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Browning and the Amadori Rearrangement

PHILIP NORDIN[1] and YEE SIK KIM
Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan, Kan.

The browning of glucose with alanine, glycine, and phenylalanine in solution was compared with the browning of the Amadori rearrangement products of glucose and the corresponding amino acids. The rates of browning and the development of ultraviolet spectra for these systems were recorded. The amount of Amadori rearrangement products formed in a browning system was measured.

THE CHEMISTRY of the Amadori rearrangement and its significance for the nonenzymatic browning of food stuffs has been reviewed (*9*). Reducing sugars are known to condense with amino acids under anhydrous conditions (*1, 7*) and in highly concentrated aqueous solutions (*2-4*). Under these conditions, products from a number of amino acids have been isolated and characterized as Amadori rearrangement products. Richards (*11*) heated glucose and glycine in the dry state at 37° C. and pH 6.7 and obtained a product identified as the enolic form of the Amadori rearrangement product.

In a previous communication, evidence was presented that the Amadori rearrangement took place in bakery products (*10*). In the above references cited, anhydrous or nearly dry conditions prevailed. It was of interest, therefore, to determine whether the rearrangement occurs in dilute aqueous solution, a condition unfavorable to the formation of the nitrogen-substituted aldosylamines, the initial products of condensation. Evidence is presented that the rearrangement occurs in solutions 0.0425*M* in glucose and amino acid and contributes significantly to browning.

Materials and Methods

Amadori Rearrangement Products. 1-Deoxy-1-glycino-D-fructose (DGF), 1-deoxy-1-alanino-D-fructose (DAF), and 1 - deoxy - 1 - phenylalanino - D - fructose (DPhF) were prepared by the procedure of Abrams, Lowy, and Borsook (*1*).

Paper Chromatography. Whatman No. 1 paper, extracted for 24 hours with absolute ethyl alcohol to remove traces of reducing substances, was used. The solvent system of Gottschalk (*7*) (40, 10, and 50; *n*-butyl alcohol, acetic acid, and water) was suitable for these compounds.

Analytical Methods. The absorbance measured at 500 mμ in a Beckman Model DU spectrophotometer was used as a measure of color formation. Development of absorption in the ultraviolet region was also followed with this instrument. Browning observations were made in solutions which were 0.0425*M* in carbohydrate and 0.1*M* in phosphate buffer (pH 6.8), and at a temperature of 93° C.

The method of Abrams, Lowy, and Borsook (*1*) for the determination of Amadori rearrangement products was used with the following modifications. The sample was reacted with 0.1% potassium ferricyanide for 30 minutes at 30 ± 0.05° C. instead of for 5 minutes at room temperature. The changes increased the sensitivity of the method approximately threefold and made it considerably more precise. A straight line was obtained when absorbance was plotted against concentration.

The method as revised is not likely to be specific for Amadori rearrangement products or reliable with a mixture of compounds. However, in this case the compounds were first separated by paper

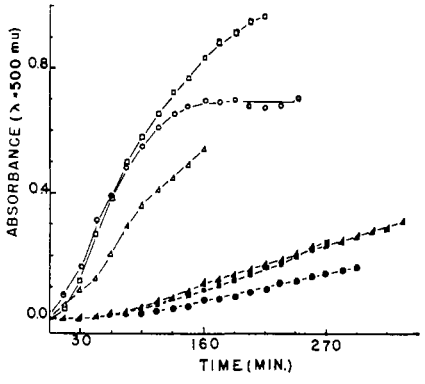

Figure 1. Browning of glucose with three amino acids compared with the corresponding Amadori rearrangement products

○ DAF ● Glucose and alanine
□ DGF ■ Glucose and glycine
△ DPhF ▲ Glucose and phenylalanine

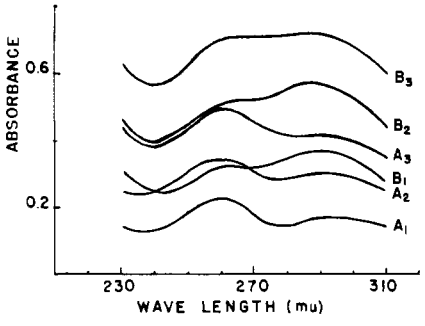

Figure 2. Comparison of ultraviolet spectra of heated DPhF (*B* curves) and of heated solution of glucose plus phenylalanine (*A* curves). Heating times in minutes: A_1-135, A_2-225, A_3-285, B_1-45, B_2-75, and B_3-165

[1] Present address, Chemistry Department, Kansas State College, Manhattan, Kan.

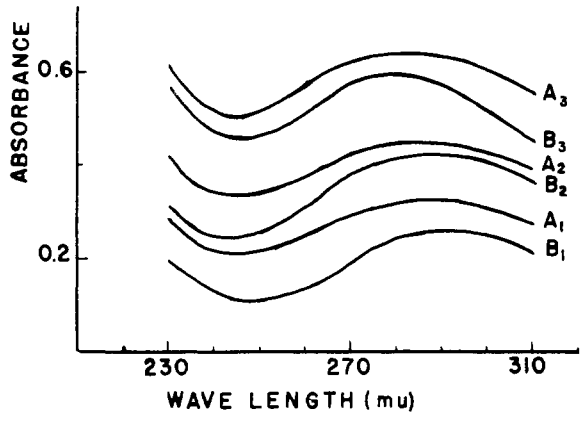

Figure 3. Comparison of ultraviolet spectra of heated DGF (*B* curves) and of heated solutions of glucose plus glycine (*A* curves). Heating times in minutes: A_1-150, A_2-240, A_3-360, B_1-45, B_2-90, and B_3-165

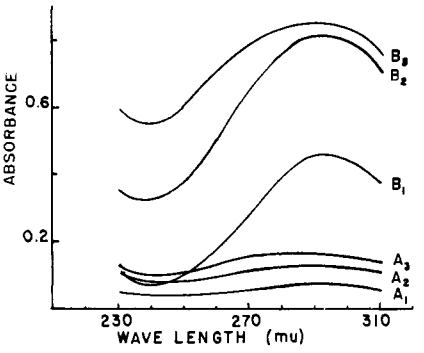

Figure 4. Comparison of ultraviolet spectra of heated DAG (*B* curves) and heated solutions of glucose plus alanine *A* curves). Heating times in minutes: A_1-150, A_2-240, A_3-300, B_1-30, B_2-75, and B_3-165

chromatography and then eluted quantitatively from the appropriate bands located by means of developing strips.

Results and Discussion

Browning of Amadori Rearrangement Products. The browning rates of 1-deoxy-1-glycino-D-fructose, 1-deoxy-1-alanino-D-fructose, and 1-deoxy-1-phenyl-alanino-D-fructose are compared in Figure 1 with those of glucose and the corresponding amino acids. The rates of browning for the three Amadori rearrangement products are similar and are very much greater than those of the parent compounds.

The ultraviolet spectra of the samples were also measured and are shown in Figures 2, 3, and 4. The points have been eliminated from the figures for easier comparison. With all three systems, a peak forms at approximately 290 mμ, which shifts to shorter wave lengths as heating is continued. With the phenylalanine system, an additional peak at approximately 260 mμ appears.

The ultraviolet and browning data have been corrected for browning of glucose alone in the phosphate buffer. A solution of glucose was heated under the same conditions and similar data were collected. The corrections were significant only in the glucose-plus-amino acid systems, reaching a maximum of about 10% for the browning data, toward the end of the heating period.

Formation of 1-Deoxy-1-alanino-D-fructose in a Browning System. One hundred milliliters of a solution 0.0425*M* in glucose and in alanine, and 0.1*M* in phosphate buffer (pH 6.8) were heated at 93° C. Samples were withdrawn at 1-, 2-, and 4-hour intervals. The samples were then examined by paper chromatography. 1-Deoxy-1-alanino-D-fructose could be detected only as a very faint spot with alkaline copper spray (*6*) and with alkaline ferricyanide spray (*7*), although there were other reducing compounds present. To obtain enough 1-deoxy-1-phenyl-alanino-D-fructose for analysis and more definitive tests, 80 λ of solution were chromatographed. The bands corresponding in R_f to that of a standard of 1-deoxy-1-alanino-D-fructose were cut out and transferred quantitatively (*8*) to a second sheet of paper and rechromatographed as a single spot. The spots were eluted (*5*) and analyzed by the method described. Results obtained without rechromatographing were erratic.

The results from this technique indicated that the glucose-alanine system contained 10.5, 11.9, and 6.9 mg. of 1-deoxy-1-alanino-D-fructose per 100 ml. of solution at 1, 2, and 4 hours, respectively. These figures are the means of three separate determinations which checked to within 10%. They have been corrected for a blank reading obtained by cutting similar-sized strips of paper from blank chromatograms developed in the same solvent.

The low levels of 1-deoxy-1-alanino-D-fructose found did not permit a precise identification. Samples concentrated by chromatography for analysis as described above, however, did decolorize 2,6-dichlorophenolidophenol in bicarbonate solution and reacted faintly with ninhydrin, as did also the authentic samples of 1-deoxy-1-alanino-D-fructose.

The results confirm that browning does proceed through the Amadori rearrangement in dilute solutions. If browning proceeds exclusively via this preliminary mechanism, then apparently the rate of browning for the glucose–amino acid system is being limited by the rate of formation of the rearrangement products. An approximately steady state should thus be reached early in the heating at fairly low levels of the rearrangement products. The traces of 1-deoxy-1-alanino-D-fructose found are consistent with this view. This steady-state level could be calculated if absorbance readings at 500 mμ were proportional to the amount of 1-deoxy-1-alanino-D-fructose which decomposed. Unfortunately this is not true. An inspection of the browning curves (Figure 1) will show that they are definitely not first order. In fact they are S-shaped, indicating that the Amadori rearrangement products were converted first to noncolored products. Also the curves tend toward different plateaus, suggesting that noncolored stable products may be formed as well. Thus other contributing mechanisms cannot be ruled out.

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