likely. Extended fermentation times beyond 98 hr possibly would have revealed a reduction in sucrose content.

M. hiemalis utilized sucrose early in the fermentation but failed to use stachyose and raffinose. *A. elegans* used stachyose after 44 hr of fermentation; the decrease in stachyose was accompanied by an increase in raffinose. Hydrolysis of stachyose after 44 hr occurred at a faster rate than did raffinose utilization and may account for the latent buildup of raffinose. Sucrose levels appeared to increase slightly over the 98-hr fermentation.

Optimal cultural conditions for the production of α-galactosidase by fungi included in this study may not have been achieved. Fatty acids with more than 12 carbon atoms have been shown to stimulate α-galactosidase production by *M. vinacea* (Kobayashi and Suzuki, 1972). In an earlier paper we reported that several of the fungi included in this study had strong lipolytic activity on full-fat Florunner peanuts (Beuchat and Worthington, 1974). A study relating free fatty acid accumulation to induction of fungal α-galactosidase production on a peanut substrate would be of interest. Other nutrient requirements, pH, temperature, and aeration were not investigated with regard to their effect on enzyme production in the peanut substrate. We can state, however, that several fungi are capable of decreasing the sucrose, raffinose, and stachyose content of peanuts. The most notable of these is *N. sitophila*, the ontjom fungus.

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Distribution of Volatile Compounds between the Pulp and Serum of Some Fruit Juices

Terence Radford, Kaoru Kawashima, Paul K. Friedel, Larry E. Pope, and Maurizio A. Gianturco*

The results of an investigation of the distribution of volatile flavor compounds between the pulp and sera of orange, grapefruit, lemon, and apple juices are reported. The trends observed with the natural juices were investigated further using model systems. The data obtained suggest that pulp may have an important effect on the flavor of certain, but not all, fruit juices. The findings are also of some relevance to the selection of methods for the isolation of flavor volatiles from juices.

As part of an investigation of certain fruit flavors, we have studied the distribution of volatile flavor constituents between the pulp and serum fractions which can be obtained from some fruit juices by high-speed centrifugation. This study was prompted by our desire to (1) determine how pulp affects headspace concentrations, and therefore aromas, of fruit juices and purees; (2) investigate statements in the literature which imply that the essential flavor constituents of orange juice are associated with the pulp; (3) determine whether centrifugation offers any advantages as a preliminary step in the isolation of flavor volatiles from fruit juices.

Corporate Technical Division, The Coca-Cola Company, Atlanta, Georgia 30301.

EXPERIMENTAL SECTION

Preparation of Fruit Juices. Juice was extracted from orange, lemon, and grapefruit by removing the flavedo and albedo from the fruit and squeezing by hand. Vesicles were removed from the juice by filtering through cheesecloth. In the case of apple, the fruit was peeled, cored, sliced, and minced through a household grinder. Juice was obtained by pressing the mash through two layers of cheese cloth.

Separation of Pulp and Serum. Freshly prepared juice (1 l.) was centrifuged at 250,000*g* using a Beckman L2-65B ultracentrifuge. The serum was filtered to separate juice vesicle remnants. The pulp was removed from the centrifuge tubes using distilled water.

Isolation of Volatiles. The serum (~960 ml) was evaporated on a Rinco rotary evaporator at 30° under reduced

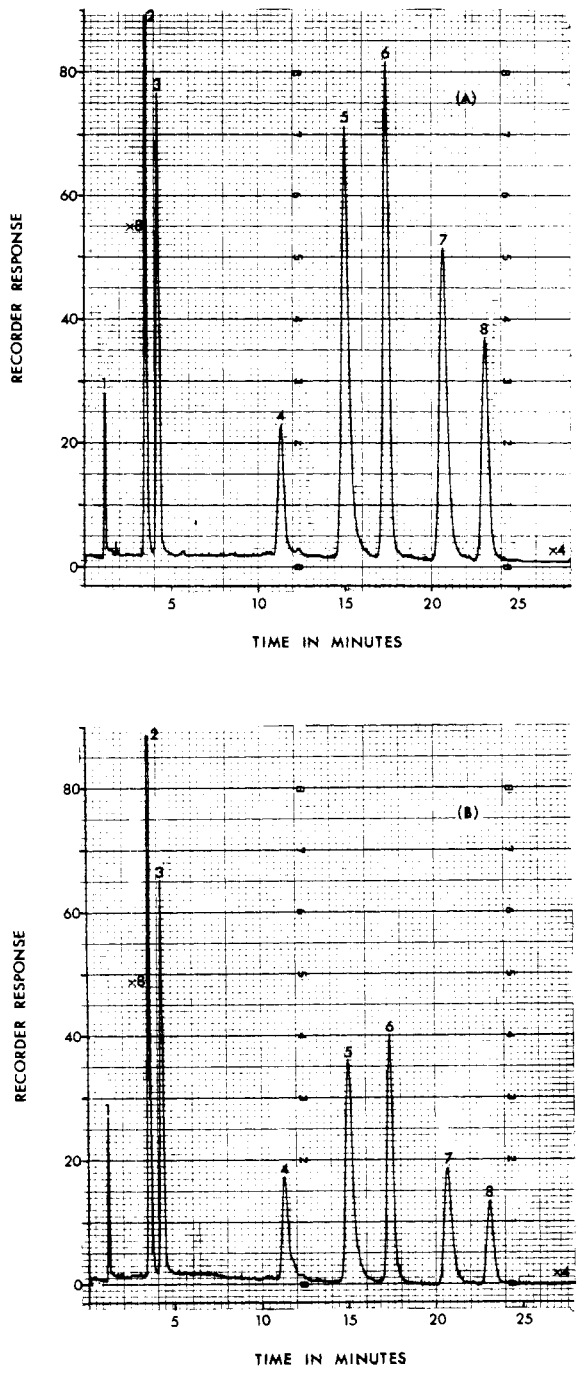

Figure 1. Headspace chromatograms of volatiles in (A) the absence and (B) the presence of orange pulp.

pressure. Evaporation was continued until a syrupy residue remained. The volatiles were condensed in traps cooled in liquid nitrogen. The condensate was thawed, saturated with sodium chloride, and extracted with methylene chloride (4 × 50 ml). The combined extracts were dried and concentrated to about 0.5 ml by evaporation through a short fractionating column. The residue was further concentrated to about 0.1 ml by slow evaporation at room temperature. The pulp, in distilled water, was subjected to the same procedure.

To evaluate the extent and selectivity of losses during the concentration step, the procedure was examined using a methylene chloride solution of ethyl butyrate, limonene, *cis*-hex-3-en-1-ol, and ethyl benzoate. For the model system, volatile retentions ranged from 73 to 76%. While material losses of the order of 25% are significant if absolute concentrations are being determined, the fact that only distribution data were being sought is considered to render the procedure viable.

Analysis of Isolates. The concentrates were analyzed using a Perkin-Elmer F30 gas chromatograph equipped with an FID and a 300 ft × 0.02 in. stainless steel open tubular column support coated with Carbowax 20M. The temperature was programmed from 60 to 200° at 2°/min. The helium flow rate was 5 ml/min. Injector and detector temperatures were maintained at 250°. Peak areas were determined with a Vidar 6300 digital integrator using an appropriate ethyl ester as internal standard, which was added in methylene chloride solution immediately before the analysis.

Due to its long retention time on the SCOT column, the values for nootkatone (Table II) were determined using a 20 ft × ⅛ in. stainless steel column packed with 5% Carbowax 20M on 80–100 mesh Chromosorb W.

Gas chromatographic peaks were identified by gc–mass spectroscopy using an Hitachi RMU 6L coupled with a Varian 1200 gas chromatograph. The gc was equipped with an FID and a 20 ft × ⅛ in. stainless steel column containing 5% Carbowax 20M on 80–100 mesh Chromosorb W. The temperature was programmed from 70 to 200° at 2°/min. Injector and detector temperatures were held at 220°. The helium flow rate was 25 ml/min. Carrier gas was removed from the effluent using a glass jet separator maintained at 220°. Mass spectra were determined at 70 eV and 80 μA, with a source temperature of 200–250°. Assignments were made by comparing the unknown spectra with those of authentic compounds which were either obtained commercially or synthesized by published procedures. Assignments were confirmed by enhancing gas chromatographic peaks with authentic compounds. The compounds, identified by gc–mass spectroscopy, were located on the chromatogram obtained with the SCOT column by enhancement.

Model Experiments. A large batch of orange juice was prepared by the procedure described above and immediately canned in 12-oz containers and stored at −40°. Immediately before each model experiment, the contents of one can were thawed and 350 ml of the juice was centrifuged using an IEC centrifuge operating at 10,000*g* for 15 min. The serum was discarded and the pulp was washed three times with distilled water to remove soluble solids. The pulp was then mixed with distilled water and the mixture was concentrated on a Rinco rotary evaporator. The process was repeated several times to ensure complete removal of volatiles. An aqueous solution of 10 ppm of the compound to be studied was prepared and checked for undissolved material by the procedure of Buttery *et al.* (1969). In all cases complete solution was achieved. The pulp was then vigorously stirred at 20° in 335 ml of the standard solution. The equilibration period for each type of compound was determined by trial experiment, but was normally about 30 min. After equilibration the mixture was centrifuged and the distribution of the compound between supernatant and pulp was determined. The supernatant was evaporated and the condensate was analyzed directly by gas chromatography using an F&M 810 instrument equipped with an FID and a 12 ft × 0.25 in. glass column containing 15% Triton X-305 on 80–100 mesh Chromosorb W. The oven temperature was programmed from 70 to 200° at 2°/min. The injector and detector temperatures were maintained at 220 and 240°, respectively. The helium gas flow was 80 ml/min. The pulp was mixed with water and volatiles were removed as before. The volume of the condensate was made up to that of the supernatant with distilled water. Gas chromatographic analysis then gave the proportion of the model compound associated with the pulp. Determinations were made for homologous series of aldehydes, esters, alcohols, and lactones.

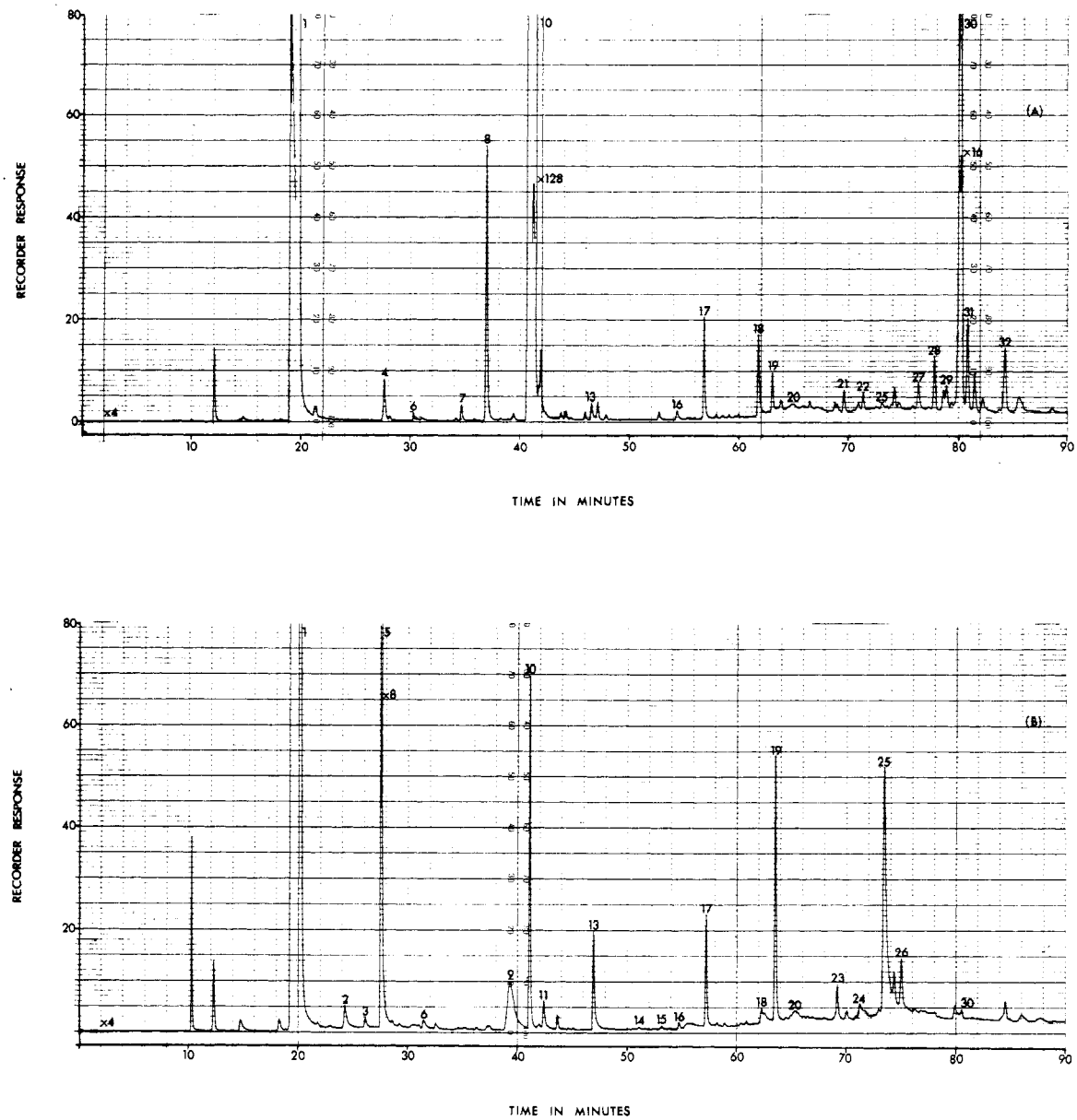

Figure 2. Gas chromatograms of extracts prepared from (A) pulp and (B) serum of orange juice.

Headspace Experiment. The techniques employed were identical with those described in connection with the model experiments. Orange pulp, freed of flavor volatiles, was equilibrated for 30 min at 20° with a solution (75 ml) containing 20 ppm each of 3-methylbutan-2-one, pentan-3-one, *trans*-hex-2-enal, octanal, ethyl heptanoate, nonanal, and ethyl octanoate. The headspace gas (3 ml) was analyzed by gas chromatography. A similar analysis was performed with a mixture which did not contain orange pulp.

RESULTS AND DISCUSSION

The chromatograms reproduced in Figure 1 show that orange pulp has a suppressing effect on headspace concentrations of volatile compounds in an air–water system. The pulp used in this experiment was freed of its own flavor volatiles prior to equilibration with an aqueous solution containing 3-methylbutan-2-one, pentan-3-one, *trans*-hex-2-enal, octanal, ethyl heptanoate, nonanal, and ethyl octanoate. These compounds represent peaks 2–8, respectively, in the headspace chromatograms (peak 1 is due to air). It will be noted that the headspace concentration of each of the compounds tested is affected by the pulp to a different extent. Thus, the presence of pulp modifies not only the intensity but also the balance of the overall aroma. These results, in conjunction with previously reported data concerning the effect of other nonvolatiles on aroma [for discussions and leading references see Buttery *et al.*, 1973 (lipids); Wientjes, 1968 (sugars); Jennings, 1965 (salts)], explain the well-known observation that a synthetic flavor must be formulated to suit the particular base in which it is to be used.

The experiments described next provide estimations of the distributions of flavor volatiles between pulp and sera in citrus and in apple juices. The juices were centrifuged and the flavor extracts prepared from the separated solid and liquid phases were analyzed by gas chromatography. The chromatograms obtained for orange juice are shown in Figure 2 (for peak identities, refer to Table I). Distribution data relating to the more abundant flavor volatiles in orange, grapefruit, lemon, and apple juices are given in Tables I–IV, respectively.

The results show that, in orange juice, hydrocarbons are almost exclusively associated with the pulp. This finding is consistent with previous observations (Blair *et al.*, 1952; Huet, 1969; Peleg and Mannheim, 1970a; Scott *et al.*,

Table I. Flavor Volatiles Identified in Orange Juice and Percentage Distribution of the More Abundant Compounds between Pulp and Serum

Peak no.	Assignment	Basis	% distribution	
			Pulp	Serum
1	Solvent			
2	Methyl butyrate	Ms, gc		
3	Unknown			
4	α-Pinene	Ms, gc	~100	Not detected
5	Ethyl butyrate	Ms, gc	Trace	~100
6	Hexanal	Ms, gc		
7	Sabinene	Ms, gc	~100	Not detected
8	Myrcene	Ms, gc	~100	Not detected
9	3-Methylbutan-1-ol	Ms, gc	Not detected	~100
10	Limonene	Ms, gc	98.0	2.0
11	Ethyl hexanoate	Ms, gc		
12	γ-Terpinene	Ms, gc		
13	Octanal	Ms, gc	12.5	87.5
14	Hexan-1-ol	Ms, gc		
15	*cis*-Hex-3-en-1-ol	Ms, gc		
16	Nonanal	Ms, gc		
17	Standard (ethyl octanoate)[a]			
18	Decanal	Ms, gc		
19	Linalool	Ms, gc	10.0	90.0
20	Octan-1-ol	Ms, gc		
21	β-Elemene	Ms		
22	β-Caryophyllene	Ms, gc		
23	Terpinen-4-ol	Ms, gc		
24	Methyl 3-hydroxyhexanoate	Ms, gc		
25	Ethyl 3-hydroxyhexanoate	Ms, gc	1.5	98.5
26	α-Terpineol	Ms, gc		
27	Sesquiterpene hydrocarbon	Ms		
28	Sesquiterpene hydrocarbon	Ms		
29	Sesquiterpene hydrocarbon	Ms		
30	Valencene	Ms, gc	99.0	1.0
31	δ-Cadinene	Ms, gc		
32	5β, 7β, 10α-Selina-3,11-diene	Ms		

[a] This compound has been reported in orange but was not detected in the investigation reported here.

Table II. Percentage Distribution of the More Abundant Volatile Flavor Compounds between Pulp and Serum of Grapefruit Juice

	% distribution	
	Pulp	Serum
Pentan-1-ol	Not detected	~100
Limonene	99.0	1.0
cis-Linalool oxide	Not detected	~100
Caryophyllene	~100	Not detected
Humulene	~100	Not detected
Ethyl 3-hydroxyhexanoate	Not detected	~100
Nootkatone	38.5	61.5

Table III. Percentage Distribution of Selected Volatile Flavor Compounds between Pulp and Serum of Lemon Juice[a]

	% distribution	
	Pulp	Serum
β-Pinene	97.9	2.1
Limonene	93.4	6.6
γ-Terpinene	92.5	7.5
Terpinen-4-ol	4.6	95.4

[a] Values were not determined for neral and geranial as interference from neighboring peaks precluded accurate integration of the appropriate peak areas. However, it is obvious from the gas chromatograms that the greater proportion of these compounds is associated with the serum.

1965). On the other hand, it is evident that oxygenated compounds are mainly contained in the serum. Since compounds of this type are considered to contribute important notes to orange aroma (for example, see Veldhuis, 1971), it may be concluded that serum volatiles play a significant role in the flavor of orange juice. This observation is difficult to reconcile with the reported success of methods proposed for the preparation of orange concentrates by centrifugation of the juice, and concentration of the separated serum without reincorporation of the serum volatiles in the finished product (Brown *et al.*, 1966; Peleg and Mannheim, 1970 a,b; Sargeant, 1968).

The results obtained for the distribution of volatiles be-

Table IV. Percentage Distribution of the More Abundant Volatile Flavor Compounds between Pulp and Serum of Apple Juice

	% distribution	
	Pulp	Serum
Butyl acetate	2.2	97.8
Hexanal	12.0	88.0
Butan-1-ol	2.9	97.1
trans-Hex-2-enal	3.6	96.4
Hexyl acetate	12.2	87.8
Hexan-1-ol	7.9	92.1

Table V. Percentage Distribution of Volatile Flavor Compounds between Orange Pulp and Water in Model Systems

	% distribution	
Compound	Pulp	Water
Pentanal	0.0	100.0
Hexanal	0.0	100.0
Heptanal	0.0	100.0
Octanal	16.8	83.2
Nonanal	42.7	57.3
Decanal	78.0	22.0
Ethyl butanoate	2.1	97.9
Ethyl pentanoate	3.6	96.4
Ethyl hexanoate	9.9	90.1
Ethyl heptanoate	32.0	68.0
Ethyl octanoate	65.3	34.7
Ethyl decanoate	82.3	17.7
Pentan-1-ol	2.0	98.0
Hexan-1-ol	2.6	97.4
Heptan-1-ol	5.5	94.5
Octan-1-ol	17.2	82.8
Nonan-1-ol	44.8	55.2
Decan-1-ol	75.0	25.0
γ-Valerolactone	1.9	98.1
γ-Hexalactone	3.2	96.8
γ-Heptalactone	2.3	97.7
γ-Octalactone	3.6	96.4
γ-Nonalactone	6.3	93.7
γ-Decalactone	12.7	87.3

tween the pulp and serum of other juices follow the pattern established for orange juice. Significantly, in the case of apple juice (Table IV), all the organoleptically important compounds are oxygenated and mainly appear in the serum. This finding is in accord with the established commercial practice of clarifying apple juice to obtain a product of improved appearance without impairment of flavor.

The trends suggested by the data in Tables I–IV are supported by the results of similar analyses of the distribution of volatiles in mango (unpublished results from this laboratory), a fruit which contains both hydrocarbons and oxygenated compounds (Angelini *et al.*, 1973; Hunter *et al.*, 1974). This suggests that the observed trends in the distribution of flavor volatiles between pulp and sera in fruit juices may well be of general occurrence.

At this point, it became of interest to determine whether pulp has the capacity to remove compounds from a true solution, or whether the association between pulp and flavor volatiles is due exclusively to entrainment brought about by centrifuging a mixture of oil droplets and pulp particles in an aqueous medium. The data given in Table V prove that volatiles in solution will in fact partition between pulp and water. The results also show that the extent to which partition occurs for a particular compound depends upon the chain length and functionality of the compound. It will be noted from Table V that hydrocarbons were not utilized in these experiments. This is a consequence of the low solubilities of hydrocarbons in water, which make accurate determination of their distributions between pulp and water impracticable.

It may also be concluded from the results reported here that in certain instances centrifugation may offer a valuable supplementary technique for the isolation of flavor compounds from fruit juices. For example, if hydrocarbons are absent in a juice, removal of the pulp will lead to only minor losses of volatile compounds. The improved flow characteristics of the pulp-free juice, however, will facilitate the isolation of flavor volatiles, and at the same time reduce the tendency for browning to occur under processing conditions. The presence or absence of hydrocarbons in a fruit juice can easily be established by a small scale preliminary experiment. Even in cases where the initial results indicate that a juice contains both hydrocarbons and oxygenated compounds, centrifugation may still be a worthwhile step in the isolation procedure. In addition to the advantages already mentioned, the pulp, having a relatively small volume, can conveniently be processed separately using a rotary evaporator. In this way a more complete removal of volatiles is achieved and losses of hydrocarbons, which often occur during large scale isolation of volatiles under vacuum, are minimized.

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