

for the apparent flavor difference between typical and atypical fruit or whether other sites of chemical difference could be detected if the higher boiling fraction and fatty acid compounds were examined. The latter did not elute from the columns used nor did basic dissociating compounds such as methyl anthranilate. Conditions were too mild for high boiling compounds to elute.

The information reported here should be of use in developing an objective test for significant compounds which may affect flavor in fruit juices.

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STORAGE CHANGE

5-Hydroxymethylfurfural in Stored Foam-Mat Orange Powders

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Deteriorative effects of storage on orange powder were studied using extracts of powders in comparison with the concentrate from which they were prepared. A solvent extraction was applied to orange concentrate and to foam-mat dried powders. Two samples were compared: one stored at -90° F. and the other at 100° F. The extracts from the concentrate and the two powder samples were compared by thin-layer chromatography. One compound peculiar to the 100° F. stored powder sample was isolated by elution and purified by rechromatographing. Comparison by infrared and ultraviolet spectrum, by R_f and color reaction with anisaldehyde- H_2SO_4 spray reagent, and by mass spectral data indicate that it is identical with authentic samples of 5-hydroxymethylfurfural. This method is rapid and reliable and may provide the basis for a quality control test.

INFORMATION concerning the effects of dehydration on chemical composition of foods is increasingly needed. Dehydration processes are widespread among the food industry, but up to now, research related to dehydration has been directed mainly toward process development, especially in the foam-mat drying process (6, 9). Information concerning the effects of dehydration on chemical components of foods and the chemical effects of storage of dehydrated foods is relatively scarce. Studies of changes which occur upon dehydration or storage could lead to improvements in processing methods, as well as quality control procedures.

Relatively nonvolatile components ap-

pear to be particularly influential in quality changes in foods which contain a very high percentage of carbohydrate where nonenzymic browning reactions may occur (4). Foods of this type often form sugar-fission or sugar-amine condensation products in the early stages of these reactions. In studies of dehydration of orange juice under various time-temperature conditions using the foam-mat process (7), it has been found that detectable flavor changes may occur in the powders after a few weeks of storage at 85° or 100° F. Since orange juice solids contain a high percentage of sugar, it appeared that the factors which cause these flavor changes might include the formation of some of the products associ-

ated with nonenzymic browning.

Since flavor and aroma can be influenced by extremely small amounts of material, an extremely sensitive method would be required to investigate these materials and any material which might serve as an index of storage history or processing treatment would have to be detected in extremely small amounts or in early stages of change to be useful in quality control. One of the most sensitive and reliable methods for analysis of relatively nonvolatile materials is thin-layer chromatography (TLC). A study was undertaken with the objective of developing this technique for analytical study of foam-mat dried orange powders and orange concentrates.

Among the chemical compounds which form during storage of high-sugar foods is 5-hydroxymethylfurfural (HMF). The measure of HMF has been suggested as an objective criterion for storage changes in tomato paste (8). Its concentration is related to the quality of stored applesauce (7), and storage at lower temperature resulted in a slower rate of HMF formation. Both processing temperature and storage temperature affect the HMF content of honey (12). These findings suggested that HMF might form during dehydration or storage of orange powders.

Experimental

Twenty pounds of orange powder were prepared on a foam-mat dryer using a 58° Brix Valencia orange concentrate, 12.5 minutes' drying time, and 77° C. drying temperature as described by Bissett *et al.* (1). To promote development of "off-flavors" during storage, the powder was deliberately made with a high moisture content (3.5%). The material was canned and divided into experimental and control lots. The experimental samples were stored at 100° F. to encourage reactions leading to a characteristic off-odor and flavor in order to provide a sample for study of the chemical changes accompanying the off-flavor. The control samples were stored at -90° F. The samples were compared by TLC with samples of the frozen concentrate (stored at -5° F.) from which the powder had been made (reference sample).

For TLC, a slurry of 30 grams of silicic acid (Bio-Sil A, 10 to 30 microns with binder) in 60 ml. of 0.02N sodium acetate solution was applied to 5- \times -25-cm. smooth glass plates using a TLC applicator (Research Specialties Co., Model 200). Plates were developed in a mixture of benzene, ethanol, water, and acetic acid at a ratio of 200:47:15:1 as described by Hay, Lewis, and Smith (3). They were dried in air and examined under ultraviolet light for fluorescent spots. These were marked; then the plates were sprayed with anisaldehyde spray reagent in H₂SO₄ as described by Stahl and Kaltenbach (10) and Bobbitt (2). The sprayed plate was heated to 160° C. for 3.5 minutes for color development.

Pure 5-hydroxymethylfurfural was obtained in an ampoule under nitrogen from Fluka A G, Buchs S G, Switzerland. The pure sample had a molecular weight of 126 and melting range of 31° to 32° C. No impurities were detectable by thin-layer chromatography, and only a few slight traces of extraneous compounds were indicated by gas chromatography. For both the commercial sample and the isolated fractions, ultraviolet absorption spectra were determined using a Cary 14 recording spectrophotometer, infrared spectra using a Perkin-Elmer 137 Infra-red, and molecular weight and cracking patterns using a Bendix time-of-flight mass spectrometer.

Procedure

A weighed amount of orange concentrate or orange powder was stirred with a

solution consisting of equal parts of acetone and water. For each gram of solids 1.7 ml. of the solvent mixture were used. Generally, 168 grams of solids were extracted at a time. This mixture was held for 30 minutes with occasional stirring at room temperature, then extracted four times with 150 ml. of diethyl ether. To the combined ether extracts was added 100 ml. of water. This mixture was evaporated under vacuum until almost all of the ether and acetone were removed, transferring most of the dissolved material to the water layer. At this point there was a precipitation of water-insoluble material which was removed by filtration. The filtrate from the experimental powder differed in odor from that of the control powder or the original concentrate.

Ten grams of sodium chloride were added to this filtrate and it was re-extracted three times with 150 ml. of diethyl ether. The combined ether extracts were washed with salt water, treated with sodium sulfate to remove remaining water, and evaporated to dryness under vacuum. The residue was dissolved in 4 ml. of a solution containing equal parts of ether and acetone. This solution was examined by TLC.

Following the procedure outlined, extracts from orange concentrate and from the two samples of orange powder were compared on the basis of gross fluorescent patterns, *R_f* values, and colors developed with anisaldehyde. Where specific TLC spots appeared of interest, further separation was achieved by scraping and eluting the section desired and rechromatographing the eluate. After repeated treatments, when TLC indicated the fraction was relatively pure, the eluate was concentrated and used for determining ultraviolet and infrared absorption spectra and molecular weight and cracking patterns by mass spectrograph.

Results and Discussion

The TLC procedure described here resulted in a highly reproducible pattern, enabling the separation of 14 spots detectable by anisaldehyde spray reagent (Figure 1). According to Stahl and Kaltenbach (10), this anisaldehyde reaction is particularly sensitive to carbohydrates and steroids. Bobbitt also described its use in identification of steroids (2). Although the exact nature of the color reaction is not established, it is believed to involve a reaction similar to the K \ddot{a} gi and Miescher reaction for steroids (5). Thus, it is likely that most of the compounds separated are in the carbohydrate or steroid class. The four yellow spots have been separated and identified as flavonoids (17). Current studies of these extracts were confined to the spot which appears to reflect a difference between the experimental powder and original concentrate or the control powder.

In the extract developed from the described procedure, there appeared to be only one spot which was peculiar to

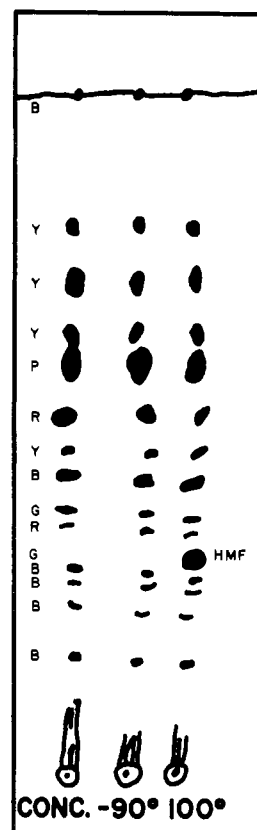


Figure 1. TLC patterns from frozen orange concentrate and orange powders stored at -90° and 100° F.

B — blue, G — green, R — red, Y — yellow, P — purple, HMF — hydroxymethylfurfural

the experimental powder, fifth from bottom, HMF in Figure 1. This fraction was eluted and purified as described above. It gave an infrared absorption spectrum identical to that of a sample of pure 5-hydroxymethylfurfural. Both the pure known sample and the eluted fraction gave identical ultraviolet absorption spectra in 95% ethanol solutions, showing absorption maxima at wavelengths of 227 and 281 μ . Mass spectrometric determination showed the eluted material to have a molecular weight of 126, the same as that of the pure sample of 5-hydroxymethylfurfural. The isolated fraction and the known sample of HMF also yielded identical mass spectrographic cracking patterns. Both the known and the unknown showed the same *R_f* values by TLC, and both turned dark green when treated with the anisaldehyde spray reagent. Both appeared as dark purple spots when observed on thin-layer plates under ultraviolet light.

A very sensitive method for the detection of hydroxymethylfurfural has been found. As little as 0.1 μ g. can be detected although the green color does not become apparent until the con-

centration reaches approximately 0.5 μg . In lower concentrations the color is a rather faint bluish gray. Since the formation of 5-hydroxymethylfurfural is one of the earliest indications of storage changes in several different types of food materials, this method should find a wide applicability in quality control and food research laboratories.

Although taste panel studies have indicated that HMF is not responsible for the flavor considered characteristic of stored orange powders, its detection may be the first indication of storage changes.

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MOISTURE DETERMINATION

Rapid Estimation of Dried Fruit Moisture by Refractive Index

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Moisture of dried prunes, raisins, figs, and apricots was estimated by the use of a refractometer. The refractive index of ground, unfiltered, dried fruit correlates with moisture as determined by vacuum oven. Statistical evaluation indicates that the precision of the refractometric method is excellent.

THE STABILITY, texture, and appearance of dried fruits depends to a large extent upon their moisture content. Frequent moisture measurements are made during production to ensure fruit of optimum quality. The literature is replete with discussions and reports of moisture analyses, but workers in the field still seek more accurate or practical methods.

A thorough discussion of water determination in foods is given by Joslyn (1). Stitt (4) discussed moisture equilibrium of dehydrated foods and evaluated several methods of moisture determination. A rapid chemical method based on oxidation with dichromate has been reported by Tomimatsu and Launer for determining moisture in fruits and vegetables (5).

The vacuum oven method is the standard or reference method for moisture determination in dried fruits. However, for commercial application where rapid, simple methods are required, the vacuum oven process is too slow and involved. This study was undertaken to determine if there is sufficient correlation between the refractive index of dried fruits and their moisture content (as determined

with the vacuum oven) to employ the refractive index to estimate moisture content.

Material and Methods

Dried prunes, raisins, figs, and apricots in the natural or low-moisture state (about 10 to 19% moisture) were obtained from commercial sources. These fruits were then hydrated to various moisture levels by immersion in boiling water, immersion in boiling water followed by cold water (2), and by cold water addition alone. Retail packages of several brands of each dried fruit (which contain about 16 to 30% moisture) were also tested.

A random sample of each lot of dried

fruit was ground and mixed well (prunes were pitted). A 50-gram portion of the ground fruit was taken for the determination of refractive index and vacuum oven moisture. The refractive index was determined on three separate portions of the 50-gram sample by placing about 50 mg. of the ground fruit directly on the prism of a Bausch and Lomb Abbe-3L refractometer. The refractometer was maintained at 25° C., and the average of the three readings was recorded. The vacuum oven moisture was determined from two 5-gram samples of the original 50-gram portion according to the procedure of Nury (3). The averages of readings for each test were used in the statistical calculations.

Table I. Statistical Relationship of Refractive Index (X) and Vacuum Oven Moisture (Y) of Some Dried Fruits

Fruit	Observations	Correlation Coefficient (r)	Regression Equation
Raisins	11	-0.9998	$Y = 591.630 - 385.625X$
Prunes	11	-0.9993	$Y = 612.324 - 399.619X$
Figs	7	-0.9922	$Y = 621.793 - 406.636X$
Apricots	7	-0.9992	$Y = 628.615 - 410.414X$