Separation of Caramel Color from Salts and Sugar by Gel Filtration

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Ion exchange was unsatisfactory in isolating the colorants of maple sirup because it failed to separate the colorant from mineral components. A gel filtration method, using cross-linked dextran, was developed and tested in this laboratory. Acid proof caramel colorant could be completely separated from ash salts and sucrose in one pass through a Sephadex column.

As with many foods, the color of maple sirup is an important factor in establishing its grade, quality, and price. In the investigation of the colorants of maple sirup it was necessary to obtain the color isolates. Ion exchange resins (5, 8) were unsatisfactory since the prescribed methods of elution introduced appreciable amounts of salts, and leaching of colored materials from the ion exchange resin introduced contaminants. In addition, some colorants were found to be bound irreversibly on the resin (3), and amino groups of anion exchangers can undergo oxidation or a Maillard-type reaction with carbonyl groups (7). While the ion-exclusion method permitted separation of the colorant of dark molasses from sucrose, it failed to separate the colorant from the mineral components (1). Gel filtration, using cross-linked dextran, has been developed for separating molecular species on the basis of their size (4).

This paper describes a method of gel filtration which permits the separation of relatively high amounts of caramel colorants from sugar and salts.

Experimental

To check effectiveness of color separation by this method, three solutions were prepared and labeled A, B, and C. Solution A, containing only commercial caramel color[1] and water, was prepared by adding caramel color dropwise to distilled water until a visual match was obtained with the color standard for medium amber maple sirup (138 MacAdam chromaticity units) (2). This solution served as a control in the flame photometric determination of the ash constituents as described below. Solution B was prepared by dissolving 2640 g of sucrose in 1360 ml of water which contained 8.0 g each of potassium malate, sodium acetate, and calcium acetate to approximate the concentrations of organic acid salts in maple sirup. Caramel color was then added to obtain a visual match with Solution A, using a Duboscq colorimeter. This solution was used to determine the effectiveness of gel filtration in the separation of the colorant. Solution C, containing 6% sodium chloride, was prepared in the same manner as Solution B by substituting a $3N$ sodium chloride solution for the organic salts. This was used for checking effectiveness of the separation of colorant from solutions having a very high ratio of salt to colorant.

To reduce viscosity, 150 ml portions of the above sirup solutions and the control solution were diluted to 200 ml before being added to the columns of dextran gel. The dextran columns were prepared by slurrying 475 g of the dextran gel (Sephadex G-25, 50–270 mesh, manufactured by Pharmacia, Uppsala, Sweden) with water, and transferring the slurry to a 120 × 5 cm i. d. chromatographic column, allowing the excess water to drain to the top of the Sephadex. The dilute solutions of A, B, and C were washed through their respective columns with 3 L of distilled water at approximately 12 ml per min., using a 4 ft hydrostatic head, and the eluate was collected in 20 ml fractions.

Each fraction was analyzed for sugar content by the anthrone reaction (6). The concentrations of potassium, calcium, and sodium in the fractions from Solution B were determined by flame photometry. The corresponding fractions from Solution A, the commercial colorant which contained sodium, were used for reference in the sodium determinations. The fraction of eluate obtained from Solution C (high concentration of sodium chloride)

[1] Twitchell Company, Camden, N.J., acid-proof grade. Mention of company and trade names does not imply endorsement by the Department over others not named.

containing the first trace of the salt was detected by precipitation of the chloride ion with silver nitrate. The color of all individual fractions was estimated by visual comparison with MacAdams chromaticity standards.

To determine recovery of caramel colorant from the dextran column, 150 ml of Solution B was passed through a Sephadex column. All of the colored eluate fractions were combined, yielding 560 ml of solution, and the color was compared to 150 ml of Solution B diluted to the same volume, using a Duboscq colorimeter. The dry weight method was not used for determining colorant because of the unknown composition of the caramel and the danger of causing degradation of the caramel on drying. Absorption spectroscopy was not used because absorption at one wavelength would not necessarily detect the removal of one or more color compounds by the Sephadex.

Results and Conclusions

By using the gel filtration method, it was possible to completely separate acid proof caramel colorant from ash salts and sucrose in one pass through a Sephadex column. A large column of Sephadex (120 × 5 cm i. d.) had to be used since smaller columns, 20 × 2 cm i. d., did not completely separate the colorant after two or more passes. The amount of colorant in MacAdams chromaticity units and amounts of cations (calcium, potassium, and sodium) in the eluate are shown in Fig. 1. The combined weights of the three cations of organic salts were used since they were all eluted in the same fractions. Colorant was completely separated from the organic salts, sucrose, and sodium chloride, as no traces of these materials were found in the colored eluates.

As indicated in Fig. 1, the first 680 ml of eluate was colorless. Apparently the dextran did not retain any colorant. The next 560 ml of eluate contained all of the colorant added to the column, most of it in the first 300 ml. The cations first appeared in the eluate fraction immediately following the elution of the colorants (1240 ml). The sugar and the chloride ion appeared later.

These results indicate that gel filtration provides a simple technique for isolating the higher molecular weight caramel colorant from solutions of sugars and salts.

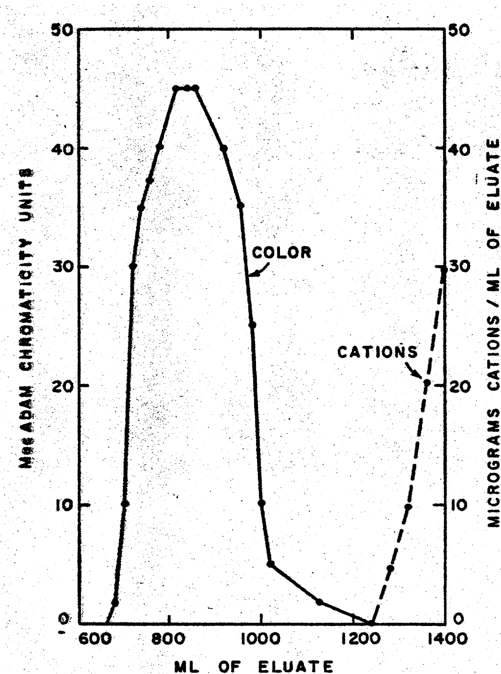

Fig. 1—**Separation of caramel colorant by gel filtration from solution containing salts and sugar.**

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