JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Distribution of Volatile Compounds in the Pulp, Cloud, and Serum of Freshly Squeezed Orange Juice

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The quantitative distribution of volatile compounds in the pulp, cloud, and serum of a freshly squeezed orange juice (cv. Naveline) was measured. Juice monoterpene and sesquiterpene hydrocarbons were primarily recovered from the pulp (74.0 and 87.2%, respectively) and cloud (7.3 and 14.9%, respectively). Esters and monoterpene alcohols were mainly found in the serum (90.4 and 84.1%, respectively). Long chain aliphatic aldehydes tend to concentrate in the pulp. The relative proportions of individual volatile compounds were similar in the pulp and cloud. Pulp and cloud alcohol insoluble residues exhibited similar compositions; half of them are made of nonwall proteins, and the rest are made of cell wall materials. Pulp and cloud total and neutral lipids had similar fatty acids distributions, although the cloud was much richer in total lipids than the pulp. No relationship was found between the retention of aroma compounds in the pulp or cloud and their AIR and lipid content or composition.

KEYWORDS: ***Citrus sinensis*; freshly squeezed orange juice; volatile compounds; pulp; cloud; serum; alcohol insoluble residues; lipids**

INTRODUCTION

Fresh, hand-squeezed orange juice is a heterogeneous two phase system consisting of the serum, a clear aqueous phase containing soluble compounds (e.g., sugars and organic acids), and a water insoluble phase made of particles ranging from 0.05 μm to a few hundred micrometers in size (*1*). These particles are complex mixtures of rag cell wall and membrane fragments, cellular organelles, oil droplets from peel oil glands that burst during extraction, carotenoid-containing chromoplasts, proteins, lipids, minerals, and flavonoid crystals formed in part by crystallization after juice extraction (*2*, *3*). The insoluble phase is classified as either pulp, consisting of the coarsest particles (>2 μm) that tend to settle upon storage or are pelletable at 360*g* (*4*) or cloud, made up of finer particles (<2 μm) (*3*), which under favorable conditions remain suspended in the serum (*5*). The clear serum is a watery pale yellow liquid possessing little orange aroma (*6*). The insoluble particles enhance the color, flavor, aroma, and body of the juice and are therefore highly desirable in the commercial product.

Even if the flavor of freshly hand-squeezed orange juices is considered a reference against which all juices are judged, most oranges are mechanically processed (e.g., Food Machinery Corporation process) to produce juices that after separation of the pulp at low speed centrifugation, are concentrated to reduce costs of transportation and storage (*7*). The disadvantage of depulping is the enormous amount of aroma compounds drawn off from the juice (*8*). Prior to commercialization, juices are reconstituted by diluting concentrates with water and adding certain aroma compounds (aqueous essence and essence oil) recovered during the concentration step (*7*). The reconstituted juice must have a stable, cloudy appearance. Cloud loss, resulting from the formation of an unattractive two phase system with a flocculent sediment in a clear serum, can render a juice nonmarketable. The flavor of such reconstituted juices differs from that of freshly hand-squeezed, pulpy juices (*9*). Nowadays, the market increasingly demands that juices have a flavor as close as possible to unpasteurized, freshly hand-squeezed juices.

The distribution of juice volatile compounds between macroscopic fragments of pulp and serum (water phase) was fragmentally studied by Radford et al. (*8*) in citrus and Yu et al. (*10*) in passion fruit. They demonstrated that some classes of volatile compounds are unequally distributed between the pulp and the serum (e.g., mono- and sesquiterpene hydrocarbons were primarily associated with the pulp in citrus juices). It remains unclear whether aroma compounds are associated with solid particles by adsorption of oil droplets on rag particles (*3*) and/or by physical entrapment inside the particle cell wall fibrillar network. Furthermore, several authors have found evidence of interactions between volatile compounds and polysaccharides (*11*) or glycopeptides (*12*).

To our knowledge, this is the first detailed quantitative study of the distribution of volatile compounds in the pulp, cloud, and serum of a freshly hand-extracted orange juice. Having

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10.1021/jf026226y CCC: $25.00
Published on Web 04/24/2003

established this distribution, the macromolecular constituents and lipids of pulp and cloud particles were analyzed so as to gain a better understanding of the partition of volatile compounds between the water phase and the water insoluble particles.

MATERIALS AND METHODS

Preparation of Fruit Juice. Sweet orange [*Citrus sinensis* (L.) Osb, cv. Naveline] juice was extracted with a Santos extractor. Juice was immediately frozen and stored at − 20 °C.

Separation of Pulp, Cloud, and Serum. All separations were conducted at 4 °C to minimize the action of pectinmethylesterases (*13*). Juice was centrifuged for 15 min at 1300*g* using a Sorvall RC 5B centrifuge to separate pulp and supernatant. The supernatant was then centrifuged for 30 min at 31 000*g* to remove cloud. The resulting serum was a bland, pale yellow solution with an optical density at 600 nm of less than 0.05. Each fraction (pulp, cloud, and serum) was then weighed. Aliquots of pulp and cloud were then added to distilled water and, after thorough mixing, centrifuged for 30 min at 31 000*g*. This washing step was repeated twice before the washed pulp and cloud were freeze-dried and weighed.

Solvents and Chemicals. The solvents (*n*-pentane and ether) were of analytical grade. Reference compounds, if available, and *n*-alkane (C_5−C_{27}) standards were from Aldrich Chimie (Saint Quentin Fallavier, France). All chemicals were of analytical grade.

Extraction of Volatile Compounds. The internal standard (4.8 μg of *n*-hexanol) was added to fractions of freshly squeezed juice and serum (10 g), pulp (1.0 g), and cloud (0.2 g). Each fraction was then homogenized using a Potter Elvejhem homogenizer with 50 mL of pentane/ether (1:1) for 5 min. Phase separation was achieved by centrifugation at 9000*g* for 5 min. The upper organic phase was recovered, dried over anhydrous sodium sulfate, and finally concentrated at 37 °C to a volume of 2 mL with a 25 cm Vigreux distillation column.

GC-MS Analysis. A Hewlett-Packard 6890 gas chromatograph was used coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer with electron ionization mode (EI) generated at 70 eV. The ion source and quadrupole temperatures were 230 and 150 °C, respectively, and the filament emission current was 1 mA. Volatile compounds were separated on a DB-Wax (column A, J&W Scientific, Folsom, CA) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film) and on a DB-1 (column B, J&W Scientific) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). The oven temperature was increased from 40 °C at a rate of 3 °C min^{-1} up to 250 °C where it was held for 20 min. The on-column injector was heated from 20 to 245 °C at 180 °C min^{-1}. The detector temperature was 245 °C. Helium was the carrier gas at 1.1 mL min^{-1}. Electron impact mass spectra were recorded in the 40−600 amu range at 1 s $interval^{-1}$. Injected volumes were 1 μL of concentrated extract. Compounds were identified on the basis of linear retention indices on both columns (DB-Wax and DB-1) (*14*) and EI mass spectra (Wiley 275.L library) from the literature or from authentic standard compounds. Because our aim was to measure concentrations of volatiles in each fraction, amounts are expressed as μg *n*-hexanol equivalent g^{-1} of fresh weight, response factors being taken as 1.0 for all compounds with reference to the internal standard. Linear retention indices were calculated with reference to *n*-alkanes (C_5−C_{27}). Concentrations are given as the average of triplicates.

Preparation of Alcohol Insoluble Residues (AIRs). AIRs were prepared by adding ethanol (8 mL) to aliquots of pulp and cloud (2 g). After it was thoroughly mixed, the slurry was boiled for 30 min and then filtered on a sintered glass crucible (porosity no. 4). The residue was then washed successively with ethanol/water (80:20) (4 mL), ethanol (2 mL), acetone (2 mL), and ether (1 mL). Finally, the residue was dried (24 h) in a vacuum oven (50 °C) and weighed.

Determination of Lipids. Distilled water (2 volumes, v/w) and chloroform/methanol (2:1, 6 volumes, v/w) were added to wet pulp and cloud aliquots. After they were thoroughly mixed with an Ultra-Turrax and phase-separated, the lower organic phase was recovered while the upper water phase was reextracted three times. The extracts were mixed, dehydrated on sodium sulfate, and brought to dryness in a rotary vacuum evaporator. The residue (total lipids) was weighed. Neutral lipids were extracted by gently crushing freeze-dried pulp (200 mg) and cloud (60 mg) in a mortar with 10 mL of hexane. After they were filtered, extracts were dried under vacuum and diluted to 1 mL with hexane. Fatty acid esterification was performed according to ref *15*.

General Methods. Neutral monosaccharides were released from AIR of pulp and cloud (5 mg) by hydrolysis with 2 M trifluoroacetic acid for 75 min at 120 °C (*16*). They were also submitted to Saeman hydrolysis, as described by Hoebler et al. (*17*), using 72% (w/w) sulfuric acid at 25 °C for 45 min and then 1 M sulfuric acid at 100 °C for 2 h. Sugars were then derivatizated into their alditol acetates (*18*) and analyzed by gas chromatography according to Hoebler et al. (*17*) with inositol as an internal standard. Uronic acids were measured without desterification after preliminary dissolution in concentrated sulfuric acid by the *m*-phenylphenol procedure (*19*, *20*). The estimation of methanol was carried out using the alcohol oxidase method of Klavons and Bennett (*21*). Proteins ($N \times 6.25$) were determined by a micro-Kjeldahl procedure (*22*) and by the Lowry method (*23*) using bovine serum albumin as a standard after precipitation with trichloroacetic acid (7.5% final concentration), centrifugation (1000*g*, 5 min), and subsequent dissolution of the pellet in 0.5 M sodium hydroxide (10 min, boiling water). Ash was determined after heating at 525 °C for 6 h. Calcium and phosphorus were determined by a modified atomic absorption method.

RESULTS AND DISCUSSION

Weight Distribution of Pulp, Cloud, and Serum. Pulp, cloud, and serum are the three native fractions that constitute freshly squeezed orange juice and represent 4.2, 0.8, and 94.4% (fw/fw) of the juice, respectively. Freeze-dried, water-washed pulp, and cloud represented 220 and 134 mg 100 g^{-1} of juice (dw/fw), respectively. Our water-washed cloud weight was similar to total water-washed cloud weights (ca. 150 mg 100 g^{-1} of juice) obtained from two ready-to-serve pasteurized orange juices (not made from concentrates) (*4*).

Distribution of Volatile Compounds. Table 1 shows the volatile compounds extracted from the freshly squeezed orange juice pulp, cloud, and serum by the pentane−ether (1/1) azeotropic mixture and then separated and identified by GC-MS. Each fraction was extracted in triplicate, with extracts from the same fraction giving similar results. The ranges in standard deviations (given in parentheses) according to concentration ranges were as follows: 5−20 μg g^{-1} fresh weight (10−18%); 20−200 μg g^{-1} (8−14%); 200−500 μg g^{-1} (6−10%); 500−1000 μg g^{-1} (4−7%); and 1000−2000 μg g^{-1} (2−5%). It must be noted that compounds with higher polarities than *n*-hexanol (ethanol, acetic acid, ethyl acetate, and ethyl 3-hydroxy-butanoate) might be underestimated due to their higher solubility in the water phase, i.e., the serum, than in the azeotropic mixture.

Monoterpene hydrocarbons constitute the main volatile group, representing 80−90% of total volatiles in all fractions apart from the serum where they represent half the total. Limonene is by far the most abundant monoterpene in juice, pulp, and cloud (∼90−94%/total), followed by *p*-cymene (∼1.6−3.0%), β-myrcene (∼1.5−2.5%), α-pinene (∼0.6−0.7%), and sabinene (∼0.5−0.8%). Similar concentrations (100 ppm of monoterpene hydrocarbons) and relative proportions of these compounds were previously found in a fresh, hand-squeezed Californian orange juice (cv. Navel) (*24*), although *p*-cymene was not detected.

Sesquiterpene hydrocarbons (4 ppm) are the second most important group in juice, pulp, and cloud, with α-selinene representing ∼60−70% and valencene representing ∼8−10% of the total. Valencene was detected at 3.7 ppm in the above quoted Navel juice (*24*).

The number of esters and their total concentration (2.5 ppm) are higher in our juice than in the Navel juice (0.9 ppm). Two

Table 1. Volatile Compounds Recovered from Juice, Pulp, Cloud, and Serum

component[a]	log P[b]	LRI[c]	LRI[d]	juice	pulp	cloud	serum	reliability of identification[e]
			monoterpene hydrocarbons					
α pinene	4.27	1010	918	0.6	13.0	5.3	tr[f]	1
β-pinene	4.35	1089	957	0.7	6.7	4.6	0.5	1
sabinene	4.69	1103	957	0.5	13.4	4.4	0.3	1
δ–3-carene	4.61	1130	992	0.5	10.9	3.7	tr	1
β-myrcene	4.88	1146	976	2.2	44.8	13.5	0.1	1
α-terpinene	4.75	1159	1001	0.1	2.0	0.8		1
limonene	4.83	1180	1010	91.7	1629.7	857.3	4.6	1
β-phellandrene	4.70	1185	1010	1.2	7.4	4.3	tr	1
γ-terpinene	4.75	1222	1044	0.2	3.4	1.0	tr	1
β-ocimene	4.80	1230	1032	tr	0.7			1
p-cymene	4.00	1244	1004	3.1	27.8	16.6	2.4	1
α-terpinolene	4.47	1259	1067	0.1	2.4	0.6		1
total				100.8	1762.2	912.3	8.0	
			sesquiterpene hydrocarbons					
α-copaene	5.71	1465	1390	0.1	1.2	0.8	tr	2
β-cubebene	6.73	1508		0.1	1.0	1.3		2
β-caryophyllene	6.30	1565	1471	0.1	1.7	1.1	tr	1
ni[g]		1607		0.1	1.0	0.5		
ni		1615		tr	0.7	0.8		
ni		1648			0.6			
ni		1661		0.1	3.3	2.3		
germacrene D	6.99	1678	1452	tr	0.9	0.5		1
ni		1684		tr	0.5	0.4		
α-selinene	6.30	1689		3.0	69.4	65.0		2
ni		1693		0.3	3.0	2.3		
ni		1703		0.1	tr	0.5		
bicyclogermacrene	6.22	1707	1467	0.1	1.3	1.1		2
valencene	6.30	1731	1482	0.4	9.2	8.6		2
ni		2099		tr	0.2	1.0		
ni		2182		0.2	4.6	2.6		
ni		2201		0.1	0.6			
total				4.7	99.2	88.8	tr	
			esters					
ethyl acetate	0.86	878		0.2	1.6	3.4	0.2	1
ethyl butanoate	1.85	1021	794	0.3	3.1	2.6	0.2	1
2-methyl ethyl butanoate	2.34	1037	1007	0.2	2.0	1.4	0.2	2
butyl butanoate	2.83	1196	969	0.1	0.5		0.1	1
ethyl hexanoate	2.83	1212	980	0.6	4.6	2.7	0.5	1
n-hexyl acetate	2.34	1251	987	tr	0.6		0.1	2
hexyl butanoate	3.81	1393	1171	0.1	0.7		tr	1
ethyl octanoate	3.81	1412	1173	0.5	4.5	1.9	0.4	1
ethyl 3-hydroxy butanoate	0.31	1484	943	tr			tr	1
linalyl acetate	4.39	1528	1231	0.1	0.6		0.1	2
ethyl 3-hydroxy hexanoate	1.29	1652	1340	0.4	0.3		0.4	1
p-menth-1-en-9-yl acetate	5.39	1791		tr	0.8	0.5		1
ethyl hexadecanoate	7.74	2220	2231	0.1	0.4		0.1	2
total				2.5	19.5	12.5	2.4	
			aliphatic alcohols					
ethanol	0.14	900		0.4		3.4	1.5	1
n-butanol	0.84	1125		0.6	5.6	3.9	0.5	1
2-heptanol	2.24	1298	877	tr			tr	1
(*Z*)–2-hexen-1-ol	1.61	1380	850	tr			tr	1
n-octanol	2.81	1534	1068	0.7	5.6	4.4	0.4	1
n-nonanol	3.30	1640	1151	0.1	1.8	1.0	tr	1
decanol	3.79	1745	1254	0.3	4.2	4.6	0.1	1
n-hexadecanol	6.73	2467		0.1		6.6	tr	2
total				2.2	17.2	23.9	2.6	
			monoterpene alcohols					
linalool	3.38	1521	1080	0.6	3.4	1.3	0.4	1
terpinen-4-ol	3.33	1572	1160	0.7	2.8	2.8	0.6	1
α-terpineol	3.33	1671	1174	0.4	3.6	3.7	0.5	1
nerol	3.47	1777	1205	0.1	0.3		0.1	1
trans-carveol	3.29	1806		tr	tr		tr	2
geraniol	3.47	1824	1236	0.1	0.2		0.1	1
total				1.9	10.3	7.8	1.7	
			aldehydes and ketones					
hexanal	1.80	1063	780	0.4	3.5	2.5	0.3	1
octanal	2.78	1264	980	0.1	1.2	0.5	tr	1
α-thujone	2.65	1364		tr	0.4	0.5	tr	2
nonanal	3.27	1367	1081	0.1	1.1	2.4	tr	1
decanal	3.76	1471	1172	0.1	4.1	3.0	tr	1

Table 1 (Continued)

component[a]	log P[b]	LRI[c]	LRI[d]	juice	pulp	cloud	serum	reliability of identification[e]
				aldehydes and ketones				
benzaldehyde	1.71	1478	924	tr	0.3		tr	1
acetophenone	1.67	1607				0.9		2
α-sinensal	5.61	2261	1711	tr	0.7		tr	1
nootkatone	4.88	2390	1746	0.7	14.1	10.6	0.3	2
total				1.4	25.2	20.3	0.8	
				carboxylic acids				
acetic acid	0.09	1418		0.4	4.4	22.5	0.2	1
hexanoic acid	2.05	1827	890		0.3			1
octanoic acid	3.03	2034	1262	0.2	1.2		0.1	1
nonanoic acid	3.52	2144	1745	0.1	0.5		tr	1
decanoic acid	4.02	2231	1996	0.3	6.5	14.0	0.1	1
total				1.0	12.9	36.5	0.4	
total				114.6	1946.6	1102.1	16.0	

[a] Concentrations in μg g^{-1} fresh weight. [b] Octanol–water partition coefficient. [c] Linear retention index from DB-Wax. [d] Linear retention index from DB-1. [e] Key for reliability of identification: 1, identified by linear retention index and mass spectrum of reference compounds; 2, tentatively identified by linear retention index and mass spectrum similar to published data. [f] tr, traces (<0.05 μg g^{-1}). [g] Nonidentified sesquiterpene hydrocarbons (M_w = 204).

compounds considered important contributors to orange juice flavor are ethyl butanoate, which possesses a low flavor threshold in water (*25*), and ethyl acetate. Ethyl butanoate and ethyl acetate are found at similar concentrations to those in the Navel juice (0.43 and 0.17 ppm, respectively) (*24*). Ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxy hexanoate have higher flavor thresholds (*26*) and are also found in noticeable concentrations.

All of the above classes of volatile compounds are more abundant in the pulp and cloud than in the freshly squeezed juice. Taking into account the percentage of the total weight of the fresh juice (fresh weight basis) that the pulp, cloud, and serum constitute (4.2, 0.8, and 94.0%, respectively), the monoterpene hydrocarbons present in pulp, cloud, and serum represent 74.0, 7.3, and 7.4% of the juice content with a total recovery of 89%. Radford et al. (*8*) have already shown that in hand-squeezed orange, lemon, and grapefruit juices, monoterpene hydrocarbons are almost exclusively found in the pulp. The percentages of limonene, β-pinene, and γ-terpinene in sera obtained by ultracentrifugation of the juice at 250 000*g* were, respectively, 1−7, 2, and 7% of the corresponding monoterpenes present in the juice of the same citrus fruit (*8*). In our juice, limonene in the serum represented 4% of total limonene. Full retention of mono- and sesquiterpene hydrocarbons in the pulpy retentate (and absence in the clear permeate) of a cross-flow microfiltered mango puree has also been observed (*27*). The relative proportions of these compounds in the pulp and cloud are similar to those in the juice. The same trend is found for sesquiterpene hydrocarbons, which, because their octanol−water partition coefficients are higher than those of monoterpenes, are absent in the serum. In the pulp and cloud, they respectively represent 87.2 and 14.9% of juice content with a total recovery of 102%. Their relative proportions are similar in the juice, pulp, and cloud. Unlike monoterpene hydrocarbons, sesquiterpenes are found at similar concentrations in both the pulp and the cloud.

The situation is different for esters, which are present in the pulp, cloud, and serum at 33.6, 4.0, and 90.4% of their initial amounts in the juice with a total recovery of 128%. Once again, our data agree with Radford et al. (*8*) who reported that esters were either found exclusively in the sera of the studied citrus juices, such as ethyl butanoate, or only poorly represented in the pulp, such as the 1.5% of total ethyl 3-hydroxyhexanoate (3.2% in our own study). Apart from ethyl 3-hydroxyhexanoate, the relative proportions of these esters were comparable in pulp, cloud, and juice.

The monoterpene alcohols showed a similar trend, 22.6, 3.3, and 84.1% of the juice content found in the pulp, cloud, and serum, respectively, with a total recovery of 110%. The percentages of linalool and terpinen-4-ol found in the serum were 60−80%, with the rest found in the pulpy fraction. Once again, 10% of the total linalool of a fresh orange juice was found in its pulp (*8*). These monoterpene alcohols were reported at similar levels of magnitude in the fresh Navel orange juice (*24*).

Aliphatic aldehydes (octanal, nonanal, and decanal), with a very low odor threshold in air and in water (*26*), are generally considered important contributors to orange juice flavor (*9*) and, as already observed by Radford et al. (*8*) using a model system that included orange pulp and water, tend to concentrate in the pulp when their number of carbon atoms and their octanol−water partition coefficients increase. The opposite trend is found for the more polar hexanal, which was mainly found in the serum.

Nootkatone is a sesquiterpene ketone that has been previously found at 1.4 ppm in fresh orange juice (cv. Valencia) (*28*). It was found at 0.7 ppm in our juice, with 85% located in the pulp fraction. Because the octanol−water partition coefficient of nootkatone (4.88) is close to that of monoterpenes, their similar distributions among the fractions are not unexpected. Conversely, Radford et al. (*8*) found that ∼60% of nootkatone was present in the serum of a fresh grapefruit juice.

Finally, the total concentration of volatiles in the pulp was 1.7 times that in the cloud. Except for monoterpene hydrocarbons and carboxylic acids, all other classes of volatiles were found at similar concentrations in both pulp and cloud.

Yield and Composition of AIR. To further investigate the differences observed in the distribution of volatile compounds between the cloud and the pulp (i.e., difference in total volatile concentrations), we examined the composition of their AIRs as shown in **Table 2**. Pulp and cloud AIR yields are, respectively, 147 and 58 mg 100 g^{-1} of the starting juice (dw/fw). The cloud AIR yield (i.e., 43% of the total freeze-dried water-washed cloud) is lower than the extracted cloud weight (defined as total cloud extracted twice with water, methanol, and dimethyl sulfoxide/2-propanol−roughly equivalent to AIR) measured on two ready-to-serve pasteurized orange juices, ca. 104 mg 100 mL^{-1} (i.e., 67% of the total freeze-dried water-washed cloud) (*4*).

Table 2. Composition of AIRs[a]

	pulp	cloud
uronic acids[b]	17.0	12.2
neutral noncellulosic polysaccharides[c]	17.7	13.9
cellulose[d]	1.7	2.4
proteins	51.9[e]/49.8[f]	56.2/54.4
ash	4.4	12.0
calcium/phosphorus	0.22/0.65	0.39/0.49
uronic acids[g]	45.2 (64)[h]	43.3 (20)
rhamnose[g]	2.3	1.9
fucose[g]	0.9	1.1
arabinose[g]	16.9	14.4
xylose[g]	1.6	0.5
mannose[g]	1.2	1.2
galactose[g]	25.2	27.1
glucose (noncellulosic)[g]	1.8	1.2
glucose (cellulosic)[g]	4.9	9.3

[a] (%/AIR dw). [b] Expressed as anhydrogalacturonic acid. [c] Neutral polysaccharides obtained by hydrolysis with diluted acid (TFA or sulfuric acid) and GC of the alditol acetates, expressed as the sum of anhydrosugars. [d] Glucose obtained from the difference between Saeman and dilute acid hydrolyses. [e] Proteins determined by the micro-Kjeldahl method ($N \times 6.25$). [f] Proteins determined by the Lowry method. [g] Mole % of constituent monosaccharides. [h] Values in parentheses are the degree of methylation calculated on the basis of uronic acid contents.

Proteins, as determined by the Kjeldahl ($N \times 6.25$) and Lowry procedures, represented roughly half of the cloud and pulp AIRs. This finding agrees with previously reported data on the clouds of commercial orange juices made from concentrates and ready-to-serve orange juices (*4*). Our cloud AIR protein content agrees with the AIR nitrogen content of sediment B (i.e., cloud) obtained by centrifugation of a pulp-free orange juice at 60 000*g* (~7.6%, i.e., $N \times 6.25 = 47.5\%$) (*2*). However, the authors of this study reported that this nitrogen was not proteic in nature since the biuret test indicated a very low protein content. In our case, when the Lowry procedure was applied to sodium hydroxide extracts of cloud AIRs, it gave a protein content similar to that of the Kjeldahl analysis (**Table 2**). This indicates that as previously reported (*4*), proteins are responsible for cloud nitrogen content. Baker and Bruemmer (*5*) also reported 45% protein content in an orange juice cloud AIR. Finally, ~50% protein content was also reported for the clouding substances in guava puree (*29*). Cloud and pulp AIRs have very similar polysaccharidic compositions, consisting of ~12−17% uronic acids (presumably galacturonic acid), ~14−18% neutral noncellulosic polysaccharides (pectic substances side chains and hemicelluloses), and ~2% cellulose. While our cloud AIR cellulose content is identical to that previously reported for an orange juice cloud (= sediment B) (*2*), our pulp AIR cellulose content differs greatly from a previously published level (sediment A; 11%) (*2*). The same authors reported an AIR pectin content of 83%, but this was determined by weighing the 80% ethanol precipitate of a neutralized 0.05 N sodium hydroxide extract and therefore measures the amount of pectins and proteins that underwent solubilization and precipitation. This artifact has already been mentioned (*5*), and it has been estimated that only 53% of the precipitate is pectic in nature; the rest of it is proteins.

The monosaccharide mole percentages in pulp and cloud AIRs are also very similar, with decreasing proportions of uronic acids, galactose, and arabinose. Cloud AIR was relatively richer in cellulosic glucose than pulp AIR. Because hemicelluloses from dicocotyledons are essentially xyloglucans (*30*), it is likely that galactose and arabinose are constituents of pectic neutral side chains. However, cloud and pulp AIRs show clear differences in their ash content and the degree of methylation of pectic substances. From their compositions, cloud and pulp AIRs appear to consist of two groups of macromolecular components. The presence of cellulose associated with pectic substances and neutral noncellulosic polysaccharides indicates that cell wall fragments originating from rag tissue are present in both the cloud and the pulp. However, because proteins constitute less than 10% of primary cell walls (*30*), most of the proteins found in cloud and pulp are of intracellular rather than cell wall origin. The sum of determined constituents reached ~93 and 97% of pulp and cloud AIRs, respectively.

Finally, it has been asserted that cloud particles constitute a distinct anatomical component of fruit rather then simply being small fragments of pulp (*2*). However, our data show that apart from their ash content and the degree of methylesterification of pectins there is no fundamental difference between pulp and cloud. The cloud can therefore be regarded as that part of the pulp possessing the smallest particle size.

Table 3. Relative Percentages of Fatty Acids in Neutral and Total Lipids of Pulp and Cloud

	neutral lipids[a]		total lipids[a]	
	pulp	cloud	pulp	cloud
$C_{16:0}$	22.8	20.9	33.2	35.0
$C_{16:1}$	5.5	5.5	tr[b]	4.3
$C_{18:0}$	3.4	2.7	5.2	8.8
$C_{18:1}$	20.5	21.8	14.7	13.3
$C_{18:2}$	33.4	34.8	30.0	23.8
$C_{18:3}$	12.4	12.9	11.7	9.7
$C_{20:0}$	2.0	[c]		
$C_{20:1}$		1.4	5.2	5.1

[a] Mole % of fatty acids identified. [b] tr, trace (<1%). [c] Not detected.

Lipids. Lipids may play a role in the retention of volatiles by pulp and cloud. Total lipids (triglycerides, free fatty acids, phospholipids (*31*), hydrocarbon carotenoids, and terpenes) were extracted from wet pulp and cloud by chloroform/methanol (2:1). Yields were, respectively, 1.8 and 13.5% of the wet pulp and cloud. Scott et al. (*2*) found that a cloud obtained from a FMC-extracted orange juice contained 25% neutral lipids and 75% so-called insolubles (i.e., AIRs). Taking into account the AIR percentage in wet cloud (7.2%), our figures are 65% total lipids and 35% insolubles.

The relative molar percentage distribution of fatty acids is similar for both pulp and cloud neutral lipids (**Table 3**): linoleic acid ($C_{18:2}$) is the major fatty acid followed by palmitic ($C_{16:0}$), oleic ($C_{18:1}$), and linolenic ($C_{18:3}$) acids. Similar distributions of fatty acids are found for pulp and cloud total lipids, where palmitic and linoleic acids dominate, followed by oleic and linolenic acids. Similar results have been previously reported for a juice made from mature Naveline oranges (*32*).

Relationship between Volatile Compounds and Water Insoluble Constituents of Pulp and Cloud. Total volatile concentrations in wet pulp and cloud were 1.95 and 1.10 mg 100 g^{-1}, representing 71 and 8% of juice volatiles (**Table 2**). No relationship was found between the retention of aroma compounds by pulp and cloud and their content and composition of water insoluble constituents.

The volatile compounds associated with pulp and cloud from a freshly squeezed orange juice represent ~80% of total juice volatiles, of which 90% are in the pulp and 10% in the cloud. Because pulp and cloud do not fundamentally differ in their chemical nature, it is possible that the orange aroma of cloudy juices reconstituted from concentrates could be enhanced by the reintroduction of finely comminuted pulp.

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Received for review December 18, 2002. Revised manuscript received March 4, 2003. Accepted March 8, 2003.

JF026226Y