PRELIMINARY INVESTIGATIONS OF THE FLAVONOID PIGMENTS IN PRUNES

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In the course of a search for rich natural sources of flavonoid pigments, the commercial prune has been investigated. Earlier work by Scarborough (1) has indicated that aqueous extracts of prunes are a good source of "vitamin P-like" flavonoid pigments. Scarborough, however, did not isolate the flavonoid pigments of prunes.

Preliminary studies in this laboratory have indicated the presence of at least one, and probably three, flavonoid pigments in a sample of prunes.

EXPERIMENTAL

Seven hundred thirty-five grams of pitted Sunsweet prunes were ground in a Waring blender and then extracted with three 200 ml. portions of boiling acetone. The third extract contained only negligible amounts of flavonoid materials as evidenced by negative qualitative flavonoid tests. The acetone was allowed to evaporate at room temperature in the hood, water being added from time to time to maintain a final volume of 150-200 ml. A light tan solid separated as the acetone was replaced with water. The resulting aqueous suspension was heated to boiling and filtered. The tan colored amorphous residue was discarded. The filtrate was evaporated to a thick syrup which contained a considerable amount of brown solids. Fifty milliliters of ethyl alcohol was added to the concentrated syrup, and the mixture was heated to boiling on the water bath. The sugar which deposited on cooling the solution was removed by filtration. The filtrate was then concentrated to approximately 20 ml. and poured into 60 ml. of boiling water. A tan colored precipitate separated from the solution on cooling. This precipitate gave strong positive tests for flavonoid material. The solid was separated by centrifugation and extracted with two 10 ml. portions of hot iso-amyl alcohol. The bright yellow amyl alcohol solution was separated from a small amount of brown, gummy residue and evaporated to dryness at room temperature. The residual yellow solid was recrystallized from ethyl alcohol by addition of water. The recrystallized product gave positive flavonoid tests with the following reagents: alcoholic ferric chloride; basic lead acetate; concentrated sulfuric acid; dilute ammonium hydroxide, alcoholic hydrochloric acid and magnesium metal (cyanidin reduction test).

The composition of the flavonoid fraction from prunes was studied by means of paper chromatography. Table I lists the solvent systems used and the R_f values obtained.

The aqueous layer of each of the above two-phase solvent systems was used for the development of the chromatograms. Three distinctive colored zones were located on each of the developed paper chromatograms.

In an attempt to separate larger amounts of the three pigments, partition chromatography on corn starch was tried. A chromatographic column, 2 cm. x 20 cm., was prepared by means of a butyl alcohol slurry of corn starch. Twelve drops of a concentrated alcoholic solution of the flavonoid fraction from prunes was deposited on the top of the corn starch column. The column was then developed with butyl alcohol saturated with water. Three bands of yellow color were formed during development. The bands were collected separately as they washed through the column. Attempts to obtain ultraviolet absorption spectra of the separated pigments, after evaporation of the butyl alcohol and solution in ethyl alcohol, were not successful. Traces of other materials which absorbed in the ultraviolet region were also removed from the starch column along with the flavone fractions.

TABLE I

R_f Values of the Three Pigments Isolated from Prunes

SOLVENT SYSTEM	R_f VALUES 1	2	3
Chloroform-isopropyl alcohol-water (20-40-40 volume %)	.80	.71	.25
Carbon tetrachloride-isopropyl alcohol-water (10-80-10 volume %)	.80	.85	.45
Petroleum ether-isopropyl alcohol-water (10-80-10 volume %)	.80	.88	.58
Cyclohexane-isopropyl alcohol-water (10-80-10 volume %)	.78	.85	.59

Investigations on the identities of the flavonoids present in prunes are in progress.

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LITERATURE CITED

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