interferes with the HBB detection even though the extract was not cleaned up.

Table I shows the levels of HBB in the dam liver and in various tissues of 17-day old pups. The HBB level found in the pup liver is approximately 3 times that of the dam and the standard errors of the mean are very small indicating the reproducibility of the determination.

CONCLUSION

The method for extracting HBB using acetonitrile/hexane partition is quantitative. It is simple and does not require cleanup of the extract. The initial extraction step is compatible with that for analysis of esterase activity since the initial aqueous homogenate can be used in both residue and enzymatic analyses. The method is efficient and time saving.

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A High-Pressure Liquid Chromatographic Method for the Quantitation of Neohesperidin Dihydrochalcone

Neohesperidin dihydrochalcone was resolved from filtered grapefruit juice by high-pressure liquid chromatography (HPLC) using a micro C-18 column and eluting with a water–acetonitrile system. Detection was accomplished at 280 nm.

Neohesperidin dihydrochalcone (NHDHC), a nonnutritive sweetening agent which is 20 times sweeter than saccharin on a molar basis (Horowitz and Gentili, 1963), is prepared from the flavonoid glycosides neohesperidin (Horowitz and Gentili, 1969) or naringin (Krbechek et al., 1968). Since there is a possibility that NHDHC may be employed in the citrus industry as a nonnutritive sweetener of grapefruit juice, a simple rapid analytical assay for this compound became desirable. This paper reports such a procedure.

MATERIALS AND METHODS

Apparatus. A Model ALC 202 high-pressure liquid chromatograph (HPLC) with a Model 6000 A pump and U6K injector (Waters Associates, Milford, Mass.) was used. The recorder was a Texas Instruments Servo/Riter II 2-pen. A Schoeffel UV–visible liquid chromatography analyzer Model SF 770 (Schoeffel Instrument Corp., Westwood, N.J.) was the detector. A Spectra-Physics Integrator (minigrator, Spectra-Physics, Santa Clara, Calif.) was used. A Waters Associates sample clarification kit with 1.2 or 0.45 μm Millipore aqueous filter system was used.

Column. A Waters Associates 30 cm × 4 mm i.d. reverse phase μ Bondapak C-18 column (octadecyltrichlorosilane chemically bonded to <10 μ Porasil packing) was used.

Reagents. The eluting system was water–acetonitrile, 75:25, v/v. Both solvents were degassed.

Sample Preparation. Fresh, hand-squeezed, processed single-strength grapefruit juice or reconstituted concentrate which had been sweetened with NHDHC was filtered.

High-Pressure Liquid Chromatographic (HPLC) Resolution and Quantitation of Neohesperidin Dihydrochalcone. An aliquot (50 μl) of the above filtered juice was injected onto the column with a flow rate of 1.5 ml/min. Detection was accomplished at 280 nm with 0.1 absorbance unit full scale. Integration was conducted at an attenuation of 1.0, peak width setting of 41, and slope sensitivity of 270. The recorder chart speed was 12 in./h.

The quantity of NHDHC in unknown samples was determined from a linear regression equation. This equation was obtained from eight standard samples of NHDHC over the range of 0.1–1.0 μg. These samples were eluted isocratically and detected under the above conditions.

Percent Recovery and Precision. The reliability of the procedure was determined by a series of recovery experiments in which a base sample of grapefruit juice was fortified with known amounts of NHDHC. Five individual samples were fortified with sufficient NHDHC to provide a concentration of 3–15 ppm of NHDHC in 3-ppm increments.

The repeatability of the method was determined by analyzing five aliquots from a grapefruit juice sample containing NHDHC.

RESULTS AND DISCUSSION

This procedure constitutes a simple, rapid, quantitative determination of NHDHC. The time required for a complete analysis was 20 min. The samples are filtered to remove particulate material which may clog the system.

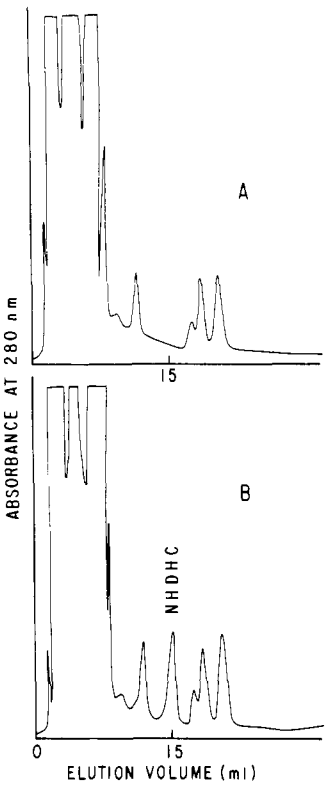

Figure 1. Chromatogram A represents fresh or processed grapefruit juice. Chromatogram B is processed grapefruit juice containing 7 ppm of NHDHC. For experimental details, see text.

The NHDHC was eluted isocratically after approximately 15 ml (10 min) (Figure 1). The number of theoretical plates for the column, using NHDHC as the reference peak, was 2650, equivalent to a plate height of 0.11 mm. The column capacity factor, k', was 4.8.

The purity of the NHDHC used as a standard was established by HPLC. The recoveries of NHDHC from the five fortified samples were all within ±8% of the NHDHC added. The range of NHDHC found in the five repeatability experiments was 7.6–8.3 ppm, with a mean of 7.9 and a standard deviation of ±0.29.

A plot of peak areas vs. micrograms of NHDHC showed linearity over the range of 0.1–1.0 μg (r = 0.993). This covers a span of 2–20 ppm, embracing the 3–15-ppm range which Fellers (1976) indicates should cover any addition of NHDHC to grapefruit juice for sweetening purposes.

Figure 1A shows that fresh, hand squeezed grapefruit juice has no interfering peaks at an elution volume of approximately 15 ml. Processed juice obtained from six Florida citrus canning plants showed the same pattern as seen in Figure 1A. Chromatogram B shows the elution volume (15 ml) for NHDHC, which represents a concentration of 7 ppm of NHDHC in grapefruit juice.

Taste panel studies (Fellers, 1976) indicated that "U.S. Grade A" grapefruit juice at a Brix to acid ratio of about 9 is not significantly sweetened when adjusted to a concentration of 6.7 ppm of NHDHC. However, at 9 ppm, the juice was significantly sweeter and highly preferred over the corresponding control juice. The threshold value appeared to be about 7.6 ppm. The taste panel studies also suggested that these values are considerably variable depending upon juice quality and individual tasters. Feeding studies on rats have, thus far, shown this sweetener to be free of ill effects (Booth and Robbins, 1968). However, NHDHC has not been approved as yet by the FDA.

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