

## STUDIES ON THE MECHANISM OF COLOR FORMATION IN GLUCOSE SYRUPS<sup>1</sup>

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### ABSTRACT

Two furans, 5-(hydroxymethyl)-2-furaldehyde (HMF) and 2-(2-hydroxyacetyl)-furan (HAF), were identified among the products produced during the heat-decomposition and coloring of a glucose syrup. The yields of both compounds increased with the rate of color formation in the syrup. The mechanism by which these furans are produced (that is, via a nitrogenous Amadori product or via an acid-catalyzed dehydration, both without fission of the carbon chain) was studied by producing HMF and HAF from a syrup which was adjusted to pH 2.5 and which contained D-glucose-2-H<sup>3</sup>. It is known that when D-glucose-2-H<sup>3</sup> is

converted to HMF and HAF via amine condensation and the Amadori rearrangement, radiochemically inert furans are formed; whereas in an acid-catalyzed dehydration reaction of the unsubstituted sugar, the resulting HMF is tritium labeled on the aldehyde carbon and HAF on the hydroxymethyl carbon. The resulting HMF and HAF produced in the syrup were essentially radiochemically inert, thus indicating that these compounds were not produced via an acid-catalyzed non-nitrogenous reaction. They are most likely produced via an Amadori compound in Maillard-type reactions.

The formation of colors and odors during food processing is of considerable industrial importance and, in the case of glucose syrup production, it is important from the aesthetic standpoint, as well as providing an index of purity. Color formation in food products is a complex, generally poorly understood reaction, due largely to the multiplicity of reactive substrates, reaction types, and

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catalysts that can be involved (1-5). In glucose syrups, the catalysts can be numerous inorganic ions, hydrogen ions, or basic amino groups resulting from protein contamination and incompletely removed hydrolytic enzymes.

Although the origin of brown pigments developed in glucose syrups is obscure, the fact that most syrups contain at least small amounts of protein, in addition to reducing sugar, indicates that such pigments might arise via a Maillard-type reaction (2-5) which could involve condensation of reducing sugars with amines, their subsequent rearrangements and decomposition to furan and pyrone derivatives, fragmentation to reductones, and polymerization to pigments. Prior work in this area supports such a concept. Lindemann (6) has shown, for instance, that coloring in starch syrups occurs in proportion to the HMF content and that amino acids are also involved in the browning of glucose syrups. Likewise, Diemair and Jury (7) have examined the role of HMF in nonenzymatic browning and have found positive correlations between HMF content and color formation. Deschreider (8) has also shown that Maillard reactions occur in glucose syrups and that these reactions contribute to syrup discoloration at pH 5.2. Wegener (9) has also concluded that coloration of glucose syrups may be regarded as a special example of the Maillard reaction. Because chromophores (10) are also produced during the heat treatment of carbohydrates in the absence of nitrogenous compounds through simple acid-catalyzed dehydration reactions, a mechanism for color development which does not involve the participation of amino groups cannot be excluded.

In summary, a number of different pathways to color formation in glucose syrups are possible. These include: a) the production of HMF and subsequent condensation with itself or other reactive aldehydes to produce nitrogen-free colored substances, b) the condensation of HMF with amino acids to form nitrogenous brown products, and, c) the condensation of glucose with amines and amino acids, subsequent rearrangement to the ketose, dehydration with and without amine elimination to furans and subsequent polymerization to pigments.

The present work was directed at studies of the mechanism of formation of certain furans (and pigments derived from them) in glucose syrups. These studies involved determinations of the dehydration products found in darkened syrups, studies of relationships between rates of production of dehydration products and color intensity and studies, using isotopically labeled D-glucose in the syrups, to determine if the dehydration products found are produced via an acid-catalyzed reaction or via amine-carbohydrate condensation products (Amadori compounds). For purposes of convenience, the 2 major dehydration products discussed herein, 5-(hydroxymethyl)-2-furaldehyde and 2-(2-hydroxyacetyl)-furan will hereafter be referred to as HMF (I) and HAF (II), respectively.

## MATERIALS AND METHODS

### General Methods

Thin-layer chromatography was run on glass plates using silica gel HF as support and chloroform-methanol (19:1, v./v.) as irrigant. Spots were visualized by either ultraviolet irradiation or by spraying with 10% ethanolic sulfuric acid followed by charring at 110°C. for 10 min. Paper chromatography was performed by the descending method using ethyl acetate-acetic acid-formic acid-

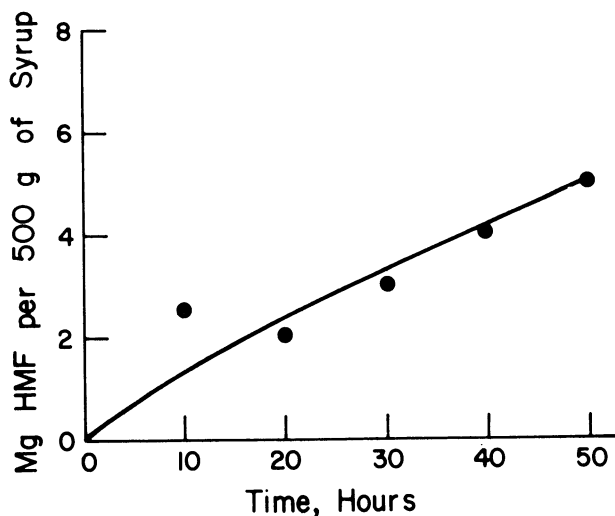


Fig. 1. Rate of formation of HMF in a glucose syrup at 80°C.

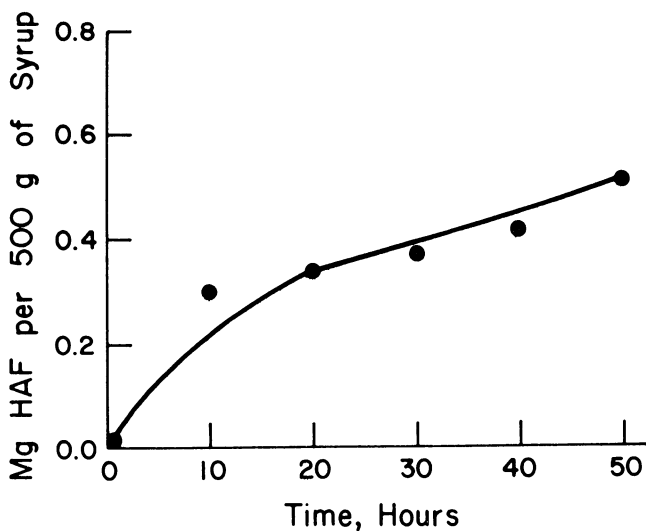


Fig. 2. Rate of formation of HAF in a glucose syrup at 80°C.

water (18:3:1:4 v./v.) as irrigant and aniline hydrogen phthalate (11) as spray reagent. D-Glucose-2- $H^3$  used in the experiments was purchased from New England Nuclear, Inc. as a chromatographically pure material. The glucose syrup was a commercially available 43 D.E. syrup that contained 20% dextrose. This syrup had a pH value of 4.92, a nitrogen content of 0.0076%, and an HMF content of 0.366 mg. per 500 g. as determined using the method outlined in this work.

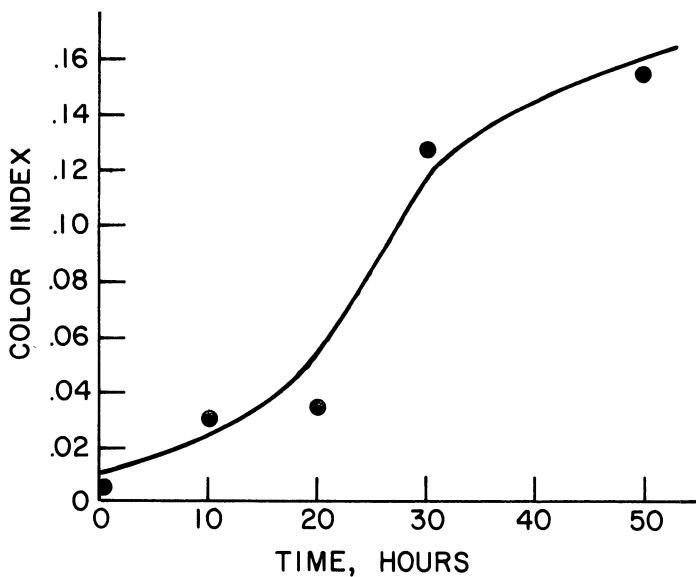


Fig. 3. Rate of color formation in a glucose syrup at 80°C.

Spectrophotometric measurements were obtained on a Coleman model 124 recording double beam grating spectrophotometer. Scintillation counting was done on a Packard Tri-carb scintillation counter using a scintillant made up of 2 parts of a solution composed of 2 liters of toluene, 8.25 g. of 2,5-diphenyloxazol (PPO), 0.25 g. of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (Me<sub>2</sub>POPOP) and 1 part of Triton X-100 (v./v.). Efficiencies were determined using an internal standard of tritiated toluene.

Analyses for furan derivatives involved streaking an aliquot on a thin layer plate and locating the desired band after development, with ultraviolet light. The band was scraped from the plate and the resulting silica gel extracted with three 10-ml. portions of water. The furan contained in the combined extracts was determined spectrophotometrically using the following reference data: HMF,  $\lambda_{\max} = 280$  nm,  $\epsilon = 14,500$ , HAF,  $\lambda_{\max} = 270$  nm,  $\epsilon = 15,000$ . Color index (as shown in Fig. 3) represents the color determined by the standard analysis of the Corn Refiners Association Handbook (12).

#### Extraction and Examination of Syrup

The following procedure was used for the qualitative determination of dehydration products as well as for rate studies. An accurately weighed sample of syrup consisting of about 500 g., after heat treatment at 80°C. for various periods of time, was diluted with 500 ml. of water and extracted with three 200-ml. portions of chloroform. The combined extracts, which contained all the 2-furaldehyde derivatives and related furans, as evidenced by spectrophotometric measurements, were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was made up to a suitable volume and either streaked or spotted on thin layer plates for qualitative or quantitative analysis.

### Dehydration Products from an Amadori Compound

For this procedure, 1-deoxy-1-*p*-toluidino-D-fructose, a representative Amadori compound, was prepared as described by Hodge and Fisher (13). This compound (3.0 g.) was suspended in a solution composed of 80 ml. water and 10 ml. acetic acid and heated at 80°C. for 1 hr. After this reaction, the solution was cooled, percolated through a column of Dowex 50 (hydrogen form), evaporated to dryness at 40°C. using a Buchler flash evaporator, and the residue, after dissolution in 1.0 ml. of methanol, used directly for thin-layer chromatographic studies.

### Isolation of HMF and HAF from a Glucose Syrup Enriched with D-Glucose-2-H<sup>3</sup>

To 500 g. of commercial dextrose syrup was added 3.5 mCi of D-glucose-2-H<sup>3</sup> and the pH was adjusted to 2.5 with dilute hydrochloric acid. After thorough mixing, the syrup was heated at 80°C. for 12 hr., diluted with 1 liter of water and the resulting dehydration products isolated by three consecutive extractions with 200-ml. portions of chloroform. After evaporation to 1.0 ml., the resulting extract was streaked on a thin layer plate and, after development, the zones corresponding to the above furans were located by UV light, removed, and the resulting silica gel extracted with chloroform. The amounts of furan in the extracts were determined spectrophotometrically and inert carrier material added to each aliquot. For purification, the solution which contained the HAF was evaporated to dryness and the resulting HAF was crystallized from hexane and then sublimed at 100°C. and 0.25 mm. prior to counting. The solution containing the HMF was evaporated to dryness, taken up in water, and converted to the crystalline phenylhydrazone. This derivative was combusted to carbon dioxide and water using a Packard Tri-carb Oxidizer and the water so obtained subjected to scintillation counting.

## RESULTS AND DISCUSSION

There is abundant evidence to show that browning, when it occurs in the presence of amines, involves the condensation of aldoses with amines to form glycosylamines which rearrange to Amadori products (1-amino-1-deoxy-2-ketoses) (14). Amadori products then undergo decomposition to reactive dicarbonyl intermediates, reductones, furans, and furanones (15). Such dehydration products undoubtedly play a part in the formation of the brown pigments subsequently produced. A variety of products are produced during the decomposition of Amadori compounds, but in aqueous, slightly acidic solution, the major product arising from a 1-amino-1-deoxy-hexose is HMF, the same 2-furaldehyde as is produced from the acid-catalyzed dehydration in a hexose (16,17).

Several studies support the concept that reactions leading to brown pigment formation involve, at least in part, the condensation of dehydration products which arise from Amadori products. Haas et al. (18), for instance, reported that ethyl acetate extracts of fruit product browning absorbs strongly at 285 nm.—nearly the same wavelength at which many 2-furaldehydes absorb. Underwood et al. (19) isolated and purified a brown pigment from maple syrup and elemental analyses indicate that it is dehydrated with respect to carbohydrate formulas.

When the glucose syrup sample used in this study was heated for 12 hr. at 80°C., visual color, HMF and HAF were produced. The latter two compounds were identified by comparisons of their thin-layer chromatographic mobilities with authentic samples and, after isolation by thin-layer chromatography, by comparison of their ultraviolet spectra with those of authentic samples.

The rates of production of HMF, HAF, and color at 80°C. were measured as shown in Figs. 1, 2, and 3, respectively. The data show that HMF, HAF and color levels all increase with time and are therefore consistent with the furans serving as precursors of colored compounds in glucose syrups.

Both HMF and HAF are readily produced from hexoses on heat treatment with aqueous acid. In order to determine if these furans can also both be produced from an Amadori product, a careful examination of the products of decomposition of 1-deoxy-1-*p*-toluidino-D-fructose was undertaken. Both HMF and HAF were observed as reaction products as evidenced by thin layer chromatographic comparisons. However, the question of whether the HMF and HAF produced in a syrup arise via an acid-catalyzed process or Maillard-type reaction remains unanswered.

In order to prove which pathway operates, the following rationale was used. It is known that, for a simple acid-catalyzed process, D-glucose-2- $H^3$  is converted to HMF and HAF via a C-2→C-1 intramolecular tritium transfer reaction, that is, the HMF is radioactive and labeled on the aldehyde carbon atom and the HAF is labeled on the hydroxymethyl carbon atom (20). Palm and Simon (21) have reported, on the other hand, that when D-glucose-2- $H^3$  is converted to an Amadori product, the isotope is lost from the molecule and is found in the reaction solution. Using this rationale, if a syrup were enriched with D-glucose-2- $H^3$ , heated to induce color formation, and the HMF and HAF so produced were isolated, a determination of their radioactivity would allow a differentiation of reaction pathways. The dehydration products arising from D-glucose-2- $H^3$  via an acid-catalyzed process would be radioactive, while those arising via an intermediate Amadori product would be inert.

When this experiment was performed using 500 g. of syrup (which contained 20% glucose, 3.5 mCi of D-glucose-2- $H^3$ , and was adjusted to pH 2.5) the amount of HAF produced amounted to 0.325 mg. as determined spectrophotometrically on a chloroform extract. This sample was diluted with 50.0 mg. of inert HAF and, after purification, 14 mg. of pure HAF was isolated. This sample corresponds to 0.090 mg. of the original 0.325 mg. sample. The 14-mg. isolated sample of HAF contained  $4.73 \times 10^{-5}$   $\mu$ Ci of activity. Since the 14-mg. sample represents 0.090 mg. of the original 0.325-mg. sample, the specific activity of the isolated material calculates to be .066  $\mu$ Ci per mmole or only 1.0% the activity of the starting glucose. These calculations are based on the glucose content of the syrup (20%) and the sample size (500 g.) which indicate that the glucose in the syrup has an activity of 6.3  $\mu$ Ci per mmole. Considering the known isotope effect (21) for the conversion of glucose-2- $H^3$  to HAF, the resulting HAF should have had a specific activity of 1.90  $\mu$ Ci per mmole if it were produced via an acid-catalyzed process. The fact that it contained negligible activity indicates that the HAF so produced in syrups arises via amine assistance (probably an intermediate Amadori product).

These data were supported by the isolation of HMF during the same experiment. The HMF isolated (55.4 mg.) was diluted with 71.0 mg. of inert

material and converted to the phenylhydrazone which had a specific activity of  $1.5 \times 10^{-2} \mu\text{Ci}$  per mmole.

The tracer experiment in this instance was performed at pH 2.5 because prior studies have definitely established (22) that, in this range of acidity, D-glucose-2- $\text{H}^3$  is dehydrated with transfer of isotope.

The data collected in this study are, thus, consistent with the formation of color in glucose syrups involving, at least in part, a process wherein free glucose reacts with traces of amines present (or residual protein contained by the corn or hydrolytic enzyme protein) to form Amadori products which then decompose to give dehydration products and reactive intermediates which condense with each other to produce pigments. It is noteworthy that this process operates under milder conditions and lower acidity than the acid-catalyzed dehydration reaction, and that the amine is regenerated unchanged after the dehydration reaction.

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