Table V. Effects of Iron Salts on Yield of Denatured Nitrosomyoglobin Obtained at 70° C.

Reactants			
MetMb	Added NO_2^- μmoles	Added Fe^{+2} μmoles	Denatured $MbNO^a$, %
+ cysteine, 40 μmoles	1		51, 46
	1	1	67, 67
	10	..	71, 71
	10	1	73, 78
+ ascorbate, 20 μmoles	1	..	60, 62
	1	1	60, 66
	10	..	65, 68
	10	1	76, 68

[a] Original metmyoglobin.

zinc ions to be effective in catalyzing nitrosohemoglobin formation, using a system consisting of methemoglobin, nitrite, and ascorbate. A patent, in which ferrous ions are reported to improve the color of meat, has been recently granted (*5*). Because ferrous salt solutions are known to be excellent trapping agents for nitric oxide gas (*9*), the activity of iron salts in this model system was tested (Table V). No differences were observed when ascorbate was used as the reductant or when cysteine was used with the high level of nitrite. The lower yields of denatured nitrosomyoglobin obtained with 1 μmole of nitrite and cysteine (50%) were, however, increased to maximum yields (60 to 70%) by the presence of 1 μmole of iron salts. This increase was obtained with either ferrous or ferric salts, probably because of the reducing activity of the system converting the ferric ion to the ferrous ion. The presence of equimolar amounts of citrate did not influence the results. These data show an apparent nitrite sparing effect by ferrous ion.

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FOOD COLOR CHANGES

Role of the Sugars in the Browning Reaction in Potato Chips

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This study was undertaken to elucidate the role of the sugars in potato chip color development, as a result of nonenzymatic browning. Both reducing sugars and sucrose react with amino acids to produce brown colors, at temperatures used to fry potato chips. Multiple correlation between potato chip color and the reducing sugar and the sucrose contents of the raw potatoes, yielded a correlation coefficient of 0.983. Both reducing sugars and sucrose must be considered in determining the suitability of a potato for chipping. The multiple regression equation may accurately predict chip color.

POTATOES HIGH IN SUGAR content are particularly susceptible to excessive browning, when processed as potato chips. Sweetman (*13*) found that potatoes stored at temperatures favoring sugar accumulation resulted in dark colored potato chips. Denny and Thornton (*3, 4*) found that the color produced during the frying of chips correlated best with the reducing sugar content of the raw stock, less well with the total sugar content, and not at all with the nonreducing sugar values. These authors substantiated their findings by demonstrating that filter paper disks impregnated with glucose and fried would brown, while filter paper disks impregnated with sucrose and fried would not brown. However, selecting and handling potato tubers to control chip color, based upon the premise that the color is a function of the reducing sugar content, have led to inconsistent results. Tubers low in reducing sugars may yield burnt or dark chips.

Patton and Pyke (*9*) observed that potato slices which had been leached until no color developed upon frying had to be impregnated with both reducing sugars and amino acids to develop color. More recently, Habib and Brown (*5*) found that multiple correlation among

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chip color, total solids, reducing sugars, and amino acids gave a correlation coefficient slightly higher than that obtained by comparing chip color with the reducing sugar content of the raw stock.

This report describes the role of the sugars in potato chip color development in the light of the nonenzymatic browning reaction (*1, 2, 6*). Data demonstrate that sucrose as well as glucose and fructose react in nonenzymatic browning systems at potato chip processing temperatures, and that both reducing sugars and sucrose are responsible for the development of potato chip color.

Experimental

Source of Potatoes. Four potato varieties, Katahdin, Kennebec, Green Mountain, and Russet Rural, were planted at East Ithaca, N. Y., in a randomized block from certified seeds. These varieties were chosen because of their contrasting potato chipping quality. Three harvests were made: an early harvest 3 months after the planting date, an immediate harvest 3 weeks after the first harvest date, and a late harvest 6 weeks after the first harvest. On the date of picking, the tubers from the randomized blocks (three) were grouped, and separated according to their specific gravity using brine solutions (*10*) to eliminate the extremes in variance.

After sampling for chemical analysis and chip color on the date of harvest, all varieties were placed in storage at 32°, 40°, 50° F., and room temperature (80° ± 10° F.). Samples for chip color and chemical analysis were taken from the 32°, 40°, and 50° F. groups at 2-month intervals. Samples were taken from the room temperature storage at 1-month intervals. The object of the varietal, harvest, and storage treatments was to create extreme ranges and ratios of sugar content, distribution, and variable potato chip color.

Chip Frying. On each sample preparation date, 1000 grams of potatoes were peeled, washed, and sliced 2 mm. thick. All tubers were sliced longitudinally, or from the basal to the apical end of the tubers. The slices were hand mixed, and about 200 grams were fried in fresh cottonseed oil using a starting temperature of 188° C. (370° F.). The chips were removed from the oil, near the end of the bubbling, and packaged in cellophane. It was established that potato chip color is not appreciably changed, if the time of frying does not vary by more than 1 minute from the subjective estimation of doneness by observing the near cessation of bubbling (water evolution).

Color Measurement. The Hunter color meter was used to measure the reflectance of the potato chips. Chips were macerated with a mortar and pestle to a particle size of about 2 mm. or less. An 8-gram sample was pressed into a 1-inch cylindrical cake on a Carver press using 10,000 pound pressure for 3 seconds. This procedure gave the chips a uniform surface, and expressed most of the oil. Hunter *Rd* values (reflectance) were recorded using a black paper mask containing a 1-inch aperture to cover the Hunter meter sample platform. All data are expressed as log $(1/Rd \times 10^3)$. This expression is a near linear function of subjective scores for potato chip color over the entire range of the brown pigment observed in chips.

Model System Procedure. The model system procedure used in this study is essentially a variation of the technique of Denny and Thornton (*3, 4*) for making artificial potato chips. Whatman No. 1, filter paper disks, 5.5 cm., were treated with solutions of sugars and of amino acids and fried in cottonseed oil for 2 minutes at 188° C. To remove the oil, the disks were washed with carbon tetrachloride. Reflectance measurements were recorded using the Hunter color meter.

Determination of Sugars. At each sampling date, two 50-gram samples of sliced and chopped potato tubers were plunged into 212 ml. of boiling 95% ethyl alcohol containing 0.25 gram of calcium carbonate. After bringing the alcohol to a second boil, the samples were simmered on a steam bath for 55 minutes, and then transferred for storage to 1-pint Mason jars.

For sugar analysis, the chopped potato sample was further extracted three times with water, and the combined alcohol and water extracts were concentrated to 200 ml.

Sugars were determined, using Nelson's (*7*) photometric method, after the extracts had been cleared using the ion exchange procedure of Williams, Potter, Bevenue, and Scurzie (*14*). Total sugars were determined by treating a portion of the extract with 1 drop of 1% invertase, prior to treatment with Nelson's reagents. Sucrose was calculated by subtracting the values for reducing sugars from the total sugar determination and multiplying the result by 0.95. The reducing sugars in potatoes are predominantly glucose and fructose, while the nonreducing sugar is predominantly sucrose (*10, 11*). All data are expressed as grams per 100 grams of potato, on a fresh basis, and are the average of single determinations on duplicate samples.

Results

Both reducing sugars (glucose and fructose) and sucrose react with amino acids to yield dark brown pigments, when filter paper disks are wet with a solution of sugar and amino acid and fried under conditions simulating potato chip processing. The reaction between glucose and sucrose with 0.2*M* glycine is shown in Figure 1. No color could be developed on filter paper with either glucose or sucrose alone. Paper chromatographic studies (*11*) showed that sucrose is hydrolyzed in the presence of amino acids (glycine) to yield glucose and fructose at temperatures as low as 150° C. Presumably, then, the amino acid condenses with invert sugar and subsequently, forms brown melanoidins (*1, 2, 6, 8*).

Simple correlation studies between the sugar content and chip color of all potato varieties from the first harvest date and various storage treatments ($n = 52$) confirmed the work of others (*3, 4, 13*). The color of the potato chips correlated best with the reducing sugar content of the potatoes ($r = 0.86$), less well with the total sugar content ($r = 0.82$), and poorly with the sucrose content ($r = 0.39$). However, all correlation coefficients were highly significant. When potato chip color was plotted against the reducing sugar content of the raw tubers in Figure 2, the relation was not linear, although

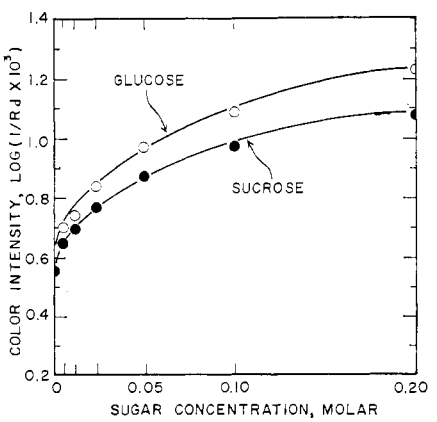

Figure 1. Nonenzymatic browning reaction between glucose or sucrose with 0.2M glycine at 188° C. for 2 minutes

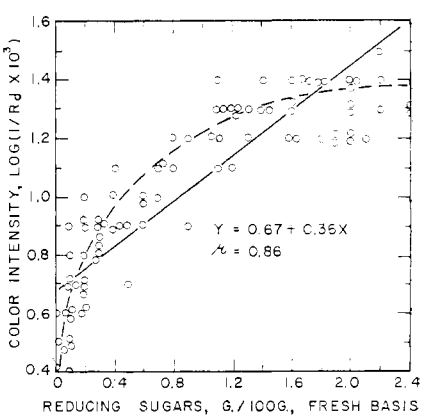

Figure 2. Relation between potato chip color (reflectance) and reducing sugar content of raw potatoes

simple correlation presupposes that the relation between any two variables is linear.

The role of the sugars in potato chip color development is best tested by means of multiple correlation. Table I shows that potato chip color is a function of both the reducing sugar and sucrose contents of the potato tubers, as indicated by the high coefficient of multiple correlation (0.983) obtained by comparing reducing sugars and sucrose together with the potato chip color. That the expression

$$y = 0.73 + 0.32\,x_1 + 0.018\,x_2$$

where y is chip color, x_1 the reducing sugar content, and x_2 the sucrose content of the tubers best fits the data obtained, was confirmed by establishing that the reduction of the residual error was highly significant, when the multiple relation was compared with the effect of either the reducing sugars or sucrose alone.

To check further the assumption that potato chip color is a function of both the reducing sugar and sucrose contents of the potato tubers, data were selected at random from all three harvest dates ($n = 156$), and calculated reflectance values for the chips were compared with the observed reflectance values. With a correlation coefficient of 0.983 the difference between the calculated and observed color of the chips should not be significantly different, regardless of the varietal, maturity, or storage treatment. An example in which nine random samples were compared is shown in Table II. The difference between the average of the nine measurements is not significant, on the basis of Student's T test, at even the 50% probability level. This finding confirms the high coefficient of correlation obtained with the data of the first potato harvest, and suggests that the multiple regression equation may accurately predict potato chip color.

Table I. Comparison of Regression Equations of Sugars in Potatoes upon Potato Chip Color (Reflectance)

[y = color, x_1 = reducing sugars, x_2 = nonreducing sugars (sucrose)]

Factors	Regression Equation	Standard Error of Estimate	Coefficient of Correlation
y vs. x_1	$y = 0.67 + 0.36\,x_1$	0.178	0.859[a]
y vs. x_2	$y = 0.80 + 0.33\,x_2$	0.953	0.386[a]
y vs. x_1 and x_2	$y = 0.73 + 0.32\,x_1 + 0.018\,x_2$	0.061	0.983[a]

[a] Significant at 1% level.

Table II. Comparison of Calculated Color Scores for Potato Chip Color with Observed Scores

[Using equation $y = 0.73 + 0.32\,x_1 + 0.018\,x_2$ where y = color, x_1 = reducing sugars, and x_2 = nonreducing sugars (sucrose)].

Potato Variety	Storage Temp., ° F.	Storage Time, Months	Harvest No.	Reducing Sugars, G./100 G. Fresh Basis	Sucrose G./100 G. Fresh Basis	Potato Chip Color, log (1/Rd × 10³) Estimated	Observed
Katahdin	40	6	2	2.22	0.42	1.46	1.52
Kennebec	...[a]	...	2	<0.01	<0.01	0.73	0.46
	40	6	2	1.80	0.72	1.31	1.36
	50	2	1	0.48	0.32	0.89	0.90
	50	8	1	0.13	0.12	0.77	0.65
Russet Rural	32	2	2	0.74	2.01	1.01	1.08
	50	2	3	0.40	0.28	0.86	0.94
Green Mtn.	80	4	1	0.10	0.19	0.76	0.55
	50	4	2	0.63	0.16	0.93	1.00
					Av.[b]	0.97	1.02

[a] Data of tubers analyzed on date of harvest.
[b] Difference between means not significant.

Discussion

The observation that potato chip color is best correlated with reducing sugars and sucrose is consistent with the finding that both reducing sugars and sucrose react with amino acids in model systems to produce brown color at temperatures used to fry potato chips. All amino acids and amides reported for the potato tuber react with reducing sugars and sucrose at temperatures used to process chips.

That sucrose may also react with amino acids to produce brown pigments may explain chip color development when the reducing sugar content of the raw stock is low. An example is shown in Table II with the variety Russet Rural stored at 32° F. Other varieties behaved similarly at this temperature. Similar examples with a high sucrose content were found with all varieties stored at room temperature and occasionally at 50° F. At 40° F. the reducing sugar content nearly always exceeded the sucrose content of the tubers.

One explanation of why total sugar values (sucrose and the reducing sugars) do not correlate with chip color as well as do the reducing sugars, while the multiple correlation between these three parameters is excellent, is that sucrose does not react in carbonyl-amine browning systems as readily as glucose does (Figure 1). Conversely, reducing sugars alone correlate fairly well with chip color, because they are more reactive and a greater portion of the reducing sugars enter into the browning reaction. Sucrose must first be hydrolyzed to participate in a nonenzymatic browning reaction (*1*, *8*). This additional step explains the slower reaction rate.

The high multiple coefficient of correlation obtained between the sugars and potato chip color suggests that the functional nitrogen of the potatoes remained relatively constant, that catalysts for the sucrose inversion and browning do not change, and finally, that the amount of color formed under conditions of constant temperature and time is proportional to the first power of the browning reactants. Analyses for amino and protein nitrogen indicate that the reactive nitrogen of potato tubers could seldom, if ever, limit the nonenzymatic browning reaction in potato chips.

The multiple regression equation derived from the data does not serve to explain chip color with a reflectance measurement less than 0.73. It would appear that the reactions responsible for light, desirable potato chips are not primarily sugar–amino acid condensation reactions.

Denny and Thornton (*3*, *4*) reported that glucose alone browns on filter paper, while sucrose does not. The authors have found that neither glucose nor sucrose will brown alone on filter paper under conditions that simulate potato chip processing, but both will brown in the presence of an amino acid. This anomaly can be explained only by assuming that the glucose used by Denny and Thornton was impure, or that the filter paper contained an impurity which would react with glucose, but would not cause appreciable hydrolysis of sucrose.

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FOOD COLOR CHANGES

Browning Reaction Initiated by Gamma Irradiation

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The browning reaction initiated by gamma irradiation in crystalline sucrose, glucose, fructose, sorbitol, and their 50% solutions was characterized for comparison with the browning reaction induced by heat. Reductones were present in irradiated crystalline sugars, but not in irradiated sugar solutions or heated crystalline sugars. The ultraviolet spectra of the irradiated sugars were those characteristic of enols or enediols. Hydrogen, methanol, and smaller amounts of formaldehyde and carbon dioxide were produced on irradiation of crystalline fructose. Hexoses could not be detected in irradiated crystalline sucrose, despite an increase in reducing value.

THE PURPOSE OF THIS INVESTIGATION was to compare the coloration–browning–produced in sugars by gamma irradiation with that produced by heat. The browning reaction in heated carbohydrates has received much attention (*9*). The browning may involve reaction of a sugar with amino acids (*6*), or may be due to decomposition of the sugar itself. It has been demonstrated (*11*) that the acid-base–catalyzed thermal decomposition of reducing sugars is, in many cases, as important a factor in the nonenzymatic browning of foods as the more commonly recognized reaction between reducing sugars and amino acids. The browning reaction is of considerable importance industrially, in problems such as the color reversion of packaged dextrose (*11*) and the discoloration of intravenous dextrose or fructose solutions after heat sterilization.

The browning reaction in irradiated sugars deserves attention, because of the potential use of irradiation for sterilizing foods and pharmaceutical products containing sugars. Previous investigators have studied the changes brought about by gamma irradiation of dilute solutions of simple sugars (*2, 8*). The irradiation of crystalline sugars and their concentrated solutions has received less attention.

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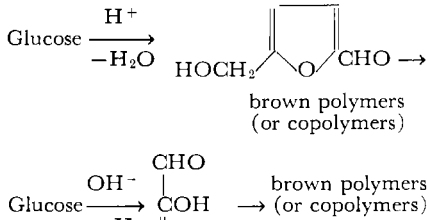
The formation of color in heated sugar solutions may proceed, in general, by two mechanisms. In one, which operates in acid media, the sugar is converted into furfural derivatives. These colorless compounds decompose, under acid catalysis, to colored polymeric products (*11*). The general reaction may be characterized as a dehydration reaction. Thermal destruction of dry sugars also proceeds by dehydration, with the formation of oxide rings in the primary products (*10*).

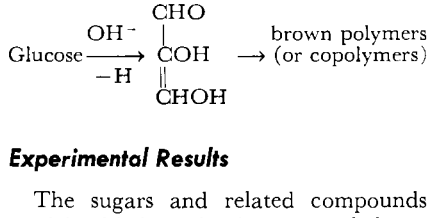
The other mechanism involves the fragmentation of the sugar molecules, with the formation of reductones or other enediols, which then condense to colored polymeric products (*1, 4*). The formation of enediols may be characterized as an oxidation-reduction reaction.

The substances chosen for the present study were reducing sugars (glucose and fructose), a nonreducing sugar (sucrose), and two polyhydroxy compounds (sorbitol and glycerol). The sugars were chosen as representing commercially important carbohydrates. Sorbitol was used so that a comparison could be made between a reducing sugar and a related polyol in which reducing groups were not present. Glycerol was used, as explained below, to provide comparison between a liquid and a solid polyol.

Examples of Nonenzymatic Browning Reactions

Sugar + amino acid → amino sugar derivatives → melanoidins (brown nitrogenous polymers)





Experimental Results

The sugars and related compounds used in this investigation were of chemically pure grade, further purified until they showed negligible ultraviolet absorption in 10% aqueous solution.

For irradiation, the sugars were exposed to a water-shielded 800-curie cobalt-60 source, which delivered approximately 3×10^5 roentgens per hour. Crystalline sugars were sealed, in vacuo, in bags made of Scotchpak (a polyethylene - polyester film combination, Minnesota Mining and Manufacturing Co.). Control experiments showed that no detectable amounts of foreign substances were introduced into the package contents from the plastic on irradi-