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FOOD COLOR CHANGES

Browning Reaction Initiated by Gamma Irradiation

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 (7) 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 (7) 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 (7). 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 (7, 4). The formation of enediols may be characterized as an oxidation-reduction reaction.

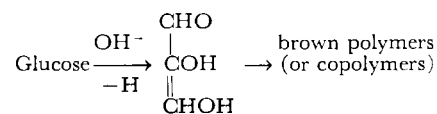
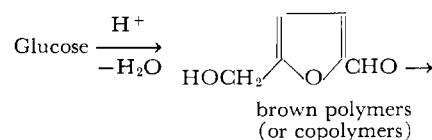
The substances chosen for the present study were reducing sugars (glucose and fructose), a nonreducing sugar (sucrose), and two polyhydroxy compounds (sorbitol and glycerol). The sugars were chosen as representing commercially important carbohydrates. Sorbitol was used so that a comparison could be made 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.

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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 irradiation.

ation. Aqueous 50% solutions of sugars were sealed in glass bottles that were swept out with nitrogen.

Samples of crystalline sugars were heated in closed glass containers in an oven at 100° C.

Portions of the irradiated or heated sugars were removed for determination of the content of reducing substances, reductone content, and ultraviolet spectra, and for paper chromatography. Reducing substances were determined by the Somogyi method (12) and reductones with Tillman's reagent (2,6-dichlorophenolindophenol) (5).

Ultraviolet spectra of dilute solutions of the sugars were obtained with a Beckman Model DK recording spectrophotometer. Distilled water was the reference standard. One-centimeter silica sample cells were used. After the spectrum of each sugar solution was measured, the solution was made slightly alkaline (approximately pH 8) with a small amount of sodium hydroxide and the spectrum was immediately measured again. Then, the solution was acidified to pH 4 with a small amount of hydrochloric acid and the spectrum was immediately measured again.

Paper chromatograms of the sugars were obtained with an *n*-butyl alcohol-ethyl alcohol-water system on Whatman No. 1 filter paper (13). Various colorimetric reagents for sugars were used for developing the chromatograms.

The gases produced on irradiation were separated and identified by gas chromatography on columns of charcoal or dinonyl phthalate-firebrick C-22.

Results and Discussion

After being irradiated, the crystalline sugars were slightly colored; glucose and sucrose were light brown, and fructose was gray. Sorbitol remained colorless. The color did not change when the irradiated compounds were stored in a desiccator for several months. The color appeared to be on the surface of the sugar crystals, because it could be removed by washing them with water.

Irradiated 50% aqueous solutions of the sugars were colorless immediately after irradiation, but usually they developed a brown color upon standing for several hours at room temperature. sorbitol solutions remained colorless.

Table I summarizes the experimental results. Only the results of irradiation at 5.0 megarep. are shown. At dosages below this, the analytical results were less consistent. The interpretation of each analytical method may be considered separately.

To the extent that the data can be compared, the results obtained by irradiating the 50% aqueous sugars are very much the same as the results reported by others (2, 8) on the irradiation of dilute solutions.

Table I. Degradation Products from Irradiated and Heated Sugars and Related Compounds

Compound	Reducing, Substances ^a Ml. 0.005N Cu/Mg.	Reductones, Meq./Mole	Ultraviolet Spectra		
			pH	Absorption, max. mμ	E ₁ ^{1%} _{1cm.}
Crystalline compounds, irradiated at 5.0 megarep.					
Glucose	7.5	0.16	3.7	None	..
			8.9	275	0.37
Fructose	7.1	0.41	4.0	283	0.23
			9.0	286	0.31
Sucrose	0.067	0.62	5.5	264	0.36
			9.0	268	0.46
Sorbitol	0.059	0.03	4.8	270 ^b	0.18
			9.0	276	0.28
Glycerol	0.020	0.00	4.0	263	0.02
			9.0	275	0.03
50% aqueous solutions, irradiated at 5.0 megarep.					
Glucose	7.4	0.00	3.9	264	0.12
			8.7	283	0.57
Fructose	6.9	0.00	4.2	265	0.49
			8.4	268	0.70
Sucrose	0.93	0.00	4.6	264	0.53
			8.7	267	0.64
Sorbitol	0.045	0.00	4.2	None	..
			8.9	273	0.05
Glycerol	0.039	0.00	3.8	246	0.10
			9.0	266	0.21
Crystalline compounds, heated 10 hours at 100° C.					
Glucose	7.9	0.00	3.5	282	0.004
			9.0	228	0.006
Fructose	5.1	0.00	4.0	282	1.12
				228	1.40
			9.0	282	1.08
				228	1.41
Fructose, 31 days after heating			4.0	282	2.31
				228	1.29
			9.0	282	2.47
				228	1.35
Sucrose	0.32	0.00	4.0	282	0.10
				222	0.09
			9.0	282	0.10
				222	0.09
Sorbitol	0.00	0.00	4.0	None	..
			9.0	None	..

^a Corresponding values for original compounds: glucose, 8.0; fructose, 7.8; sucrose, 0.000; sorbitol, 0.000.

^b Shoulder.

Reducing Substances (Somogyi).

Crystalline sucrose showed a regular increase in reducing value with increasing irradiation, up to a Somogyi value of 0.067 ml. of 0.005N copper per mg. at 5 megarep. At 20 megarep., the value was 0.21. Crystalline sorbitol showed a similar increase, to a value of 0.059 at 5 megarep. Anhydrous glycerol, irradiated at 5 megarep., had a reducing value of 0.020.

Both crystalline glucose and crystalline fructose showed a decrease in Somogyi value with increasing irradiation, although there was little change until relatively high dosages were used, indicating the possibility of an induction effect. At 8.1 megarep. the value for crystalline glucose was 7.7, at 20 megarep. the value for crystalline fructose was 6.7.

The irradiation of the compounds in 50% aqueous solution produced similar changes in reducing value. None of the compounds studied showed any

postirradiation changes in reducing value, up to 14 days.

Reductones (Tillman's Reagent).

The irradiation of the crystalline sugars and crystalline sorbitol led to the formation of reductones, whereas the irradiation of 50% aqueous solutions of these did not. Nor did heating the crystalline compounds for 10 hours at 100° C. produce detectable amounts of reductones.

The reductone content increased with increasing radiation dosage. At 20 megarep. the reductone content of crystalline fructose was 0.76 meq. per mole and that of crystalline sucrose was 0.97 meq. per mole.

There was some decrease in reductone content when the crystalline sugars were kept at room temperature in a vacuum desiccator. The reductone content of crystalline glucose irradiated at 5 megarep. dropped from 0.16 to 0.10 meq. per mole after 25 days. On the other hand, the reductone content of

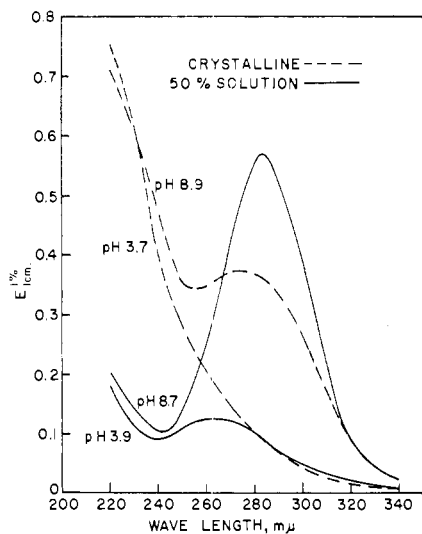


Figure 1. Ultraviolet spectra of glucose irradiated at 5.0 megarep.

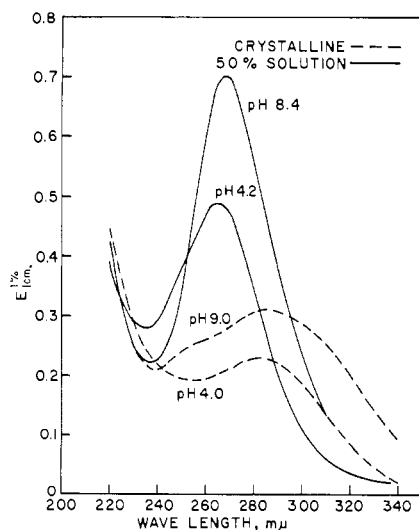


Figure 2. Ultraviolet spectra of fructose irradiated at 5.0 megarep.

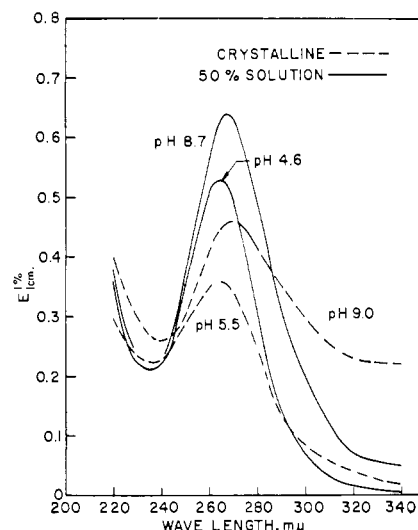


Figure 3. Ultraviolet spectra of sucrose irradiated at 5.0 megarep.

crystalline sorbitol irradiated at 5 megarep. increased from 0.03 to 0.14 meq. per mole after 28 days.

Ultraviolet Spectra. The data in Table I show that irradiated sugars are characterized by ultraviolet spectra different from those of heated sugars. The spectra of heated sugars, with absorption maxima at 282 and 222 to 228 $m\mu$, are those characteristic of furfural derivatives. These spectra are invariant with changes in pH. On the other hand, the irradiated sugars had ultraviolet spectra characteristic of reductones, enediols, or enols. At pH values above 7, the spectra of these compounds show an increase in the height of the absorption maximum, or a shift of the absorption maximum toward longer wave lengths, or both (7).

Figures 1 to 4 show the ultraviolet spectra of irradiated glucose, fructose, sucrose, and sorbitol. There appears to be no consistent pattern in the type or amount of ultraviolet absorbing materials produced from the different compounds by irradiation.

Paper Chromatography. With the exception of fructose, paper chromatograms of all the sugars and polyols that were irradiated at 5 megarep. both dry and as 50% solutions, showed only spots that were characteristic of the nonirradiated materials. Heating at 100° C. for 10 hours also did not change the chromatograms. Chromatograms of irradiated 50% fructose and heated fructose were similarly unchanged. The one for irradiated, crystalline fructose showed a second spot (R_f 0.35), presumably due to a material produced from the fructose by the radiation. Attempts to isolate and characterize this material by desorption from the paper gave inconsistent results that indicated it was either unstable or possibly a mixture. By comparison with

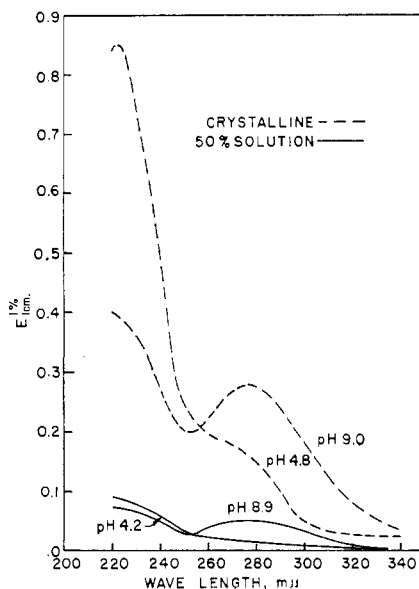


Figure 4. Ultraviolet spectra of sorbitol irradiated at 5.0 megarep.

their chromatograms, it was found that it was not glucose (R_f 0.20), arabinose (R_f 0.22), or methylglyoxal (R_f 0.86), and probably not gluco-reductone (R_f 0.41) (7).

Paper chromatograms of irradiated crystalline sucrose showed the absence of hexoses, which would have been detected by the method used, if they had been present in only 0.4% of the sucrose.

Gas Chromatography. This technique showed that the gases produced by irradiation of crystalline sugars, crystalline sorbitol, and anhydrous glycerol contained appreciable amounts of hydrogen. No hydrocarbons or oxygen could be detected.

The gases from crystalline fructose irradiated at 21 megarep. were chromatographed on a dinonyl phthalate-firebrick C-22 column. The

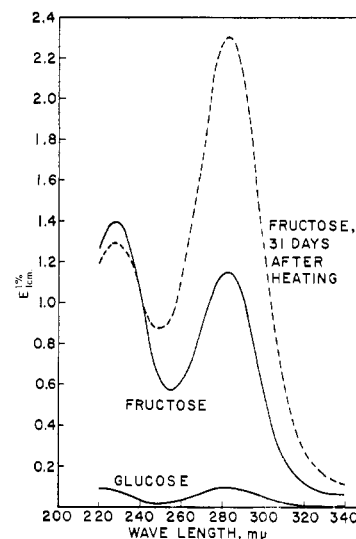


Figure 5. Ultraviolet spectra of heated sugars

chromatogram showed that in addition to hydrogen, a considerable amount of methanol was present. Comparatively smaller amounts of carbon dioxide and formaldehyde were found. The ratio of methanol to formaldehyde was about 40 to 1. The identity of the methanol was confirmed by its infrared spectrum.

Conclusions

The heated sugars gave ultraviolet spectra characteristic of furfural derivatives (Figure 5), indicating that thermal degradation occurs via dehydration. The ultraviolet spectra of irradiated sugars were those characteristics of reductones, enediols, or enols, indicating that irradiation degradation occurs via dehydrogenation. The spectra of irradiated sugars were more like the spectra of enols than like those of reductones; the absorption maximum of a reductone spectrum shifts with pH, but the height of the maximum remains

approximately the same, whereas, for an enol spectrum and the spectra of the irradiated sugars, the maximum is higher at the higher pH value.

Reductones were shown to be present in irradiated crystalline sugars, but not in irradiated sugar solutions. These results suggest that any reductones or their precursors formed by irradiation of sugars in aqueous solution may be destroyed as a result of the greater mobility of the sugar molecules in solution as compared to the crystal. Of course, it is not known whether reductones exist as such in the irradiated sugar crystal, or whether they are formed from a precursor, such as a stable free radical, when the crystal is dissolved. Irradiation of anhydrous (liquid) glycerol produced no reductones.

The spectra of irradiated crystalline sugars do not resemble that of glucoreductone $\left(\begin{array}{c} \text{CH}=\text{C}-\text{CHO} \\ | \quad | \\ \text{OH} \quad \text{OH} \end{array} \right)$, which has

absorption maxima at 263 $m\mu$ below pH 5 and at 293 $m\mu$ above this value, with a molecular absorbance coefficient of 1.8×10^4 . However, it was shown by calculation that the ultraviolet absorption due to any glucoreductone present would be masked by the observed absorption bands. This calculation was based on the assumption that the reductone content of irradiated crystalline sugars, measured by titration with Tillman's reagent, was glucoreductone.

The presence of hydrogen and methanol in irradiated fructose also indicates that irradiation degradation involves a dehydrogenation. Enediols are formed on irradiation of hexonic acid lactones by dehydrogenation (3).

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FEED ADDITIVES ANALYSIS

Colorimetric Determination of 3,5-Dinitrobenzamide in Feeds by the Janovsky Reaction

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An adaptation of the Janovsky reaction for dinitro compounds is presented for the determination of microgram quantities of 3,5-dinitrobenzamide. The method is intended for use with animal feeds containing this medicament. Extraction of the feedstuff with acetone followed by a reaction with concentrated ammonium hydroxide to produce a colored solution is the basis for the method. An alternative method, which also produces a stable color, using anhydrous liquid ammonia is described.

THE INCREASING USE of organic chemicals as medicaments in the feed industry has created the need for methods of analysis which are specific for a compound.

The compound, 3,5-dinitrobenzamide, is now being marketed as a medicinal additive for poultry feeds. It was necessary to devise a method for rapid routine assay of microgram quantities of this material, because it is subject to control under the Texas Feed Control Law.

In 1886, Janovsky and Erb (8) investigated the various bromo and nitro derivatives of azobenzenes and produced color in acetone with potassium hydroxide. Janovsky (7) in 1891 extended this work to include a study of dinitro compounds. He reported on the colors that formed when dinitrobenzenes were dissolved in acetone and treated with an alkali. Through the years several investigators have experimented with this reaction (3, 6, 7, 10, 11). Various reactions mechan-

isms have been proposed (1, 5, 9) and special conditions suggested for making the test.

A variety of other methods, for the determination of other specific compounds, mainly dinitrophenols, have been published. These, however, do not fall within the scope of the colorimetric reaction being studied here and are not cited individually. They include titrimetric, gravimetric, and colorimetric methods.

Dinitrobenzamides were not included in the above tests. A new application of the Janovsky reaction is reported and also a modification which may present a broader application of the color test.

A method for the analysis of 3,5-dinitrobenzamide (4) has been proposed and subjected to study by a group from the Association of Official Agricultural Chemists. This method uses diethylamine reagent and dimethyl sulfoxide to produce a colored product.

Experimental

The first attempt at a color test for the compound 3,5-dinitrobenzamide utilized the classical approach of dissolving 1 to 2 mg. in 10 ml. of acetone, followed by the addition of 2 to 3 ml. of a 5% sodium hydroxide solution. This resulted in the formation of a blue color which faded immediately.

Previous information suggested that the presence of water might be detrimental to the formation of a stable color. To add an alkaline medium in an anhydrous condition, liquid anhydrous ammonia was added to an acetone solution of 3,5-dinitrobenzamide. The first few drops of liquid ammonia had no effect on the solution, because of the volatilization of the ammonia. Upon further addition of the ammonia a magenta color formed. The tube was stoppered lightly to allow the escape of gas and then was set aside. The color faded slowly to light pink, but as the