New Quantitative Approach to the Study of Nonenzymatic Browning

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Simple mixtures of amino acids and sugars are adsorbed on filter paper disks and fried in deep fat to simulate potato chip frying. The course of the reaction is monitored by an amino acid analyzer by placing the filter paper disks directly on the resin columns. The browning reaction intermediates and the residual amino acids are separated and measured. The amino acids involved decrease in concentration (at different rates), while the ninhydrin-active intermediates increase to a maximum and then decrease.

One of the most important reactions in the food industry is that of nonenzymatic browning. In the frying of potato chips, for example, the reaction produces the golden brown color desired for a high quality product, but under other conditions, it produces a dark-colored, bad tasting chip. During browning, food values are lost owing to destruction of sugars and amino acids as reported by Fitzpatrick *et al.* (1965), Fitzpatrick and Porter (1966), and Schormueller and Andraess (1962). A recent review by Reynolds (1963) of the Maillard reaction covers most of the reports to that date. Numerous model systems have been described. Many of these systems employ solutions for carrying out the reaction, but in actual practice, the moisture content of some food products is often quite low during browning.

The frying of potato chips was simulated by Shallenberger *et al.* (1959). They impregnated filter paper disks with solutions of sugars and amino acids. After air-drying, these impregnated disks were fried like potato chips, and the reaction was followed by the amount of color developed. About the same time, Ingles and Reynolds (1958) began studying the extracts of browned, freeze-dried apricots. The extracts were analyzed by elution chromatography on ion exchange columns. Ninhydrin-active peaks were found, in addition to those of the normal amino acids, which were attributed to intermediate compounds between the amino acids and sugars. These intermediates were reported to give the same color yield on a molar basis as the parent amino acid. However, the resulting picture was extremely complicated owing to the numerous overlapping peaks detected. Fitzpatrick (1967) found this to be true when he applied the procedure to potato chips. Recently, Campagne (1963) spotted solutions of sugars and amino acids on sheets of chromatographic paper and heated the entire sheet. The resulting products were separated by electrophoresis.

The authors have simplified the study of the Maillard reaction by combining the above techniques and carrying them a step further. Small filter paper disks were treated with combinations of amino acids and sugars in solution. After drying, they were fried in deep fat and the fat washed out with carbon tetrachloride. The dry disks of paper were then placed directly onto the ion exchange column of an amino acid analyzer, and changes in the ninhydrin-active compounds were determined. Since automatic sugar analyzers are becoming commercially available, it should be possible to study the changes in the sugar components in the same way. In one case, the authors have been able to follow the aldehyde formation from the amino acid, using gas chromatography to monitor the reaction (Filipic *et al.*, 1967; Buttery and Teranishi, 1963). This model system can also be used to simulate storage problems in dehydrated foods under controlled conditions. Dehydrated potato samples have become unusable during storage for a period as short as 3 weeks as a result of the interaction between sugars and amino acids (Willard, 1966).

EXPERIMENTAL

Nine-millimeter disks of filter paper (Whatman No. 1) were placed in groups on a sheet of water-repellent plastic such as polyvinylidene chloride or Saran Wrap. Hoover (1967) has suggested the use of thicker paper, such as Whatman No. 3MM, or possibly a glass fiber paper. Also, he suggested that the moisture content may be adjusted to higher values such as 80%. The 9-mm. disks probably can be purchased commercially but the authors punched them from larger circles by means of a punch (Evans and Convery, Philadelphia, Pa., M. C. Mieth, Port Orange, Fla., Style No. 448, 23/64-inch diameter). The disks were treated individually with measured quantities of water solutions of the materials to be tested and allowed to air-dry between applications. Each disk was loaded with 10 μl. of a 0.1338M solution. Six of these loaded disks gave a suitably sized sample for an amino acid analysis. For applying the solutions, a 0.5-ml. capacity Hamilton gastight syringe fitted with a repeating dispenser unit, which produces a 10-μl. sample per stroke, was employed (Thomas *et al.*, 1965). As soon as the paper disks were air-dry, the sets for each sample were folded into circles of filter paper and stored in a box. Some were left to stand at room temperature. Others were fried in deep fat using small baskets (2.5 × 2 × 1 cm.) made of stainless steel screening. A set of disks was placed in the basket, the basket picked up with tweezers and placed in the hot fat in a domestic-type, deep fat food fryer. After the frying operation, the basket and contents were placed

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on a small Hirsch type funnel (bottom diameter 11.5-mm. I.D.) on a suction flask. With the suction off, carbon tetrachloride was added until the funnel was nearly full. The basket was removed after transferring the disks to the funnel with tweezers. Suction was applied, and the set of fried circles was stacked so as to cover the holes of the funnel. The disks were held in place with a pair of tweezers and slowly washed with more CCl_4 from a 5-ml. syringe until the fat was removed. The disks were air-dried by suction and repackaged in filter paper to await analysis. All analyses were made using a Phoenix precision automatic amino acid analyzer, Model K-5000 modified for computer calculation of results.

For analysis, the buffer was removed from above the resin column in the normal fashion, and six disks of a set of filter paper disks were placed on top of a disk of porous Teflon which had been placed on the resin bed. The disks were pushed nearly into place with a piece of polyethylene tubing and then completely with the long-needled syringe used to add rinses and buffer to the column. Three rinses of the appropriate buffer were added in the usual way; the column was filled and the pumps started. (Old disks were removed before adding a new sample by means of a stainless steel screw welded to a stainless rod.) The elution program was the same as that reported by Zacharius and Talley (1962a) for the normal amino acid analysis.

The results of the analysis were calculated in the usual manner (Porter and Talley, 1964), using the calibration constant of the parent amino acid for the new peaks.

RESULTS

The work done to date has been primarily of an exploratory nature, but the results shown illustrate the scope of the method. Figure 1 illustrates the type of analog record obtained with threonine and glucose (the long path, 570-mμ trace only is shown). The large peak at about 160-ml. effluent volume results from threonine itself, and the main intermediate appeared at about 72 ml. This sample was heated for 8 minutes at 103° C.

Figure 2 shows the changes resulting when threonine and glucose were heated in equimolar quantities for different periods of time. The small peaks are not shown because they would appear as traces. The maximum value for the peak at 72 ml. occurred at about 15 minutes of heating at 103° C. and then decreased with longer heat treatment. The curve showing the residual amino acid is based upon the amount recovered at the normal effluent volume. It does not include any of the amino acid which has reacted with some other constituent or which has decomposed. Most of the amino acid is not recoverable after 8 hours of heating. The intermediate peaks have also essentially disappeared at this point.

With valine in the presence of glucose, two fairly large intermediate peaks were found. The curves had the same general shape as with threonine, except that the loss of valine was somewhat faster. (The effluent volume of valine itself was 370 ml. with the first main intermediate at 97 ml. and the second at 101 ml. The two intermediates were not resolved when the concentration of the first was low and the second high.) The first intermediate

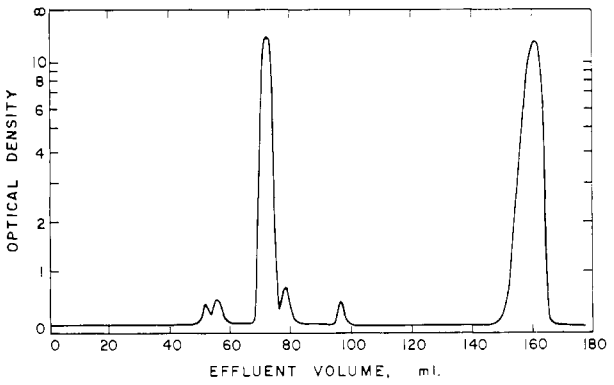

Figure 1. Effluent peaks obtained with glucose heated with threonine at 103° C. for 8 minutes—neutral and acidic analyzer column, 570-mμ trace only

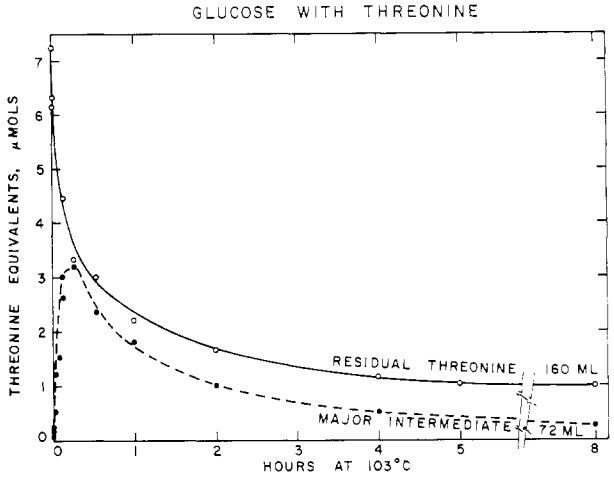

Figure 2. Threonine and glucose—changes in concentration of threonine and major intermediate with time of heating

attained its maximum value at about 2.0 μmoles after about 5 minutes' heating at 103° C., and the second intermediate had the value of about 2.4 μmoles after about 20 minutes. All the values were lower than those with threonine at the same time of heating.

Isoleucine and glucose were tested at 0.10 and 0.05*M* concentration levels (equimolar ratios of amino acid and sugar). Here only one major intermediate was found, as in the case of threonine. The concentrations of the components at various heating times were about proportional to the original concentration levels.

Lysine with glucose and fructose gave similar patterns with fructose reacting considerably slower. Two major intermediates were found in each case, 94- and 129-ml. effluent volumes to be compared with lysine at 258 ml. (basic column). The glucose intermediates peaked at about 4 minutes' heating, while the fructose ones peaked at about 20 minutes. Sucrose was very much slower reacting. The intermediates were present in trace amounts, and around 80% of the original lysine was recoverable as lysine after 8 hours' heating at 103° C. Lysine heated on paper without added sugar showed only a slight loss (about 4 to 5% in 8 hours).

DISCUSSION

Preliminary investigations were carried out by treating standard, commercial-sized disks of filter paper with solutions of mixtures of single amino acids and sugars and drying them on plastic sheets. For analysis, small 0.9-cm. circles were cut out with a cork borer. However, it was difficult to obtain check results owing to nonuniform distribution of the sample components over the disk area. Use of the 0.9-cm. circles gave good reproducibility. The plastic material is not wetted by water and, therefore, the entire sample remains in the filter disk. It may be possible to heat the disks in an oven or other device, but the deep fat treatment does not expose the disk sample to air during the heating period. If the peaks being eluted are suitably spaced, efficiency of operation and analysis can be increased by adding a second sample to the same ion exchange column at a suitable point in the same run.

As shown by the results obtained, the interactions of amino acids and sugars are complicated, even when only one of each is involved. However, study by this procedure lowers the possibilities and interpretation becomes manageable. This technique allows changes to be followed under conditions that are encountered in practice.

The results, to date, confirm the view that amino acids combine with sugars to form intermediate compounds. The concentration of these intermediates builds up to a maximum and then decreases as further heating occurs. The concentration of the intermediate compound, at any particular moment, is probably the resultant of synthesis of the compound and its loss as it enters into further reactions in the system. At the maximum shown, these reaction rates would be equal. The trace peaks noted may be due to compounds forming further along the reaction chain. They may have a low ninhydrin color yield or even may be nonnitrogenous materials similar to those reported by Zacharius and Talley (1962b). The available amino acid is decreased as the reaction proceeds.

It should be possible to isolate and characterize the intermediate compounds by using the scaled-up ion exchange procedure of Talley and Porter (1960). If and when these are isolated, they can be employed as starting materials, and further information can be obtained as to the ultimate course of the reaction.

The treated disks of filter paper can be stored under different conditions of moisture, atmosphere, temperature, etc., to check the various factors that may be involved in the processing and storage of foods. Possibly the results may be different if the papers are heated in air rather than under the more or less anaerobic conditions of frying in deep fat. Various compounds may be added to test for possible inhibitory effect. Of course, the disks may be fried at various temperatures. For the most part, 103° C. has been used in the tests discussed since, in potato chip frying, the temperature of the piece is close to that of boiling water until the water is gone. This temperature also causes a fairly rapid reaction, yet the reaction is slow enough that one may follow the steps involved. Higher temperatures, such as near the temperature of the frying oil used in chip plants, may be tested readily and possibly may indicate different intermediates (Hoover, 1967). [Campagne's (1963) conditions were sufficiently severe to destroy the intermediate compounds and only the final products were found.] For storage applications, room temperature or lower might be more useful.

The technique illustrated here allows tests to be conducted under conditions that are encountered in practice. The picture is simplified to the point where the changes may be followed readily. Controlled conditions over a wide range may be used. After the simple combinations are worked out, additional factors may be added to make the final conditions as complicated as needed to study all the interactions which may be involved. Thus, the technique should be very useful for studying processing and storage of foods.

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