

Alpha-and beta-pinene, d-limonene, and gamma-terpinene gave green spots which changed to blue, while myrcene gave a purple spot which changed to green.

Among the aldehydes and ketones studied, citral gave a permanent blue spot. Carvone gave a pink spot which changed to yellow, and methyl heptenone gave a pink spot which changed to a bright green. The latter reaction is apparently common to this structure as the alcohol methyl heptenol behaved in a similar manner.

As mentioned above, those compounds reacting with vanillin were easily differentiated from the saturated aliphatic compounds which showed no reaction. For instance, octanol, decanol, hexyl acetate, butyl butyrate, octanal, decanal, and similar compounds gave no spots with vanillin.

Using TLC, evidence has been obtained that the esters ethyl caproate, ethyl caprylate, linalyl acetate, citronellyl butyrate, and octyl caprylate are present as trace constituents of orange essence. Also, there was some evidence for the presence of geranyl or neryl acetates. Esters other than ethyl butyrate were present in only very small quantities. It has also been possible with this technique to identify methyl heptenone, a ketone not mentioned in previous publications, as an orange essence constituent, and to confirm the identifications of 2-octenal and the alcohols methyl heptenol, nerol, geraniol, and carveol, which had been tentatively identified previously (3, 4).

Experimentation with the two procedures for transferring the sample from the GLC effluent to the chromatoplate showed that the technique involving condensation of the sample in a glass

U-tube gave a better recovery than that of transferring the sample directly from the effluent to the plate. The chief advantage of the latter technique is that it is faster.

SUMMARY

A study of the use of thin layer chromatography as a supplemental technique to gas-liquid chromatography in the analysis of complex mixtures of orange flavor constituents has been carried out. It was found to be useful for the identification of esters, a class of compounds not forming convenient derivatives, and for the partial elucidation of the molecular configuration of several types of compounds.

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COMPARISON OF ORANGE CONCENTRATE AND FOAM-MAT POWDERS USING CHROMATOGRAPHIC PROCEDURES¹

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The foam-mat drying method is finding increased utilization in the fruit and vegetable processing industries (6). Other dehydration procedures are becoming more widespread among the food industry as well. Up to the present time the principal emphasis of research in these areas has been directed toward development of the processes (8). While there has been some investigation of the effects of dehydration on chemical components of foods, the information in this area is relatively scarce. This is especially true

with regard to foods dehydrated by the foam-mat process. Recently, commercial representatives have expressed increased interest in studies of fundamental changes which might occur during the dehydration process. A question of primary concern which has often arisen is that of whether the dehydration process gives rise to any unnatural or undesirable compounds. Also, there is the question of whether artifacts are formed during storage under adverse conditions.

The laboratory has been especially concerned with the foam-mat process and its application to citrus products (1, 3). In past studies of foam-mat dried citrus, the question has often arisen as to whether any fundamental changes occur during foam-mat drying. Foam-mat powders from citrus juices have been found to develop certain characteristic off-flavors upon prolonged storage at elevated temperatures. More information was needed concerning the chemical nature of changes which accompany these off-flavors. An analytical study of foam-mat dried powders in comparison to their parent concentrate might lead to elucidation of some of these questions. Any indication of chemical changes or differences between powder and concentrate might provide the basis for an index of storage history or powder quality. Thus, the present study was undertaken.

The purpose of this report is to indicate progress and findings to date in the chromatographic study of some of the non-volatile components. An extraction and separation procedure was used to obtain comparable fractions with samples of an orange concentrate and with powders made from it by the foam-mat process. Two different powder samples were compared. These were a reference powder kept at low temperature and an experimental sample which had developed a definite characteristic off-flavor and odor. Following the method developed, a fraction was separated from the experimental powder which differed in odor from a comparable fraction derived from concentrate or the reference powder. This fraction, which showed an organoleptic difference, was studied analytically by thin-layer chromatography techniques (TLC). A few of the flavonoids which gave several clues to their identity and which appeared as if they could be easily isolated were separated and identified. However, no attempt was made to isolate or identify the majority of substances that did not indicate differences between the three samples. One substance which does appear to indicate a difference has been isolated and is under study.

EXPERIMENTAL

Samples

Twenty lb of orange powder were prepared on the belt type foam-mat dryer. A 58° Brix orange concentrate prepared from late season Valencia oranges was used. This concentrate was dried, using 12.5 min drying time and a temperature of 76.7°C (170°F) according to the procedure described by Bissett, *et al.* (3). The powder had an original moisture content of about 3.5%. It was packaged in No. 2 cans, under nitrogen (168-g powder per can). The samples were stored with an in-package desiccant. The canned product was then divided into two lots. One lot was stored at 37.7°C (100°F) and the other at -68°C (-90°F). After two months, the samples stored at 37.7°C had developed an off-odor and flavor that is characteristic of orange powders stored for prolonged periods at elevated temperatures. These samples were then extracted and compared with the concentrate and reference samples according to the procedures described below.

Extraction Procedure

In preliminary studies, an extraction of ether solubles was followed by a fractionation according to acidity. This was carried out by extracting the ether extract successively with solutions of 5% sodium bicarbonate, 0.5% sodium hydroxide and 5% sodium hydroxide, reacidification of these extracts and re-extraction of the acidified solutions with ether. However, it was found that the separation accomplished by this procedure was not complete, fractions were similar, and there were indications that the strong base solutions were producing artifacts. A different extraction procedure was then developed in which the ether extraction was followed by water extraction.

The most satisfactory extraction method developed was as follows: A weighed amount of orange concentrate or orange powder was extracted with a 1/1 acetone/water solution. For each g of solids, 1.7 ml of the solvent was used. Generally, 168 g of solids were extracted at a time. This solution was shaken and held for thirty minutes with occasional stirring at room temperature to establish equilibrium and to allow precipitation of pectins and settling of cellulosic materials. After this time, 150 ml of diethyl ether were added and the mixture was stirred for 4 min on a counter rotating mixer. The upper layer was decanted off and the ether extraction was repeated three more times. To the combined ether extracts were added 100 ml of water. This

mixture was then evaporated under vacuum until all ether and most of the acetone was removed. At this point there was a precipitation of water insoluble material in the water layer. The water extract was then filtered through paper and again through Hyflo Supercel to remove the precipitated substances and carotenoids. At this point in the extraction procedure, the fraction from the experimental powder had a different odor from that of the control powder or the concentrate.

Ten g of salt were added to the water extract and then it was extracted three times with 150 ml ether. The combined ether extracts were washed twice with salt water, treated with sodium sulfate to remove remaining water, and then taken to dryness under vacuum. The residue was taken up in 4 ml of a 1/1 ether/acetone solution. This solution was then examined by thin-layer chromatography.

Thin-Layer Chromatography

A slurry of silicic acid (Bio-Sil A, 10-30 microns with binder) in .02 N sodium acetate solution was applied to 5 x 25 cm smooth glass plates using a thin-layer applicator (Research Specialties Co., Model 200). Thirty g of silicic acid were used with 60 ml of sodium acetate solution. These plates were activated by heating in an oven at 100°C prior to use.

The solvent system found to give the best separation in this study was a mixture of benzene, ethanol, water and acetic acid at a ratio of 200/47/15/1, using the top layer as described by Hay, *et al.* (4). After development, the plates were dried in air and then examined under UV light for fluorescent spots. These were marked, then the plates were sprayed with anisaldehyde spray reagent in H₂SO₄ and acetic acid as described by Stahl and Kaltenbach (7), and by Bobbitt (2).

Results of our investigations have shown that when the sprayed plate is heated to 160°C rather than 105°C as described in previous references (7, 2), the sensitivity of the reagent is enhanced considerably. Several spots were detected which had not been seen at the lower temperature and a wider diversity of color reactions were encountered.

Following this chromatographic procedure, the extracts from orange concentrate, and from the two samples of orange powder were compared. This comparison was made on the basis of gross fluorescent pattern, Rf values and colors developed with anisaldehyde. Fluorescent patterns were also examined and compared after expo-

sure to ammonia fumes, and, after exposure to HCl fumes. In a few cases where tentative identifications were suggested by Rf value and fluorescence or anisaldehyde reaction, further separation was achieved as follows: A thin-layer plate was spotted or streaked across the bottom of the plate, developed, the section of the plate which was of interest was scraped and eluted with 1/1 ether/acetone, the eluent was concentrated, re-spotted, and rechromatographed. After repeated treatments, when the fraction appeared relatively pure, the eluate was concentrated and used for determination of a UV absorption spectrum using a Cary 14 recording spectrophotometer, or an IR spectrum using a Perkin-Elmer 137 infracord. These data were then compared with similar data on known compounds.

RESULTS AND DISCUSSION

The extraction and chromatographic procedure described in this study resulted in a highly reproducible TLC pattern. The development system used enabled the separation of 17 spots detectable by fluorescence and 13 spots detectable by anisaldehyde reagent. The positions of these are shown in Figure 1. It should be pointed out that these are sketches of the same plate as observed under fluorescent light and after treatment with anisaldehyde spray reagent. Thus, there is some overlapping of spots detectable by the two methods, and some are detectable under UV which are not reactive to anisaldehyde spray reagent, although some are detectable by both procedures.

According to Stahl and Kaltenbach (7), the anisaldehyde reaction is particularly sensitive to carbohydrates and steroids. Bobbitt (2) describes its use in the identification of sterols and steroid compounds. Although the exact nature of the color reaction has not been elucidated, it is believed to involve a reaction similar to the K_ägi and Miescher reaction for steroids (5, 7).

Three of the spots (Nos. 1, 2 and 3 in Figure 1) appeared to be flavonoids, and were situated such as to be relatively easy to separate. These were isolated and identified. Another, number 5 in Figure 1 appeared to be the same as the material which dropped out of solution in abundance at the second stage of extraction. The fact that spot number 5 was the same as the precipitated fraction was verified by Rf and color reactions. This compound was separated and identified by IR and UV data.

Table 1 gives supporting evidence for the identity of these spots. Spots number 1 and 2

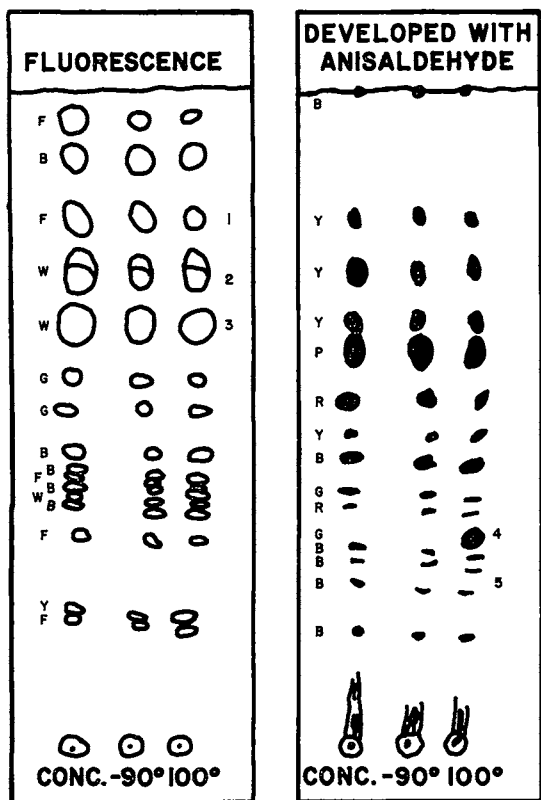


Figure 1.—TLC chromatograms of extracts from orange concentrate and foam-mat dried orange powders stored at -90°F and at 100°F . F—fluorescent; W—white; B—blue; Y—yellow; P—purple; R—red; G—green.

have been identified as 3,5,6,7,8,3',4'-heptamethoxy-flavone and nobiletin, respectively, by comparison of Rf values to those of known compounds, by their behavior under fluorescent light, by changes in fluorescence after subjection to HCl fumes, by color reactions with anisaldehyde spray reagent and ultraviolet absorption spectra. Spot number 3 has been identified as sinensetin by similar evidence, plus the change in fluorescence when subjected to NH_3 fumes. Spot number 5 has been identified by Rf, color with anisaldehyde, and by the fact that it has an identical infrared absorption spectrum to that of β -sitossterol d-glycoside. This material was separated in fairly large quantities during the second major stage of the extraction procedure when the combined ether extracts were added to water and the ether removed under vacuum. At this point, the precipitate which formed and was removed by filtration contained a large amount of this material.

From the evidence to date, at least 24 different compounds have been separated and detected by either fluorescence or color reaction with anisaldehyde. Of these, 23 appear to be identical in the extracts from the concentrate, the -90°F stored powder and the 100°F stored powder. However, one compound does appear to be found only in powder stored at 100°F . This is the compound indicated as number 4 in Figure 1. This material has an Rf of .32 and develops a dark green spot with anisaldehyde reagent. Under short wave fluorescent light of 2537-Angstrom units, it absorbs the light, and appears as a dark purple spot. These dark spots are not apparent with 3660-wave-length light. This material has not been detected in extracts from the concentrate or the -90°F stored powder under repeated extractions and increased concentrations. Therefore, it must be something which has formed upon storage, or has increased very greatly in concentration upon storage. Thus, it has been shown that at least 23 different substances appear to be unchanged during the foam-mat dehydration of orange juice, and during high temperature storage. One substance has been isolated which does appear to reflect a chemical change during storage.

SUMMARY

A fraction of non-volatile materials which are soluble in ether, acetone and water has been separated from orange concentrate and from foam-mat dried powders prepared from the concentrate. Control powder stored at -90°F has been compared to powder stored at 100°F . A procedure has been developed by which at least 24 different compounds can be detected and separated, four of which have been identified. At least 23 of the substances separated appear to be unchanged during drying and storage at 100°F . One compound has been detected which appears to form upon storage at 100°F and this material is under further investigation.

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Table 1. Evidence for identity of compounds separated from extracts of orange concentrate and foam-mat powders.

Compound	Rf	UV fluorescence			Color anis-aldehyde	Other evidence
		orig	acid	base		
3,5,6,7,8,3',4'- Heptamethoxy- flavone	.80	f	ro		y	UV ^a /max. 341,270,253
Spot #1	.80	f	ro		y	UV max. 340,270,253
Nobiletin	.71	w	dy		y	UV max. 333,270,250,209
Spot #2	.71	w	dy		y	UV max. 329,269,249,208
Sinensetin	.63	w	by	bw	y	UV max. 328,264,240
Spot #3	.63	w	by	bw	y	UV max. 328,265,240
β-sitosterol glycoside	.23				b	IR spectrum of known
Spot #5	.23				b	and unknown identical

^a/

All UV spectra determined in ethanol.

Fluorescence and color code: f - fluorescent, w - white, ro - red orange, dy - dark yellow, by - bright yellow, bw - blue white, y - yellow, b - blue.

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BULK DENSITY AND RECONSTITUTION RATES OF FOAM-MAT DRIED CITRUS POWDERS¹

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The foam-mat process is a dehydration method which is finding increased usage among proces-

sors for the production of high quality fruit and vegetable powders. It is presently being utilized commercially for the production of tomato and lemon powders in two plants in California (5, 6). Processors have shown much interest in its use for other products. One of the advantages of foam-mat dried powders is the reduction in shipping weight. Also, shipping and storage requires no refrigeration. If the bulk density of these dried powders could be increased, the additional advantage of reduced shipping volume could be realized.

In previous studies it has been found that upon reconstitution of juice from these powders, a milky, unnatural appearance sometimes occurs

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