined using AACC method 02-52. A Colormaster V with green broadband filter and white vitrolite tile standard was used to determine color reflectance of 20 g of cookie crumbs, ground to pass a 20-mesh screen. Two measurements at 90° angles were taken.

TABLE I
Values of Untransformed Variables

	Independent Variables						Dependent Variables		
Sample No.	X_1 Thickness (mm)	X_2 Time (min)	X_3 Temp (°F)	X_4 Excess Soda (mmol)	X_5 NH_4HCO_3 (mmol)	X_6 Rest Time (min)	Y_1 Thiamin % Retained (100 C/C₀)	Y_2 Color (Absorbance)	Y_3 pH
1	9.50	7.6	359	1.0	12.8	43	88.5	0.2409	7.5
2	5.56	8.0	388	1.0	7.10	19	87.5	0.2771	8.0
3	9.50	12.5	360	1.0	7.10	19	76.7	0.3658	7.6
4	5.56	11.9	364	1.0	12.8	43	60.4	0.5330	6.7
5	9.50	8.3	404	1.0	7.10	43	73.0	0.3785	7.7
6	5.56	8.1	410	1.0	12.8	19	74.3	0.4543	7.0
7	9.50	12.5	414	1.0	12.8	19	52.7	0.6804	6.6
8	5.56	12.7	408	1.0	7.10	43	22.9	0.9069	6.5
9	9.50	7.9	361	10.7	12.8	19	61.5	0.2741	9.1
10	5.56	8.3	398	10.7	7.10	43	49.3	0.3180	8.7
11	9.50	12.6	360	10.7	7.10	43	33.5	0.4665	8.0
12	5.56	12.1	374	10.7	12.8	19	27.5	0.5839	7.4
13	9.50	7.9	413	10.7	7.10	19	41.7	0.3689	8.4
14	5.56	8.1	396	10.7	12.8	43	31.3	0.4987	8.0
15	9.50	12.5	413	10.7	12.8	43	19.2	0.6942	7.4
16	5.56	12.3	415	10.7	7.10	19	11.3	0.8608	6.7
17	6.97	9.8	399	3.27	9.94	31	70.4	0.4186	7.6
18	6.97	9.5	393	3.27	9.94	31	71.6	0.3977	7.7
19	6.97	10.2	377	3.27	9.94	31	67.8	0.4593	7.5
20[a]	6.97	...	...	3.27	9.94	31	66.9	0.4547	7.8
21	5.01	9.6	384	3.27	9.94	31	63.6	0.5227	7.4
22	10.66	10.0	387	3.27	9.94	31	75.9	0.3433	8.0
23	6.97	9.8	379	0.30	9.94	31	75.2	0.4923	7.0
24	6.97	9.2	369	35.2	9.94	31	6.0	0.5870	7.9
25	6.97	9.8	373	3.27	9.94	65	63.6	0.4791	7.5
26	6.97	10.0	369	3.27	9.94	12	73.5	0.4031	7.6
27	6.97	10.0	385	3.27	9.94	65	71.6	0.4455	7.5
28	6.97	8.3	378	3.27	9.94	31	75.0	0.3304	7.9
29	6.97	7.9	359	3.27	9.94	31	81.9	0.3556	7.5
30	6.97	12.2	383	3.27	9.94	31	56.7	0.6073	7.0
31	6.97	9.8	334	3.27	9.94	31	81.0	0.3070	7.9
32	6.97	9.9	409	3.27	9.94	31	53.6	0.5907	7.1
33	6.97	9.4	432	3.27	9.94	31	49.8	0.6291	7.1
34	6.97	9.5	372	3.27	9.94	31	75.0	0.3678	7.8
35	6.97	8.1	386	35.2	9.94	31	7.8	0.4182	10.0

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

TABLE II
Transformations and Levels of Independent Variables

			Levels				
Symbol	Variable	Transformation	−2	−1	0	+1	+2
X_1	Thickness	$-8 \ln 1n \text{ mm} + 5.36$	12.3 mm	9.2 mm	7 mm	5.6 mm	4.6 mm
X_2	Baking time	$4.34 \ln \text{ min} - 9.88$	...	7.7 min	9.7 min	12.2 min	...
X_3	Temperature	$-2.08 \times 10^4 \, {}^0K^{-1} + 44.4$	348°F	366°F	385°F	404°F	425°F
X_4	Soda excess	$0.84 \ln \text{ mmol} - 1$	0.30 mmol	1 mmol	3.29 mmol	10.8 mmol	35.5 mmol
X_5	Ammonium bicarbonate	$-.352 \text{ mmol} + 3.50$	...	12.78 mmol	9.94 mmol	7.10 mmol	...
X_6	Rest time	$-0.0833 \text{ min} + 2.58$	55 min	43 min	31 min	19 min	7 min
X_7	Color (absorbance)	$6.37A - 3.01$					

Vitamin Extraction and Assay

An extraction procedure was developed to permit determination of thiamin and niacin on a single extract. Comparison of this single extraction procedure with AACC standard methods 86-80 and 86-51 (1976), using unenriched, enriched, and fortified flour as test samples, gave equivalent results at $P = 0.3$ or less for thiamin and free (unbound) niacin. Subsequent treatment of the extract was necessary to hydrolyze bound niacin in the extract and make it available to the microbiological assay organism. Comparison of the single extraction method plus second hydrolysis with the AACC method gave equivalent results for total niacin in unenriched flour.

Before extraction, the cookies were ground to 12 mesh and treated 4 hr with petroleum ether (Skelly F) in a Soxhlet flask to remove fat. Five grams of fat-free samples were mixed with 80 ml of 0.1*N* H_2SO_4 and heated in a boiling water bath 30 min. After cooling, the pH was adjusted to about 4.5 with 3.75 meq Na acetate and 5.6 meq NaOH. Two milliliters of 5% taka-diastase (Park-Davis) were added to digest starch and convert thiamin pyrophosphate to free thiamin during overnight incubation at 37°C under toluene. Samples were made up to volume and filtered through Whatman No. 1 filter paper; the first few milliliters of filtrate were discarded to avoid errors due to any adsorption of vitamin on the paper. Aliquots of filtrate were assayed microbiologically for thiamin after appropriate dilution with pH 6.1, 0.05*M* phosphate buffer.

The extract, which contained niacin in the free and bound forms (Clegg 1963), was then treated to convert bound niacin to the free form by adjusting an aliquot to pH 11 with NaOH and autoclaving 30 min. The extract was diluted with pH 6.1 0.05*M* phosphate buffer for microbiological assay.

Thiamin assay was performed using *Lactobacillus viridescins* (ATTC 12706), with 4 g of acetate added per liter of Difco medium (Pearson 1967). Niacin was assayed with *L. plantarum* (ATTC 8014), using Difco medium (Difco 1971). Five-milliliters total volume per assay tube was used for all vitamins. Calculations were based on Finney's parallel line assay (1964) as described by Schatzki and Keagy (1975). A minimum of two replicates was determined on each sample.

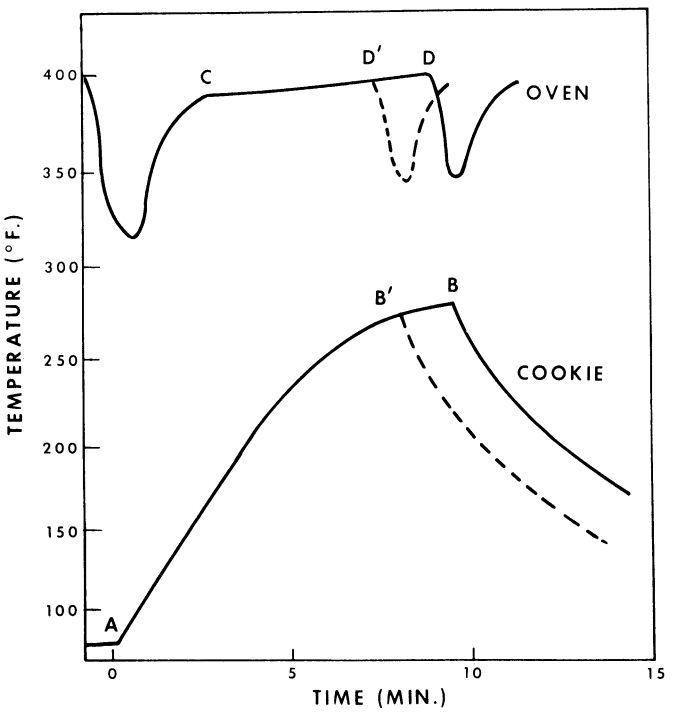

Fig. 1. Oven and cookie thermocouple profiles of center point bake. The upper curve is from a thermocouple placed in air space. Oven temperature = time-temperature average from **C** to **D**. Bake time = time from **A** to **B**. **D'** and **B'** show that at a shorter baking time, the cookie temperature is lower.

RESULTS AND DISCUSSION

Thiamin Retention

Thiamin retention ranged from 6 to 88%, with average retention in the center point cookies of 70%. Table IV 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 thiamin retention. Less significant terms were combined with the residual. Excess soda (linear and squared terms), baking time, and baking temperature are significant at $P = 0.01$ and thickness is significant at $P = 0.05$. Changing the amount of ammonium bicarbonate or dough rest time had no measurable effect in this experiment.

Predicted thiamin values (expressed as lnln of the ratio of initial vitamin concentration, C_0 [dough value], to the final vitamin concentration, C [sample value]) may be calculated by substituting the transformed, coded values of the independent variables (Table II) and the regression coefficients (Table IV) into the model:

$$\text{lnln}(C_0/C) = -.93 + .11\,X_1 + .48\,X_2 + .24\,X_3 + .58\,X_4 + .28\,X_4^2$$

The positive coefficients indicate that as the design levels of the variables increase (ie, bake time, bake temperature, and excess soda increase and thickness decreases) lnln (C_0/ C) increases and percent retention of thiamin decreases. No interactions were significant for thiamin retention.

The coefficient of determination (R^2) is .92, indicating that 92% of the total variation in lnln (C_0/ C) is accounted for by the model.

Thermal Effects

Predicted values of thiamin retention were determined as a function of time and temperature, while the other variables in the equation were held constant at the control levels (Fig. 2). The data

TABLE III
Control Cookie Formula

Cream Mass[a]		Dough[a]	
29.3	Shortening	100.0	Flour (9.8% protein, 0.58% ash)
51.7	Sugar	0.72	Sodium Bicarbonate
1.4	Salt	0.79	Ammonium bicarbonate
2.1	Nonfat dry milk	0.88	Acid salts (neutralizing value = 50)
4.1	Eggs	21.0	Water
4.3	Invert sugar syrup	92.9	Cream mass
0.0001	Ethyl vanillin		

[a]Based on flour.

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

Source of Variation	Coefficient	MS[a]	df[b]
Regression		3.96	5
Constant	−0.93		
Excess soda, X_4	0.58	8.89[c]	1
Bake time, X_2	0.48	3.98[c]	1
Soda × soda, X_4^2	0.28	2.96[c]	1
Bake temperature, X_3	0.24	2.49[c]	1
Thickness, X_1	0.11	0.30[d]	1
Residual		0.061	28
Lack of fit		0.070	22
Error		0.027	6
Corrected total		0.652	33
$R^2 = 0.92$			

[a]MS = Mean square.
[b]df = Degrees of freedom.
[c]Significance at $P = 0.01$.
[d]Significance at $P = 0.05$.

has been plotted in degrees F, time in minutes, and percent thiamin retention, rather than in the transformed functions; therefore the response function appears curved. The graph shows that thiamin retention decreases as time and temperature increase. Seventy percent thiamin is retained by baking 8 min at 409° F, 10 min at 373° F, or 12 min at 344° F. An additional 10% decrease in retention results if the cookie is baked 1.9 min longer than 10 min or at a temperature 29° F higher than 373° F.

The derivation of the model given in the Appendix (equation 3) and data from other model systems (Feliciotti and Esselen 1957, Mulley et al 1975a) suggest a comparison of the regression coefficients obtained for thiamin retention (lnln $[C_0/C]$) with values derived from kinetic theory. Equation 3 indicates that the coefficient of the ln time term (X_2) should be 1, but the experimental value is 2.08 (0.48 × 4.34; Tables II and IV). This apparent discrepancy may be explained by reference to Fig. 1. The cookie temperature profile (lower curve) shows that the cookie does not maintain a fixed temperature during baking. The baking time from points A to B is 9.5 min. If the cookie had been removed from the oven at an earlier time, B′, the baking time would be 1.6 min shorter, but the cookie would have a slightly lower temperature; therefore vitamin destruction would be less than that expected for the reduced time alone. As a result, the first order rate equation, which supposes constant temperature, is not truly applicable and a higher power for time is to be expected.

Similarly, if the oven temperature setting is increased, the oven profile (upper curve) increases more than the cookie temperature profile; therefore the regression should predict a lower activation energy (E_a) than that derived from equation 2. Feliciotti and Esselen (1957) and Mulley et al (1975a) reported activation energy for thiamin destruction in foods to be about 27 Kcal/mol, but Dwivedi and Arnold (1972) suggested a lower value for pH 7–8. The regression coefficient for 1/T corresponds to E_a = 10 Kcal/mol. In principle, these effects could be taken into account by integrating the lower curves of Fig. 1 for each experiment. Curves taken from two cookies during a given experiment did not match closely enough to make this feasible, however.

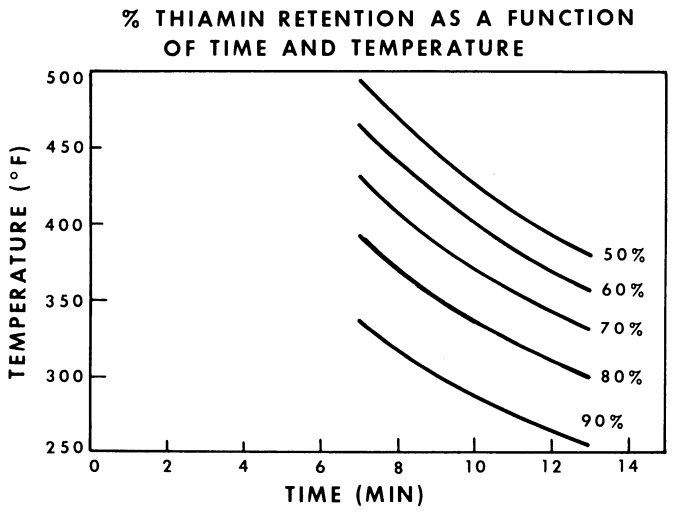

Fig. 2. Prediction curves of percent thiamin retention as a function of time and temperature.

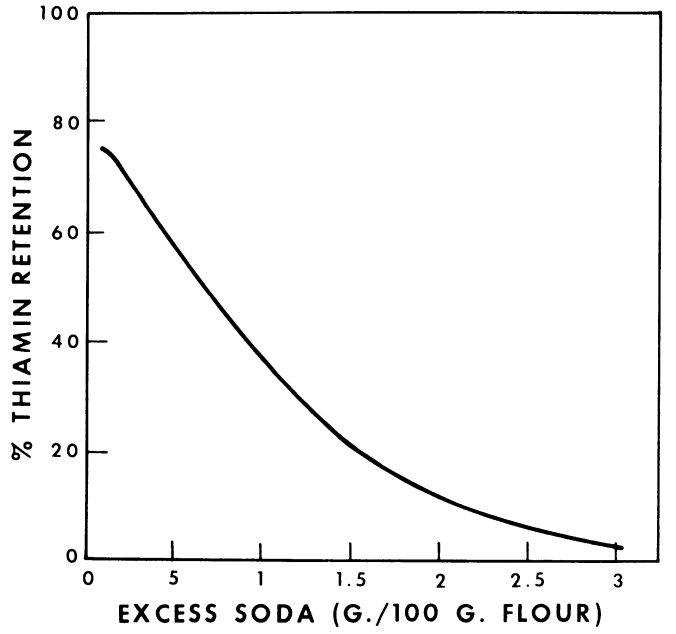

Fig. 3. Prediction curve of percent thiamin retention as a function of excess soda.

pH Effects

Excess soda profoundly affects the amount of thiamin retained in the final product, as indicated by the linear and quadratic regression terms. Figure 3 illustrates the effect of excess soda when all other variables are constant at the control levels. This regression predicts 75% retention with 0.084 g of excess soda per 100 g of flour, 67% with 0.3 g, and 40% with 0.9 g, the amount in the original commercial formula. Thus the easiest method of retaining thiamin is to minimize excess soda.

The chemical properties of thiamin in relation to pH have been reviewed by Dwivedi and Arnold (1973), Metzler (1960), and Mulley et al (1975b). Felicotti and Esselen (1957) studied the dependence of ln k (lnln $[C_0/C]$ in our terms) as a function of temperature and pH in the 4.5–7.0 range. They indicated a break in the slope of lnln (C_0/C) vs pH at pH = 6.2, but replotting of the data indicates that a quadratic function of pH would fit at least as well over the entire range. In agreement with his results, linear and quadratic terms of ln excess soda are found from regression.

Color Predicted by Processing Variables

Table V presents the significant regression results when cookie color (absorbance) is expressed as a function of the processing variables. Baking time, temperature, thickness, time-temperature interaction, and soda squared are significant at the 1% level. All other effects were combined with the residual.

The results allow a processor to predict the extent that processing changes will effect color in a standard product. As expected, increasing time and temperature or decreasing thickness increase

TABLE V
Color (Absorbance) Predicted by Processing Variables: Analysis of Variance for Regression

Source of Variation	Coefficient	MS[a]	df[b]
Regression		0.14	5
Constant	0.45		
Bake time, X_2	0.14	0.36[c]	1
Bake temperature X_3	0.06	0.15[c]	1
Thickness, X_1	0.05	0.06[c]	1
Time × temp., $X_2 \times X_3$	0.05	0.06[c]	1
Soda × soda, X_4^2	0.03	0.03[c]	1
Residual		0.004	28
Lack of fit		0.005[d]	22
Error		0.001	6
Corrected total		0.025	33
$R^2 = 0.87$			

[a] MS = Mean square.
[b] df = Degrees of freedom.
[c] Significant at $P = 0.01$.
[d] Significant at $P = 0.05$.

browning and the resulting absorbance. Soda changes must be extreme to affect color. This small but significant effect should be expected, as nonenzymatic browning increases with pH (Johnson and Miller 1961).

Thiamin Retention Predicted by Color

The similarity of processing variables that predict thiamin and color suggest a relationship between color and thiamin. Table VI presents the results of color absorbance as an independent variable predicting thiamin retention. (The transformation listed in Table II is used for color.) All of the heat-related factors are replaced by color, leaving excess soda as the only additional modifying factor. This means that for a single cookie formulation with a constant soda level, color may be used as a quality control factor to monitor thiamin retention. Figure 4 shows experimental values of thiamin retention and color for two levels of excess soda (samples 1–16).

pH as a Function of Processing Variables

The relationship of thiamin destruction and alkalinity were discussed with respect to the soda used in dough formulation. Several investigators attempted with limited success, to predict thiamin retention in baked products from final product pH (Barackman 1942, Briant and Hutchins 1946, Briant and Klosterman 1950, McKin and Moss 1943, Pace and Whitacre 1953). Table VII indicates that, in addition to soda, heat-related factors of time, temperature, and thickness greatly influence product pH. Two minutes more bake time will lower pH as much as will a threefold decrease in soda. This is probably because organic acids form as sugar caramelizes and Maillard reaction browning occurs (Johnson and Miller 1961). Further support for this explanation is the improvement in R^2 shown in Table VIII, in which color replaces the temperature-related factors in predicting product pH.

Niacin Retention

The stability of niacin during baking was confirmed in this study. The control cookies had an average niacin retention of 95.5%. The average retention of all the samples was 97.2%; SD was 3.2%, indicating that no significant loss occurred. These results are similar to those of Bednarcyk et al (1975), which showed a 5% mean loss of niacin in 63 commercial cookie and cracker products.

TABLE VI
Thiamin Retention (1n1n [C_0/C]) Predicted by Color: Analysis of Variance for Regression

Source of Variation	Coefficient	MS[a]	df[b]
Regression		6.79	3
Constant	−0.85		
Color, X_7	0.49	7.79[c]	1
Excess soda, X_4	0.53	7.46[c]	1
Soda × soda, X_4^2	0.20	1.48[c]	1
Residual		0.039	30
Lack of fit		0.042	24
Error		0.027	6
Corrected total		0.652	33
$R^2 = 0.95$			

[a]MS = Mean square.
[b]df = Degrees of freedom.
[c]Significant at $P = 0.01$.

TABLE VII
Cookie pH Predicted by Processing Variables: Analysis of Variance for Regression

Source of Variation	Coefficient	MS[a]	df[b]
Regression		2.92	4
Constant	7.61		
Excess soda, X_4	0.42	4.85[c]	1
Bake time, X_2	−0.49	4.25[c]	1
Thickness, X_1	−0.18	0.75[d]	1
Bake temperature, X_3	−0.13	0.67[d]	1
Residual		0.162	29
Lack of fit		0.198[d])[e]	23
Error		0.024	6
Corrected total		0.497	33
$R^2 = 0.71$			

[a]MS = Mean square.
[b]df = degrees of freedom.
[c]Significant at $P = 0.01$.
[d]Significant at $P = 0.05$.
[e]Time-soda interaction and ammonium bicarbonate terms were not significant at $P \leq 0.05$. Their inclusion eliminates lack of fit, but they have little effect on the prediction and add little useful information. They were therefore combined with the residual.

TABLE VIII
Cookie pH Predicted by Color: Analysis of Variance for Regression

Source of Variation	Coefficient	MS[a]	df[b]
Regression		4.59	3
Constant	7.47		
Color, X_7	−0.48	7.51[c]	1
Excess soda, X_4	0.45	5.46[c]	1
Soda × soda, X_4^2	0.16	0.99[c]	1
Residual		0.088	30
Lack of fit		0.103[d]	24
Error		0.024	6
Corrected total		0.497	33
$R^2 = 0.84$			

[a]MS = Mean square.
[b]df = Degrees of freedom.
[c]Significant at $P = 0.01$.
[d]Significant at $P = 0.05$.

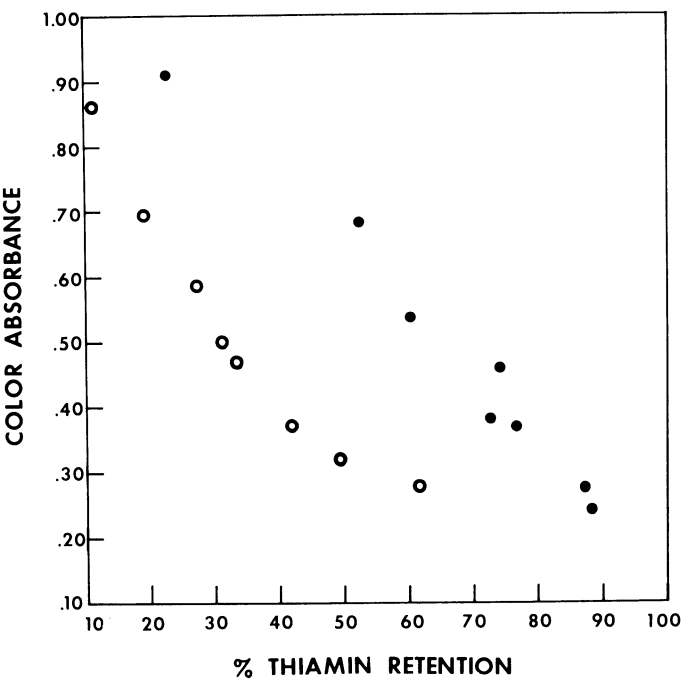

Fig. 4. Experimental thiamin retention values as a function of color (absorbance) of the samples. • = −1 excess soda levels; o = +1 levels.

SUMMARY

We have presented regression equations relating thiamin retention, processing variables, final product color, and pH. These equations and resulting graphs should allow more informed predictions of thiamin, color, and pH results during product development. In addition, product color is shown to be a valuable indicator of thiamin content for quality control purposes.

APPENDIX

Thiamin Kinetics

Farrer (1955), Feliciotti and Esselen (1957), and Mulley et al (1975a, 1975b) showed that thermal destruction of thiamin follows first order reaction kinetics given in equations 1 and 2 (Moore 1960),

$$\ln (C_0/C) = kt \quad (1)$$

$$k = Ae - E_a/RT \quad (2)$$

where C_0 is the initial concentration, C is the final concentration, k is the rate constant, t is time, A is an integration constant, E_a is the activation energy, R is the molar gas constant, and T is the absolute temperature (°K). Combining equations 1 and 2 and taking the natural logarithm gives equation 3:

$$\text{lnln} (C_0/C) = \ln A - E_a/RT + \ln t \quad (3)$$

This expression forms the basis of the transformation of vitamin concentration, time, and temperature into independent linear variables.

Excess Soda

The leavening systems commonly used in cookies are summarized by equations 4, 5, and 6 (Bohn 1957).

$$2NaHCO_3 \xrightarrow{\Delta} Na_2CO_3 + CO_2 + H_2O \quad (4)$$

$$NaHCO_3 + HX \text{ (acid salt)} \rightarrow NaX + CO_2 + H_2O \quad (5)$$

$$NH_4HCO_3 \xrightarrow{\Delta} NH_3 + CO_2 + H_2O \quad (6)$$

Equation 4 leaves Na_2CO_3 as an alkaline residue, which reacts further at cookie pH according to equations 7 and 8:

$$CO_3^{-2} = + H_2O \rightarrow HCO_3^- + OH^- \text{ pKa } 10.3 \quad (7)$$

$$HCO_3^- + H_2O \rightarrow H_2O + CO_2 + OH^- \text{ pKa } 6.37 \quad (8)$$

The hydrogen ion content of a dough system above pH 7 should be related to the amount of unneutralized soda that reacts according to equations 4, 5, 7, and 8. As thiamin loss in pure systems is usually expressed as a function of pH ($-\log [H^+]$), then the transformation relating thiamin loss to soda is log mmoles soda not neutralized by acid in the leavening combination.

Ammonium Bicarbonate

The total leavening gas available was held constant at the level in a commercial sugar cookie formula. The defining relationship, expressed in mmoles of reactants, is: 2×9.94 mmol NH_4HCO_3 + 8.52 mmol $NaHCO_3$ = 28.4 mmol total gas as $(CO_2 + NH_3)$/100 g of flour.

Each variation in the amount of NH_4HCO_3 required a change in the concentration of $NaHCO_3$ and an adjustment of the acid salts to achieve the required excess soda. Variations in NH_4HCO_3 were not expected to effect vitamin retention, but the model did test for such a possiblity.

Thickness

Higher temperatures and subsequently greater nutrient loss occurs at the surface of a cookie than in the interior. A thick cookie has less surface area per gram of dough and proportionately less vitamin loss than a thin one does. A reasonable approximation for this effect would be the surface/volume ratio; thus, for a short-cylinder-shaped cookie, vitamin retention (C/C_0) is proportional to $1/\lambda$ (where λ is the thickness), and $\text{lnln} (C_0/C)$ is proportional to $\text{lnln } \lambda$.

Centering Transformation

Data used for multiple regression analysis is usually coded into small numbers of equal value to improve the accuracy of least squares calculations, to remove correlations between linear terms and the constant term, and to reduce correlations of linear terms with quadratic terms, other linear terms, and interaction terms. The data used here were first transformed to the theoretical form and then centered as follows.

$$\frac{\text{transformed actual level} - \text{transformed center level}}{\text{increment between transformed levels}} = \text{coded level}$$

For example, the -1 level of time (X_2) is arrived at as follows:

$$(\ln 7.7 \text{ min} - \ln 9.7 \text{ min}) / 0.23 \ln \text{ min}$$

$$= 4.34 \ln 7.7 - 9.88 = -1.$$

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