Application of Method

No minimum absorbance that will produce a fancy canned product has been proposed because of differences between colorimeters, cell sizes, and individual processor preferences. Using an Evelyn colorimeter with a 515-mμ filter, a raw fruit absorbance of 90 or higher for DSC gives a processed product with deep color that an industry panel graded fancy. Bings will attain somewhat more color than Lamberts. Raw fruit absorbancy of 25 or higher for PP likewise gives a processed product with good color that an industry panel graded fancy.

Raw fruit absorbance has proved useful in this laboratory for evaluation of unfamiliar varieties, strains, or selections for processing. Certain DSC varieties have a larger percentage of pigment near the skin as ripening develops. Processing releases this pigment into the sirup and remainder of the flesh, resulting in lighter color than would be expected from the external appearance. The amount of color in PP is difficult to estimate visually once the surface of the fruit has become colored. Increases in anthocyanin pigment content are not reflected by corresponding changes in visual color or Hunter color and color difference meter values.

Raw fruit absorbance coupled with field observations could be used to determine when commercial harvests should start and which orchards should be picked first. Normally, a few days' delay will increase the color of fruit from borderline maturity to a satisfactory level. Raw fruit absorbance can be used to produce a pack standardized with respect to color for each grade.

Each processor will decide what amount of color he wishes his products to have. A trial pack will establish these levels in terms of absorbance of fresh fruit extracts and serve as a standard for subsequent harvests.

Literature Cited

(1) Allen, F. W., Calif. Agr. Expt. Sta. Progress Rept. (mimeo), 1941.
(2) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 9th ed., p. 272, 1960.
(3) Clore, W. J., Neubert, A. M., Carter, G. H., Ingalsbe, D. W., Brummond, V. P., Wash. State Univ., Agr. Expt. Sta., Tech. Bull. **48** (1965).
(4) Dickinson, D., Gawler, J. H., *J. Sci. Food Agr.* **7,** 699 (1956).
(5) Ernest, Jane V., Birth, G. S., Sidwell, A. P., Columbic, Califin, *Food Technol.* **12,** 595 (1958).
(6) Fisher, D. V., *Proc. Am. Soc. Hort. Sci.* **37,** 183 (1939).
(7) Gerhardt, F., English, H., Smith, E., *Ibid.*, **42,** 247 (1943).
(8) *Ibid.*, **46,** 205 (1945).
(9) Hartman, Henry, Oregon State Coll., Agr. Expt. Sta. Circ. **75** (1926).
(10) Hartman, Henry, Bullis, D. E., *Ibid.*, **247** (1929).
(11) Karrer, P., deMeuron, G., *Helv. Chim. Acta* **15,** 507 (1932).
(12) Sondheimer, E., Kertesz, Z. I., *Anal. Chem.* **20,** 245 (1948).
(13) Swain, T., Hillis, W. E., *J. Sci. Food Agr.* **10,** 63 (1959).
(14) Tucker, L. R., Verner, Leif, Idaho Univ., Agr. Expt. Sta. Bull. **196** (1932).
(15) Wiley, R. C., Ph.D. dissertation, Oregon State University, Corvallis, Ore., 1953.
(16) Wiley, R. C., Worthington, O. J., *Food Technol.* **9,** 381 (1955).

Received for review March 5, 1965. Accepted July 30, 1965. Reference to a company or product name does not imply approval or recommendation of others that may be suitable. The U. S. Fruit and Vegetable Products Laboratory is a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

GRAPE FLAVOR AND ODOR

Detection of an Undesirable Anomaly in Concord Grape by Gas Chromatography

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Concord grape essence was analyzed by gas-liquid chromatography. Compositional criteria established were used for the determination of an organoleptically detectable anomaly. Atypical group grapes were defined by a tenfold increase in *n*-valeraldehyde content, which can be detected organoleptically. The sensation recorded is one of an increased sweet and fruity flavor.

A STANDARD of high quality in fresh fruit is important in the preparation of all fruit products. Any anomaly in fresh fruit which could lower this standard is viewed with concern. In 1958, in the Niagara Peninsula, Ontario, Canada, grapes which were small, light in color, and different in flavor from typical Concord grapes were found on a small percentage of vines (*7*), and the juice from such grapes was inferior to that from normal ones. This fruit grew on seemingly healthy vines and in most cases adjacent vines bore typical normal grapes. The atypical grapes remained in this state until they dropped because of overmaturity. Total acidity, Brix, and pH of the atypical grapes (*4*) indicated that no consistent variation in any of these factors could be associated with the abnormality; therefore, a comparison by gas chromatography of some components of normal and atypical grapes was initiated to discover possible sites of flavor differences.

Holley, Stoyla, and Holley (*1*) used classical chemical methods to identify eight volatile constituents of Concord grape, but no gas chromatographic study of whole grape juice has been reported. Thus, methods were needed for gas chromatographic analysis of volatile components and identification of some of these compounds before a comparative study could be made.

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Experimental

Identification of Some Volatile Components. Frozen grape juice from normal typical grapes harvested in 1960 was used. The juice had been pasteurized and stored at −22° C. It was thawed and passed through a high speed separator at 1300 × g to remove chloroplasts and tartrates. The essence from 2000 ml. of cleared juice was extracted into ethyl chloride. Complete removal of taste and odor, as judged by a panel of five expert tasters, was facilitated by fourfold extraction using 200 ml. of ethyl chloride (*3*) each time. The four extracts were combined and dried 12 hours

over anhydrous sodium sulfate. The solvent was removed by fractional distillation through a helices-packed, 30-cm. Graham condenser. The water in the jacket was maintained at 12.5° C. by a Haak thermostat type F pump. The residue was described by the taste panel as having typical Concord grape aroma. The pale yellow, oily, aromatic residue was evaporated onto a U-tube suspended in liquid air by passing a stream of nitrogen (50 cc. per minute) through a stainless steel capillary which reached down into the flask containing the extract. The extract had been reduced to a standard volume in a calibrated tip of the flask, which corresponded to 0.5 ml. of water at 20° C.

An Aerograph A 700 preparative-scale gas chromatograph was used for preliminary separation of the total yield of the volatile constituents from 2000 ml. of juice in a one-step process; this yield in each case was the sample analyzed. The apparatus was equipped with a thermal conductivity detector and an automatic fraction collector. The injector temperature of this machine was set at 200° C., the detector temperature at 210° C., and the collector temperature at 205° C. The filaments were operated at 160 mv. Recorder chart speed was 0.5 inch per minute; gas flow was regulated at 200 cc. of helium per minute. The column temperature was constant at 75° C. for the first 40 minutes, and was then raised by nonlinear temperature programming so that the final temperature of 145° C. was reached at the 83rd minute. Temperatures at 10-minute intervals beyond the 40-minute mark were not recorded, but the heating rate was reproducible. A 20-foot by $^3/_8$-inch aluminum column packed with 30% Carbowax 20 M on C-22 firebrick, 30- to 60-mesh, gave the best separation. The sample was injected onto the column by inserting the liquid air–cooled U-tube, charged with extract, into the carrier gas flow system. Once inserted, a three-way valve was manipulated to divert the helium flow through the U-tube. The cooling bath was removed, and the U-tube immediately and rapidly heated to 450° C. for 40 minutes, attaining an interior temperature of 300° to 315° C. Fifteen fractions were collected in the especially designed collector U-tubes, which were cooled in a dry ice-chloroform bath at −80° C. The stopcocks of the collector U-tubes were closed immediately after the desired fraction was collected.

To accomplish an analytical separation each fraction was passed through a Pye-Argon chromatograph equipped with an ionization detector using a radium-226–coated foil with a dosage of 80 μc as a source of radiation. The following column packings were used in the 4-foot by $^1/_4$-inch glass column of this machine.

1. 40% Carbowax 550 on C-22 firebrick, 30- to 60-mesh
2. 10% diethylene glycol succinate (DEGS) on C-22 firebrick, 60- to 80-mesh
3. 15% tetraethylene glycol dimethyl ether (MEEE) on C-22 firebrick, 60- to 80-mesh

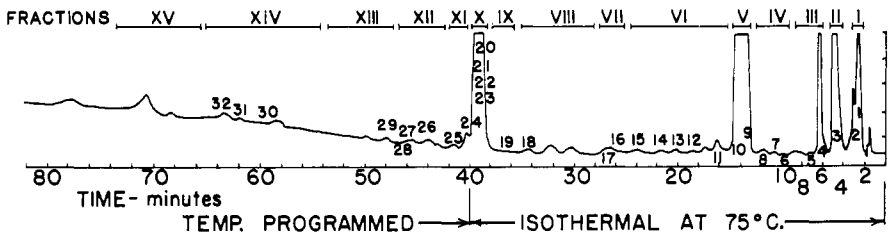

Figure 1. Chromatogram of Concord grape essence

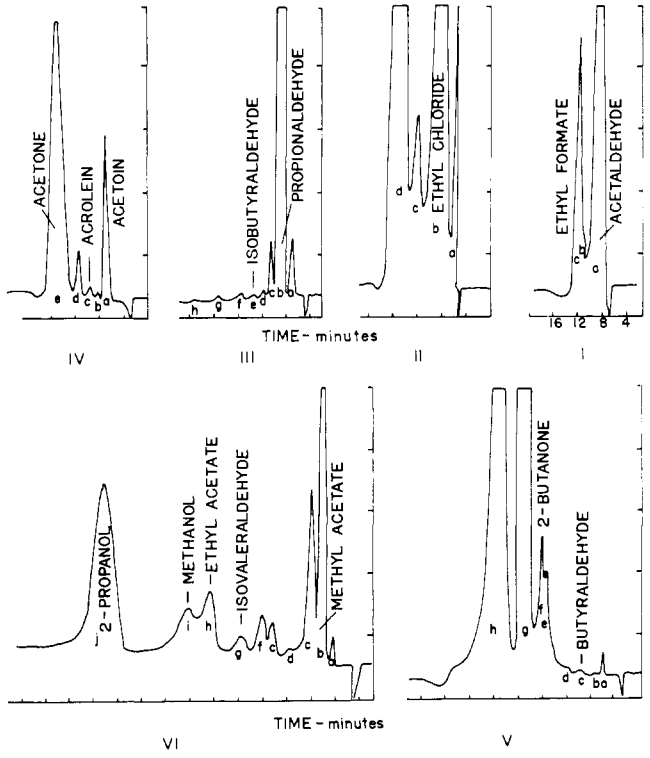

Figure 2. Representative Pye-Argon chromatograms of fractions I to VI, from preparative runs, showing peaks tentatively identified

The column temperature was set at 50° C. for the first 11 fractions and raised to 75° C. for the last four fractions. A gas flow of 45 cc. of argon per minute was maintained. The detector voltages were 1250 or 1500 volts as required to give reasonably sized peaks on the 10-mv. recorder (Leeds & Northrup). The fractions were injected by inserting the collector U-tubes into the Pye-Argon gas flow system. When inserted, the U-tube stopcocks were opened, and then the three-way stopcocks were manipulated to divert the argon flow through the U-tube, which was simultaneously heated to 200° C.

Comparison of Typical and Atypical Grapes. Fruit harvested from 15 vineyards in the Niagara Peninsula was selected to give a wide variety of soil and climatic conditions. A single vine was considered an experimental unit if it yielded at least 15 pounds of fruit. Vines were chosen so that two normal ones flanked one which was atypical. Fruit was harvested from each of three vines in each vineyard during a 2-day period within the commercial harvest season. To determine the effect of fruit maturity, five vines in one vineyard were harvested on five occasions, 3 days apart, centering about the peak of the commercial harvest. Included were two atypical vines.

Harvested grapes were cooled for at least 2 hours at 0° C. before they were crushed, and the juice was removed by "cold press" using a Bucher-Guyer hydraulic press at 325 p.s.i. The juice (2500 to 3000 ml. from grapes of a single vine) was placed in a gallon flask and cooled rapidly by passing a slow stream of liquid nitrogen through it. When the first ice crystals had formed, the flask was tightly sealed and the juice stored at −25° C. until needed. The frozen grape juice was thawed in its atmosphere of nitrogen at 10° C. until all ice had melted. As melting neared completion,

Table I. Tentative Identification by Relative Retention Times

No.	Compound	Preparative Column, Carbowax 20M			Absorbing Substrates in GLC Column, Analytical Columns						Relative Amounts[b]
					Carbowax 550		DEGS		MEEE		
		Unknown	Known	Fraction[a]	Unknown	Known	Unknown	Known	Unknown	Known	
1	Acetaldehyde[c]	0.081	0.080	Ia	0.081	0.081	0.076	0.078	0.080	0.082	L
2	Ethyl formate	0.096	0.095	Ic	0.095	0.095	0.089	0.090	0.095	0.097	M
3	Ethyl chloride	0.14	0.13	IIb	0.14	0.14	0.100	0.100	0.12	0.14	..
4	Propionaldehyde	0.25	0.25	IIIb	0.25	0.26	0.21	0.19	0.24	0.27	L
5	Isobutyraldehyde	0.27	0.27	IIIe	0.28	0.27	0.25	0.24	0.27	0.29	T
6	Acetoin	0.38	0.39	IVa	0.37	0.37					T
7	Acrolein	0.39	0.40	IVc	0.39	0.40	[d]		[d]		T
8	Acetone[c]	0.46	0.45	IVe	0.45	0.44	0.39	0.40	0.46	0.47	S
9	Butyraldehyde	0.50	0.50	Vc	0.50	0.51	0.46	0.47	[d]		T
10	2-Butanone	0.54	0.53	Vf	0.52	0.53	0.49	0.48			S
11	Methyl acetate[c]	0.61	0.61	VIb	0.60	0.60	0.55	0.56	0.61	0.62	L
12	Isovaleraldehyde	0.69	0.69	VIg	0.69	0.70	0.64	0.63	0.70	0.71	S
13	Ethyl acetate[c]	0.81	0.80	VIh	0.79	0.80	0.74	0.75	0.82	0.81	M
14	Methanol[c]	0.85	0.84	VIi	0.84	0.83	0.85	0.84	0.87	0.84	M
15	2-Propanol	0.91	0.91	VIj	0.91	0.91	0.91	0.91	0.92	0.91	L
16	Valeraldehyde	0.98	0.98	VIIa	0.97	0.96	0.92	0.91	0.97	0.95	T
17	Ethanol	1.00	1.00	VIIc	1.00	1.00	1.00	1.00	1.00	1.00	M
18	Pentanone	1.29	1.30	VIIId	1.28	1.27	1.25	1.25	1.30	1.31	
19	Acetyl acetone	1.35	1.35	IXd	1.34	1.33	1.30	1.31	1.35	1.36	T
20	Crotonal	1.46	1.46	Xc	1.46	1.46	[d]		[d]		S
21	3-Heptanone	1.47	1.47	Xe	1.47	1.47					S
22	Ethyl propionate	1.50	1.50[e]	Xi	1.50	1.51	1.48	1.47	[d]		L
23	Diacetyl	1.52	1.51	Xj	1.53	1.52	1.50	1.50			T
24	Propanol	1.54	1.53	XIb	0.86	0.87					M
25	Ethyl isobutyrate	1.58	1.58	XId	0.88	0.88	[d]		[f]		S
26	Isobutyl isobutyrate	1.65	1.65	XIIa	0.93	0.90	0.90	0.91			S
27	Phenyl ethyl butyrate	1.75	1.76	XIIf	0.98	0.97	0.96	0.96			M
28	Isoamyl acetate	1.77	1.78	XIIh	1.77	1.77					M
					1.00[g]	1.00	1.00	1.00			
29	2-Butanol	1.85	1.86	XIIIa	1.06	1.04	1.03	1.03			S
30	Amyl propionate	2.30	2.29	XIVa	1.30	1.31	1.26	1.24			S
31	Benzyl formate	2.46	2.45	XIVc	1.36	1.35	1.31	1.30			S
32	Isoamyl alcohol	2.47	2.48	XIVd	1.41	1.40	1.41	1.40			L

[a] Sections represent fractions analyzed on Pye-Argon chromatograph and small letters refer to peaks lettered in order of retention times.
[b] Estimated from analytical runs on Pye-Argon chromatograph. T Trace. S small. M medium. L large.
[c] Previously identified by Holley *et al.* (*1*).
[d] Components could not be separated.
[e] Retention times estimated.
[f] MEEE column not useful at this temperature.
[g] Because of change to higher isothermal column temperature isoamyl acetate was related to ethanol at 50° C. All others then related to isoamyl acetate at 75° C. equal to unity.

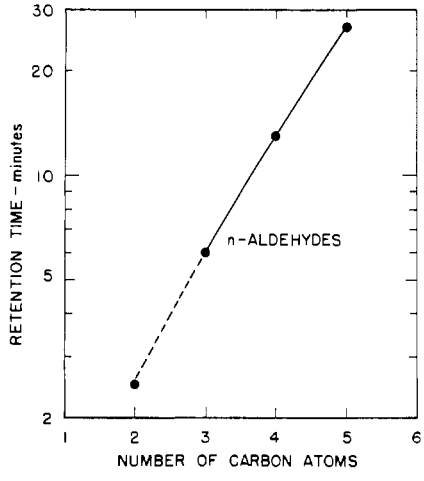

Figure 3. Log retention time *vs.* carbon number plot for *n*-alkanals

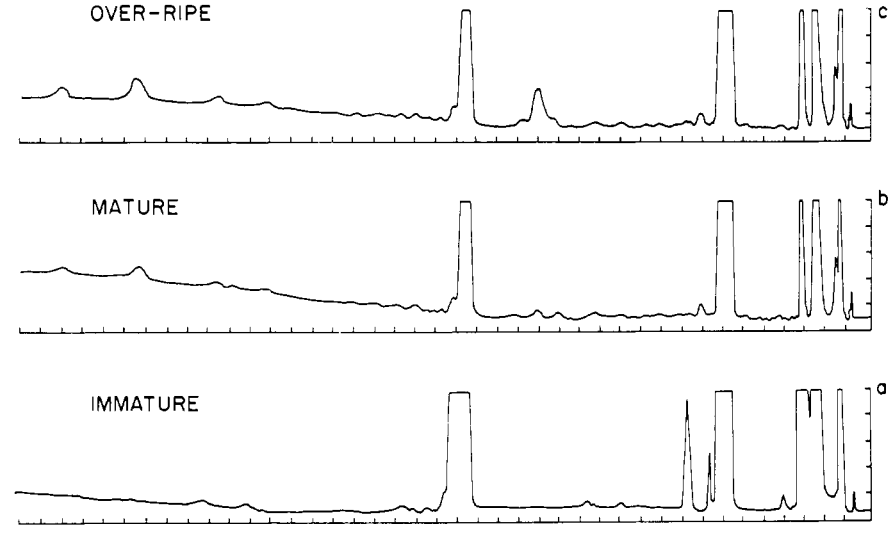

Figure 4. Chromatograms of essence from typical Concord grapes harvested at three stages of maturity

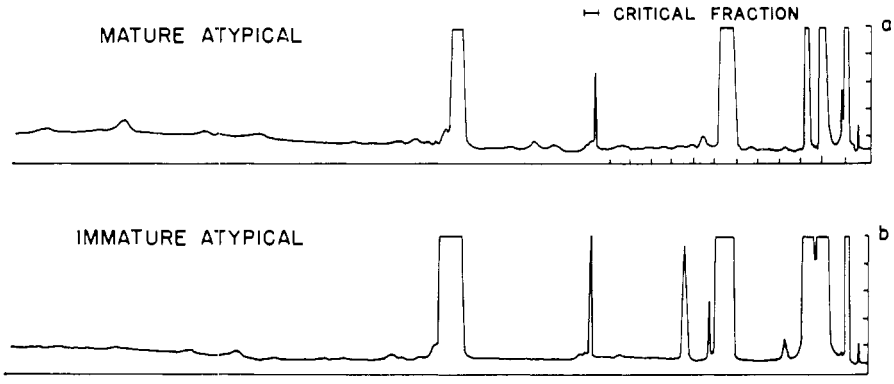

Figure 5. Chromatograms of essence from atypical Concord grapes harvested at two stages of maturity, both of which display anomaly

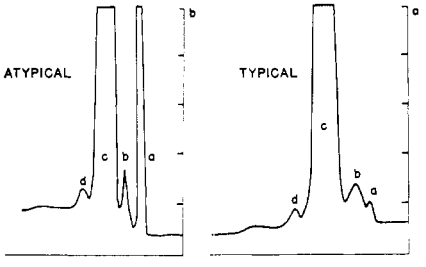

Figure 6. Gas chromatographic profiles of "critical" fractions of grapes

c. Normal
b. Atypical

Table II. Peak Areas of Critical Fractions from Typical and Atypical Grapes

(Ott planimeter units)

No. of Run	a	b	c	d
		Typical		
1	3	8	106	2.5
2	2	6	82	1.0
3	4	11	148	3.5
4	1.5	8	109	2.0
5	1.5	8	110	1.5
6	2.5	9	122	2.5
7	3	10	133	3.0
8	3.5	10	137	3.0
9	2	8.5	114	2.0
10	3	9.5	128	2.5
11	1	8	107	2.0
12	1	8	105	2.0
13	1.5	8	110	2.5
14	2.5	8.5	115	2.5
15	4	11	153	3.5
		Atypical		
16	15	8	104	2
17	19	9	121	2.5
18	16	8	109	2
19	23	9	119	3
20	20	9.5	132	3
21	19.5	11	152	3.5
22	18	10	136	2.5
23	16	8.5	112	2

the contents of the jug were agitated to assure a uniformly low temperature throughout. When thawing was complete, the seal was broken and the juice cleared and extracted. The volatile constituents were then analyzed as discussed above.

Results

A preparative chromatogram considered typical of normal fruit, and obtained from the essence of 2000 ml. of grape juice, is shown in Figure 1. Results from the analytical runs using the Pye-Argon chromatograph on the 15 fractions from the preparative chromatogram were included in the tentative identification of the juice components. As examples of these further separations, chromatograms of the first six fractions are shown in Figure 2. Some idea of the identity of compounds represented by some of the peaks was ascertained by comparison of their retention times on different columns, with those of known compounds on the same columns as listed in Table I. Relative amounts of the various identified components of the essence were indicated using a four-step scale developed by Webb and Kepner (*6*). Peak areas from the chromatograms, measured by an Ott planimeter with suitable attenuation of the large peaks, were used to arrive at relative amounts. The order of magnitude of the concentration of the substances identified ranged from 0.1 p.p.m. of isobutyraldehyde, representing trace components, to 70 p.p.m. of ethanol, representing large components. The concentrations were determined from appropriately diluted authentic samples run under the same conditions. The values are relative, since, although the extracted juice was completely tasteless, the efficiency of extraction when standard recovery procedures were run was only 92% for diacetyl compared to 99% for alcohols and aldehydes.

Of particular interest in subsequent work was peak 16, thought to represent *n*-valeraldehyde. To confirm this supposition, the log retention times of these peaks (Nos. 1, 4, 9, and 16), thought to represent the homologous series of straight-chain aldehydes, were plotted against their respective carbon numbers. A straight-line graph was obtained as shown in Figure 3. James, Martin, and Keulemans (*2*) suggested that such straight-line behavior is typical of compounds of a homologous series and can be taken as further confirmation of earlier findings that peaks 1, 4, 9, and 16 represent acetaldehyde, propionaldehyde, *n*-butyraldehyde, and *n*-valeraldehyde, respectively.

Essences prepared from normal Concord grapes, harvested at different stages of maturity, produced the profiles of Figure 4, *a*, *b*, and *c*. Changes in volatile constitution were found for grapes of varying maturity. Insufficient investigation was carried out to determine to what extent the process of ripening of grapes can be followed by these techniques; sufficient information was, however, obtained to define Figure 4*b* as representing normal typical mature Concord grapes.

When essence obtained from grapes of atypical appearance was examined, a pronounced anomaly in profile was observed, manifested by appearance of a major response curve (Figure 5*a*, "critical" section). This peak was recorded for all grapes of atypical character, even immature grapes (Figure 5*b*).

In a detailed investigation of this anomaly, individual runs on 15 normal and eight atypical units of grapes were made. The gas chromatographic fraction representing the "critical" section of the essence from these units was collected and analyzed on a Carbowax 550 column in the Pye-Argon chromatograph. The chromatogram of Figure 6*a* was produced by the 15 fractions from the normal grapes, the remaining fractions from atypical grapes producing Figure 6*b*. These profiles indicated a variation in concentration of a compound represented by peak *a*. Areas of the four peaks in Ott planimeter units are given in Table II for the 23 fractions analyzed. To eliminate the effect of instrumental variables, ratios of peak areas *a*, *b*, and *c* were reduced to their simplest values with respect to peak *d*, which was set at unity. Statistical analysis showed the apparent difference between the concentration of the compound represented by peak *a* of the normal and peak *a* of the atypical to be highly significant, but *t* tests (*5*) on the differences between any of the other peaks obtained from atypical and normal fruit showed no significance. The compound responsible for peak *a* was earlier identified to be *n*-valeraldehyde. It appears, then, that one site of chemical difference between atypical and normal grapes is variation in *n*-valeraldehyde concentration. The observed concentration increase is approximately tenfold, from 0.3 to 3.0 p.p.m. In aqueous solution this change can readily be detected organoleptically. The sensation is best described as sweet and fruity. It is difficult to ascertain whether the concentration difference of this compound is solely responsible

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.

Literature Cited

(1) Holley, W. H., Stoyla, B., Holley, A. D., *Food Res.* **20,** 326 (1955).

(2) James, A. T., Martin, A. J. P., Keulemans, A. I. M., "Gas Chromatography," p. 27, Reinhold, New York, 1959.

(3) Matthew, J. S., Sugisawa, H., MacGregor, D. R., *Food Sci.* **27,** 355 (1962).

(4) Neudoerffer, T. S., "Investigation of the Colour and Flavour Components of Concord Grape, to Determine Differences within its Variety," unpublished M. A. thesis (M. D. Smith), University of Toronto, 1964.

(5) Snedecor, G. W., "Statistical Methods," p. 45, Iowa State University Press, Ames, Iowa, 1961.

(6) Webb, A. D., Kepner, R. E., *Am. J. Enol. Viticult.* **13,** 1 (1962).

(7) Zubeckis, E., Hort. Expt. Sta. Prod. Lab., Vineland, Canada, Rept. **89** (1958).

Received for review September 21, 1964. Accepted September 20, 1965.

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 appear 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 associated 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.