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Role of Pulp in Flavor Release and Sensory Perception in Orange Juice

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This work elucidates the role of suspended solids in sensorial perception and flavor release in orange juice. The coarsest pulp (insoluble particles with a diameter of >2 μm) accounted for two major physicochemical effects in orange juice samples: it retained large amounts of aroma compounds, including terpenes and aldehydes, and modified the rheological properties of the juice matrix. These phenomena strongly affected the chemical composition of the vapor phase in the juice samples. On the other hand, orange juice cloud (finest insoluble particles with a diameter of <2 μm) also showed a strong retention effect on ethyl butanoate or hexanal, probably due to the occurrence of molecular interactions with cloud macromolecules. The amount and the size of the suspended solids critically modified not only the texture perception but also the odor and the overall flavor perception, including the "freshly squeezed" and the "artificial flavor" descriptors. The addition of a natural pulp to low-pulp juices increases the fresh orange juice character, a finding that is explained by both physicochemical (fresh pulp contains high amounts of key aroma compounds, including acetaldehyde and mono- and sesquiterpenes) and cognitive effects, mainly due to the tactile properties of the pulp.

KEYWORDS: Orange juice; viscosity; texture; mouthfeel; flavor release; retention; SPME; sensory analysis; sensory profile; aroma

INTRODUCTION

Due to its pleasant aroma and "healthful" properties, orange juice is the most appreciated juice beverage worldwide. Its high quality, which is the key of consumer demand, is greatly dependent on the characteristic "fresh orange juice" flavor. Even if the flavor of freshly hand-squeezed orange juice is considered as a reference for all orange juices, most oranges are mechanically processed to produce juices that, after separation of the pulp, are concentrated to reduce costs of transportation and storage. The drawback of depulping is the enormous amount of aroma compounds drawn off from the juice (*1*). Prior to commercialization, these juices are reconstituted by diluting concentrates with water and by adding aqueous and oil essences. The flavor of such reconstituted juices, however, dramatically differs from the flavor of a freshly hand-squeezed, pulpy juice (*2*). Conversely, the current market increasingly demands juices with a flavor as close as possible to that of unpasteurized, freshly hand-squeezed juices. This explains food industry attempts to develop new technologies—the so-called "invisible technologies"—to obtain juice products with both high nutritional and high organoleptic qualities (*3*).

Orange juice is a heterogeneous, two-phase system consisting of the serum, a clear aqueous phase containing soluble compounds, and a water insoluble phase made up of particles ranging from 0.05 μm to a few hundred micrometers in size. These insoluble particles enhance the color, flavor, aroma, and body of the juice; as such, they are highly desirable in the commercial product. These solids contribute mouthfeel to orange juice and may or may not be desirable, depending upon consumer preference. Suspended pulp, which is also called sinking or bottom pulp, contributes to the opaqueness and smooth mouthfeel typical of citrus juices. Screened pulp, or floating pulp, consists of the large juice vesicle particles (juice sacs), and imparts a distinct tactile sensation in the mouth (*4*).

Cloud is the finest insoluble fraction of orange juice (<2 μm), which is rich in insoluble pectins as well as proteins and lipids (*5*, *6*). Although cloud represents a very minor percentage of fresh orange juice (0.7% w/w), it contains a non-negligible amount of aroma, including terpenic compounds (*7*). Although its finest particles are not perceptible in the mouth—humans are able to detect only food particles with diameters of ≥ 5 μm (*8*)—they contribute to increasing the viscosity of the juices.

Radford et al. (*9*) showed a clearly defined partitioning of the volatile components of orange juice between the pulp and the serum. These authors highlighted the fact that hydrocarbons

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are almost exclusively associated with pulp, whereas oxygenated compounds are more closely associated with the serum.

Similar results were recently obtained by Brat et al. (*7*), who investigated the quantitative distribution of volatile compounds in the pulp, cloud, and serum of a freshly hand-squeezed orange juice. These authors extracted volatile compounds from the differents matrices using solvent extraction. Jordan et al. (*10*) compared the qualitative and quantitative characteristics of the volatile compounds found in the headspace of freshly extracted orange juices, with insoluble solid contents of 3 and 10−15%. In their study, the analysis of volatile components was carried out using polydimethylsiloxane and polyacrylate solid-phase microextraction (SPME) fibers and steam distillation−extraction.

To date, the influence of these suspended solids on sensory perception has received little attention, despite the fact that they may be a key to improving orange juice quality. An early study from Ahmed (*11*) showed the influence of acid, sugar, and pectin on the flavor threshold of limonene in a water system, at the concentrations normally present in orange juice. A combination of these nonvolatile components was found to increase the retronasal threshold of limonene. To our knowledge, no study has dealt with both physicochemical and sensory effects of pulp and cloud fractions on orange juice flavor, especially with respect to a real juice system.

We previously investigated the chemical composition of pulp and cloud in order to gain insight into the partition of volatile compounds between the water phase and the insoluble particles (*7*). On the basis of our findings, we put forward the hypothesis that the reintroduction of cloud or pulp could enhance the orange aroma of processed juices. The aim of this work was, therefore, to elucidate the role of these suspended solids on both sensory perception and flavor release in orange juice. We made juices with increasing pulp contents in order to carry out the descriptive profiling of texture, taste, odor, and aroma. At the same time, flavor release studies through SPME as well as physicochemical measurements were performed to obtain a combined interpretation of chemical and sensory results.

MATERIALS AND METHODS

Orange Juice. Fresh orange juice (Naveline, Spain) was hand-squeezed at 4 °C to minimize the activation of pectin methyl esterase (PME).

Separation of Pulp, Cloud, and Serum. Separation of pulp, cloud, and serum was performed following the method of Brat et al. (*7*). The juice was centrifuged for 15 min at 1300*g* using a Sorvall RC5B centrifuge to separate the pulp and the supernatant. Cloudy supernatant was submitted to a 3100*g* centrifugation to obtain the cloud and the limpid serum fractions. Pulp and cloud represented 12 and 0.7% (w/w), respectively, of the total fresh orange juice. Centrifugations were carried out at 4 °C.

Pulp Deodorization. Flavor compounds were removed using a rotative evaporator (30 °C) on a pulp aliquot. Pure water aliquots were periodically added to the pulp to avoid complete dehydratation and to restore the original percentage of moisture. The deodorization steps were repeated until no noticeable odor or aroma was detected.

Juice Formulation. Seven juices were made, as reported in **Table 1**. Immediately after preparation, samples were pasteurized in 500 mL glass bottles with a Simaco benchtop system (92 °C for 2 min) to deactivate PME. The effect of the thermal treatment on orange juice odor properties was assessed by comparing the aroma compound composition of fresh and pasteurized orange juices: gas chromatography−olfactometry (GC-O) was performed on the SPME extracts in order to highlight the eventual odor differences between fresh and pasteurized juices (*12*). The juice bottles were stored at 4 °C in the dark for 1 month before sensory and instrumental analysis.

Table 1. Reformulated Orange Juice Samples[a]

W	serum
SN	supernatant
SN+P/2	reformulated juice with supernatant and half pulp (6% pulp)
SN+P	reformulated juice with supernatant and whole pulp (12% pulp)
J	whole juice (12% pulp)
J+P	whole juice enriched with pulp (24% pulp)
J+PD	whole juice enriched with deodorized pulp (24% pulp)

[a] All samples were immediately submitted to pasteurization (92°C for 2 min).

Color Measurement. A Minolta CR-A70 colorimeter was used for color measurement. The orange juices were placed in 10 mL tubes and measured for *L*, *a*, and *b* values. An increasing *L* value indicates increasing lightness ($L = 0$, black; $L = 100$, white). An increase in *a* represents an increase in redness ($-a$, green; $+a$, red), and an increase in *b* indicates an increase in yellow tone ($-b$, blue; $+b$, yellow). The color differences assessed by the Minolta colorimeter could also be detected by sight, so during sensory analysis they were masked by means of red light.

Rheology. Thirty milliliter aliquots of SN+P/2, SN+P, J, J+P, and J+PD juices were sampled from the same batches used for sensory analysis. Immediately after sensory sessions, apparent viscosity (mPa·s) was measured using a computer-controlled rotary viscometer RM 180 (Rheometric Scientific) equipped with a coaxial cylinder geometry (test time = 120 s; shear rate $D_{\max} = 1200\ \mathrm{s}^{-1}$). Test temperature was set at 30 °C, which is the mean temperature measured in the assessors' mouths during orange juice tasting.

General Physicochemical Methods. The most common physicochemical analyses were run on the juice samples. Refraction index (°Brix) was measured at 25 °C on limpid serum using an Otago refractometer.

Free sugars and organic acids were quantified by ionic exchange chromatography. The supernatant (1 mL) from centrifuged juice J (10 mL) was passed through a SepPak C18 filter (conditioned with 5 mL of methanol and 10 mL of water) and then analyzed by a Hitachi HPLC equipped with an Aminex HPX87H column and a differential refractometer. Six millimolar H_2SO_4 was used as eluent at a flow of 0.6 mL/min, at ambient temperature. External calibration was done with solutions of D-sucrose, D-glucose, D-fructose, and citric acid (Sigma-Aldrich) at different concentrations in water.

The total acidity (expressed as citric acid) was also measured by basic titration using 0.1 N NaOH.

Sensory Analysis I: Triangle Test. The sensory panel was made up of 36 untrained people, selected from a group of 48. Selection of the panel was based on a discrimination test, a verbal creativity test, an olfactory sensitivity test (*13*), a ranking test, and two bitterness sensitivity tests using Naringin and Prop (6-*n*-propyl-thiouracile).

Triangle tests were performed once on three pairs of orange juice samples: SN/W, J/SN+P, and J+P/J+PD. Each pair was independently tested for differences in odor (orthonasal evaluation) and overall flavor and texture (in-mouth evaluation). Ten milliliter juice samples were served to assessors in sealed white plastic cups of 80 mL (serving temperature = 17 ± 1 °C) in individual boxes at a room temperature of 20 ± 1 °C. To mask the color differences detected in the products, sensory sessions were performed under red light. For each test three samples were presented including two identical samples and one different; the order of presentation was balanced over all of the panelists. Assessors were asked to carefully stir samples for 10 s and remove the cap just before sample evaluation. The panel carried out a triangle test first on odor (orthonasal evaluation) and, after a 20 min break, on global in-mouth perception (retronasal evaluation). Different orders of presentation and different codes were used between orthonasal and retronasal tests.

Sensory Analysis II: Descriptive Profiling. Sensory profiles (odor, aroma, texture, and taste) were obtained for SN+P/2, SN+P, J, J+P, and J+PD juice samples. The sensory panel consisted of the 13 members (12 women and 1 man, 22−55 years old) selected from the first assessor group. Panelists were trained over 10 sessions using experimental orange juices **(Table 1)** and six commercially available juices (Tropicana 100% pure orange juice with and without pulp,

Table 2. Descriptors Resulting from Training Sessions and Used To Create Orange Juice Sensory Profiles[a]

odor	aroma	texture	taste
freshly squeezed orange	freshly squeezed orange	fluidity	sweet
artificial flavor	artificial flavor	particle size	acid
orange peel	orange peel	pulp quantity	bitter
grapefruit	grapefruit		
lemon	lemon		
jam	jam		
vegetal	vegetal		
fermented	fermented		
cooked fruit	cooked fruit		
tropical fruits			
mandarin			

[a] Each descriptor corresponds to one sensory characteristic precisely defined by the panel.

Tropicana "Pulpissimo" orange juice, Minute Maid orange juice from concentrate, Carrefour 100% pure mandarin juice, and Andros 100% pure orange juice). Panelists created a list of descriptors **(Table 2**) by consensus. Then, they were instructed to describe the juices by scoring attribute intensities using an unstructured line scale, anchored with appropriate terms for each descriptor. The panel was also aided in their consensus by evaluating pure substances poured into 100 mL brown glass bottles, that is, orange peel for the "orange peel" descriptor; freshly hand-squeezed orange, grapefruit, and lemon juices for the "freshly squeezed orange", "grapefruit", and "lemon" descriptors, respectively; orange juice boiled for 3 h (Tropicana 100% pure orange juice) for the "cooked" descriptor; marmalade (Carrefour) for the "jam" descriptor; orange soft drink (Oasis) for "artificial flavor"; and tropical juice (Carrefour) for "tropical fruits". Profile measurements were organized in two sessions (repetitions) in individual boxes.

Experimental conditions were as follows: juice bottles were opened just before the sensory test. Thirty milliliter samples were served to assessors in sealed white plastic cups of 80 mL (service temperature = 17 ± 1 °C) under red light (room temperature = 20 ± 1 °C). A dummy product, that is, a repeated whole juice (J), was added to the plan to avoid the first product effect on sensory evaluation. Apart from the dummy product, the six products were presented according to a Williams's Latin-square design, to control for presentation order and first-order carry-over effect (*14*). Instructions for each sample were (a) to rinse the mouth with water before starting, (b) to stir samples for 10 s before each assessment, (c) to uncover cups only for evaluation. (d) to smell and score odor descriptors, (e) to sip and evaluate taste, (e) to sip and evaluate texture, and (f) to sip again and rate flavor descriptors. Panelists were asked to score descriptor intensities on a 20 cm unstructured line scale ranging from "very low" to "very intense", later converted to scores ranging from 0 to 100.

Data were collected and statistically analyzed by the Fizz program (Biosystems, Dijon, France). Normality of score distribution was determined by visual inspection of the normal probability plot. Analysis of variance was used to determine differences in descriptor intensities using a model with product and assessor as two main effects plus their interaction (model: descriptor = product + assessor + product × assessor), considering "assessor" as a random effect. The Newman–Keuls test (NK test) was used for mean comparison ($p < 0.01$).

A principal component analysis (PCA) based on the correlation matrix was performed on the juice samples (excluding the dummy product) using as variables the mean intensities (over the 13 panelists and 2 sessions) for texture, taste, aroma, and odor descriptors, which significantly varied according to the product.

A correlation matrix was calculated on sensory and flavor release data, to obtain the coefficient of correlation "r" of each possible pair of odor descriptors and aroma compounds.

Flavor Release by Headspace-SPME Analysis. Volatile compounds from the headspaces of W, SN, SN+P/2, SN+P, J, J+P, and J+PD juices were sampled by a Stableflex 50/30 μm DVB/CAR/PDMS SPME fiber (Supelco, Bellfonte, PA). The extraction methodology (5 min thermal equilibrium at 40 °C + 1 min fiber exposure) was chosen on the basis of previous experiments (*15*). Two and a half milliliter sample aliquots contained in 10 mL glass vials (Supelco, Bellefonte, PA) were analyzed in triplicate. Volatiles were automatically injected by the Combipal system (Gerstel, Germany) into an HP 6890 gas chromatograph equipped with an MSD 5973 mass detector (Agilent Technologies, Palo Alto, CA). Operating conditions were as follows: DB-Wax column (J&W Scientific, i.d. = 0.32 mm, 30 m, film thickness = 0.5 μm) held at 40 °C for 5 min and then increased at 5 °C·min^{-1} to 240 °C. Helium was used as carrier gas at a linear velocity of 40 cm·s^{-1}. The source was kept at 200 °C. The transfer line and the detector were maintained at 250 °C. Mass spectra in the electron impact (EI) mode were generated at 70 eV; they were collected from m/z 29 to 450, at 3.45 scans·s^{-1}. Mass spectral identification was done using NIST (Gaithersburg, MD) and INRAMASS (France) mass spectral libraries, the second one being realized by injection of pure reference compounds in the same mass spectrometric conditions. Linear Retention indices of authentic compounds were also used to confirm identifications.

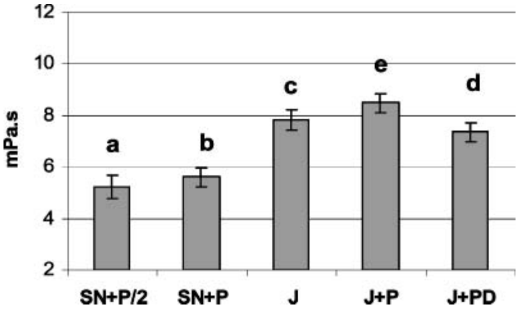


Figure 1. Apparent viscosity of juice samples measured by RM150 viscometer at 30 °C (mouth temperature).

Identification of Odor Active Compounds in Orange Juice. GC-O analysis was performed on whole juice (J) SPME extract to identify the active odor compounds. Operating conditions were as follows: DB-Wax column (J&W Scientific, i.d. = 0.32 mm, 30 m, film thickness = 0.5 μm) held at 40 °C for 5 min and then increased at 5 °C·min^{-1} to 240 °C. Hydrogen was used as carrier gas with a linear velocity of 37 cm·s^{-1}. The GC effluent was split 1:1 between the flame ionization detector and the sniffing port (250 °C). Five panelists evaluated SPME effluent enriched with purified, humidified air (100 mL·min^{-1}). For each odor stimulus, panelists recorded the detection time and gave an odor description (*15*).

RESULTS AND DISCUSSION

We produced orange juice samples with increasing pulp content for sensory and aroma release analyses. Juices ranged from 0% (SN sample) to 24% (J+P and J+PD) pulp amount; a sample of serum (W), totally lacking in suspended solids, was also analyzed. The experimental design also included SN+P and J+PD samples in order to take into account the effect of reconstituting juices as well as the effect of adding further aroma compounds with a naturally flavored pulp. We characterized samples for the most important physicochemical and viscosity properties and then performed parallel sensory profiling and aroma release studies.

Rheological Measurements. Orange juice is a Newtonian fluid. The apparent viscosity of juice samples used for sensory analysis was measured at constant shear rate and at 30 °C (tasting temperature), as shown in **Figure 1**.

Viscosity increases with increasing pulp content, passing from SN+P/2 (6% pulp w/w) to J (12%) and to J+P (24%). This is coherent with the work of Hernandez et al. (*1*), which showed that pulp and suspended solids appreciably contribute to increasing the apparent viscosity of orange juice. This is principally influenced by high pectin amounts. Interestingly, samples reconstituted with supernatant (SN, SN+P) exhibit

Table 3. Triangle Test Results for Orthonasal and Retronasal Perception[a]

	orthonasal evaluation		in-mouth evaluation	
tested pair	correct answers (*n*/total)	*p* value	correct answers (*n*/total)	*p* value
SN/W	19/36	<0.05	21/36	<0.01
J/SN+P	17/36	<0.06	20/36	<0.01
J+P/J+PD	14/36	NS*	8/36	NS*

[a] Significance of detected differences was calculated using the binomial law table.

significantly lower viscosity in comparison with whole juices with or without added pulp (J, J+P, and J+PD). In particular, J and SN+P, although they contained the same amount of pulp (12%), showed noticeable differences in viscosity. Most probably, the centrifugation and reconstitution processes, applied to SN+P, did not produce the original texture characteristics. Moreover, J+PD was characterized by a significantly lower viscosity when compared to the J+P juice. The same amount of pulp was added to both samples, but in the former, the pulp aliquot underwent a deodorization step. This process could thus account for differences in pulp structure, leading to a lower apparent viscosity.

Sensory Analysis: Triangle Test. Triangle tests were performed on three pairs of orange juice samples. Each pair was independently tested for differences in odor (orthonasal evaluation) and overall flavor and texture (in-mouth evaluation) (**Table 3**).

Serum (W) was obtained from supernatant (SN) by removing the cloud fraction by centrifugation. Assessors found significant differences between W and SN during both orthonasal and in-mouth evaluation. However, not all panelists noticed a difference. In particular, their comments indicated that they perceived higher overall flavor in SN. This shows that cloud plays an important role in orange juice flavor. Judges also found sensory differences in-mouth between whole (J) and reconstituted (SN+P) orange juices. They attributed these differences to differences in aroma and, additionally, when they evaluated samples in-mouth, they detected differences in texture. The differences already detected by rheology probably caused this perception. Finally, samples J+P and J+PD did not show any perceptible difference during either nasal or in-mouth evaluations. This means that the addition of deodorized pulp to the whole juice induced the same effect as natural pulp on overall perception (no effect of deodorization).

Sensory Profiles. The panel of 13 trained assessors evaluated texture, taste, aroma (retronasal), and odor (orthonasal) profiles of SN+P/2, SN+P, J, J+P, and J+PD using descriptive profiling.

Figure 2 shows texture and taste profiles. Assessors discriminate juices well according to their pulp content as shown by the "pulp quantity" profile: each sample belongs to a different statistical group except J+P and J+PD, which contain the same amount of pulp. These samples obtained very similar texture notations, confirming the results observed in the triangular test.

This means that the deodorization process did not affect perception of juice texture. Interestingly, whole juice (J) was noted with a significantly higher "pulp quantity" than the reconstituted juice (SN+P), thus confirming results obtained in both triangular and rheological tests. As previously stated, probably the centrifugation and reconstitution processes, applied to SN+P, did not produce the original texture characteristics. As expected, the intensity of the "fluidity" descriptor significantly decreased with increasing pulp amount. Surprisingly, the "particle size" attribute changes according to the juice matrix: in samples made from SN, judges perceived pulp particles of smaller "size" compared to the samples taken from the whole juice. Neverthelesss, all of the pulp added came from the same batch.

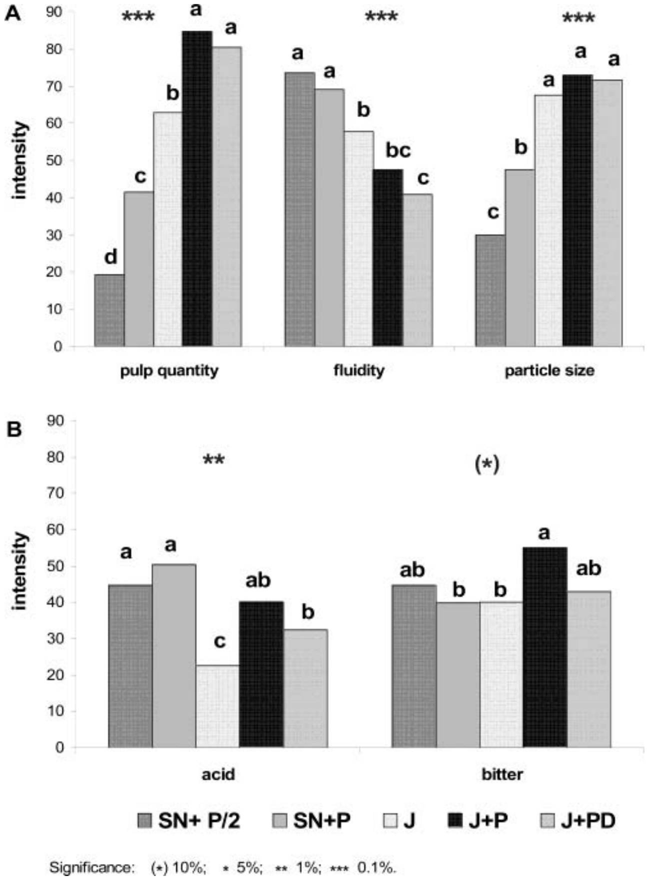


Figure 2. In-mouth description of orange juices: (**A**) texture profile; (**B**) taste profile. Juices with the same letter are not statistically different ($p < 0.05$). The sweet descriptor did not show any significant differences between samples.

No great differences were found in taste profiles (**Figure 2B)**. Whole juice (J) was noted as the least "acid" sample, whereas juice with added pulp (J+P) was perceived as being the most bitter—this increase being significant in J and SN+P. Probably the perception of bitterness could be due to high supraliminal limonene amounts that can be responsible for a bitter flavor as already reported (*16*). No significant differences were found for the "sweet" perception (data not shown).

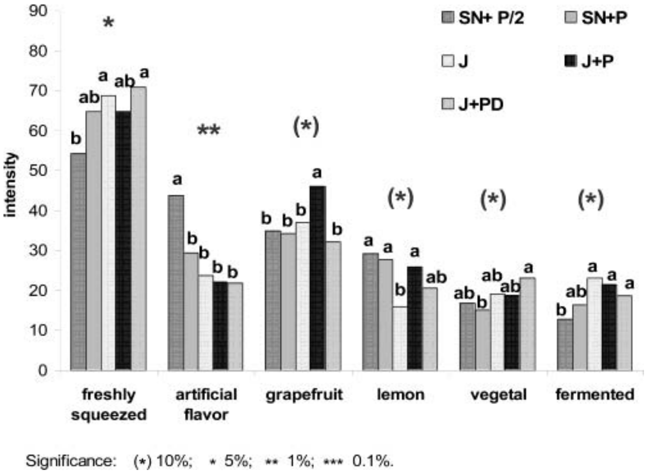
Figure 3 shows aroma profiles obtained by retronasal evaluation of the five juices. The orange juice containing the lowest amount of pulp (SN+P/2) differed strikingly in comparison to the other juice samples. In particular, this low-pulp juice was perceived as having the highest "artificial flavor" and the lowest "freshly squeezed" attributes.

On the other hand, the addition of pulp to orange juice increased the perception of the "grapefruit" aroma descriptor in J+P. Conversely, this enhanced perception of grapefruit was not detected when the added pulp was previously deodorized. This may be taken as good evidence that certain aroma compounds in pulp could be responsible for this perception.

In-mouth evaluation of food results from the complex interaction among various sensory modalities—texture and taste substantially contribute to the overall flavor perception (*8*).

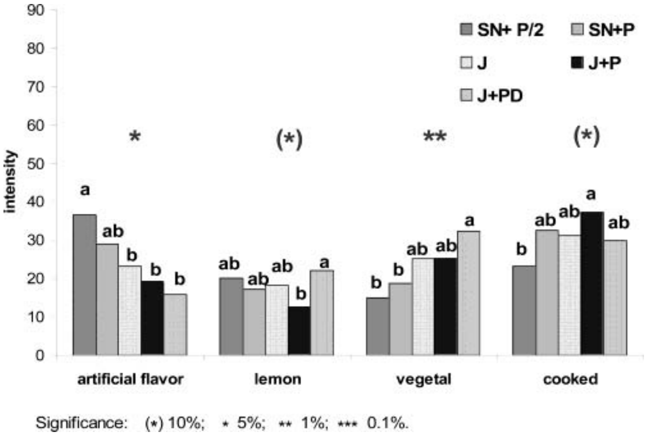
Table 4. Physicochemical Results Obtained for Formulated Orange Juices

	SN+P/2 (mg g^{-1})	SN+P (mg g^{-1})	J (mg g^{-1})	J+P (mg g^{-1})	J+PD (mg g^{-1})
pH	3.66 ± 0.02	3.67 ± 0.02	3.68 ± 0.02	3.69 ± 0.02	3.75 ± 0.02
sucrose, by HPLC	43.67 ± 0.02	43.55 ± 0.02	37.57 ± 0.02	36.36 ± 0.02	34.49 ± 0.02
glucose, by HPLC	27.45 ± 0.02	24.41 ± 0.02	20.83 ± 0.02	21.24 ± 0.02	19.14 ± 0.02
fructose, by HPLC	28.41 ± 0.02	25.42 ± 0.02	22.02 ± 0.02	22.19 ± 0.02	20.48 ± 0.02
total sugars	99.53 ± 0.02	93.38 ± 0.02	80.41 ± 0.02	79.8 ± 0.02	74.12 ± 0.02
organic acids (citric), by HPLC	9.33 ± 0.02	8.22 ± 0.02	6.6 ± 0.02	6.6 ± 0.02	5.94 ± 0.02
organic acids (citric), by titration	8.68 ± 0.001	8.53 ± 0.001	7.63 ± 0.001	7.75 ± 0.001	7.68 ± 0.001



Significance: (*) 10%; * 5%; ** 1%; *** 0.1%.

Figure 3. Aroma profile obtained by in-mouth evaluation of juice samples. Juices with the same letters are not statistically different. “Orange peel”, “jam”, and “cooked fruit” aroma descriptors did not show any significant difference between samples.



Significance: (*) 10%; * 5%; ** 1%; *** 0.1%.

Figure 4. Odor profile obtained by nose evaluation of juice samples. Juices with the same letters are not statistically different ($p < 0.05$). “Freshly squeezed”, “orange peel”, “grapefruit”, “jam”, “fermented”, “mandarin”, and “tropical fruits” odor descriptors did not show any significant difference between samples.

Orthonasal evaluation was the method used to overcome this bias. Results of this analysis are shown in **Figure 4**. Only four odor attributes varied significantly in relation to pulp content. Odor intensity of the “artificial flavor” descriptor decreased, passing from the low-pulp juice (SN+P/2) to the juices with or without added pulp (J, J+P, and J+PD). On the other hand, perception of the “vegetal” odor gradually increased with pulp amount, J+PD receiving the highest intensity score. These two odor descriptors follow the same trend observed in the aroma profile. Conversely, the odor profile for the “lemon” descriptor was substantially different from its aroma counterpart; in this case, a possible confusion with the “acid” taste might have occurred (correlation coefficient = 0.95). The “cooked” odor intensity also changed depending on juice type; the perception of this note significantly increased from the lowest (SN+P/2) to the highest (J+P) pulp juices. Interestingly, the effect of pulp amount on the “freshly squeezed” perception was less pronounced and thus no longer statistically significant when the juices were orthonasally evaluated.

Physicochemical Analysis of Juice Samples. Limpid serum was characterized by a 10 °Brix index of refraction. The physicochemical results concerning the reformulated juices are reported in **Table 4**. Samples were characterized by slightly different free sugar amounts. In particular, SN+P/2 and SN+P had higher sugar contents than the other juice samples, likely owing to their lower perceived pulp quantity. Both HPLC and basic titration gave very similar free acidity values, namely, showing slightly higher acidity values for SN+P/2 and SN+P samples. These results could explain why the acid taste was more strongly perceived in SN+P/2 and SN+P juice samples during taste profile analysis.

Flavor Release by HS-SPME. Insoluble solids (cloud and pulp) present in juice samples might interact with aroma compounds, causing a modification in aroma release and thus in perception. To verify this hypothesis, we studied aroma release from all of the samples used during sensory analysis. **Table 5** shows the general effect of pulp on the most abundant odor compounds. These molecules were chosen on the basis of their odor impact as assessed by GC-O. The headspaces of W, SN, SN+P/2, SN+P, J, J+P, and J+PD juices were sampled using a SPME method, which reduces distortion in extract odor quality and minimizes fiber saturation problems (*15*). To overcome eventual bias due to triphasic equilibria (juice matrix/headspace/SPME fiber), results were compared to those obtained by static headspace analysis; although absolute peak areas were lower in headspace extracts than in SPME extracts, relative amounts of flavor compounds were substantially the same (data not shown).

Brat and collaborators showed that the volatile compounds associated with pulp and cloud from a freshly squeezed orange juice represented ∼80% of total juice volatiles, of which 90% are in the pulp and 10% in the cloud (*7*). They found that the monoterpene hydrocarbons present in pulp, cloud, and serum represented 74.0, 7.3, and 7.4% of juice content. We found that the removal of both insoluble fractions effectively depressed the release of aroma compounds from the serum (W), in particular, terpenic compounds, which were quite entirely absent from the headspace of W. On the other hand, ethanol, acetaldehyde, 2-propanone, ethyl butanoate, and hexanal—very early eluting or hydrophilic compounds—abounded in the headspace of W. Moreover, the simple presence of cloud in SN caused a substantial increase in terpene and ester release. Introduction of pulp further augmented terpene concentration in the headspace of SN+P/2. Excluding that of hexanal, aroma recoveries from SN+P/2 and SN+P headspace were, however, very

Table 5. Flavor Release from Orange Juice Samples^{a}

compound	RI^{b}	odor descriptor^{c}	W		SN		SN+P2		SN+P		J		J+P		J+PD	
			amount	stat	amount	stat	amount	stat	amount	stat	amount	stat	amount	stat	amount	stat
aldehydes																
acetaldehyde^{d}	677^{e}	fresh, fruity	4855 ± 58	a	3836 ± 349	c	4097 ± 118	bc	3771 ± 194	c	4832 ± 81	a	4778 ± 173	a	4385 ± 206	b
hexanal^{a}	1083	orange, floral	1573 ± 183	c	942 ± 64	e	1108 ± 71	de	1480 ± 88	c	1331 ± 163	cd	2371 ± 93	a	2045 ± 209	b
octanal^{d}	1293	orange			1816 ± 118	c	2595 ± 143	a	2340 ± 117	b	2247 ± 89	b	2131 ± 250	b	1743 ± 256	c
total			6428 ± 183		6594 ± 349		7800 ± 143		7591 ± 194		8410 ± 163		9280 ± 250		8173 ± 256	
ketone																
2-propanone^{d}	814^{e}	fruity	882 ± 55	a	637 ± 12	c	842 ± 45	a	761 ± 36	b	517 ± 55	d	489 ± 18	d	474 ± 41	d
esters																
ethyl acetate^{d}	893^{e}	orange	7076 ± 374	d	14559 ± 273	b	15423 ± 400	a	14941 ± 703	ab	8828 ± 216	c	6144 ± 158	e	3416 ± 220	f
ethyl butanoate^{d}	1041	fruity, orange	28478 ± 2163	a	16905 ± 388	c	16683 ± 172	c	18585 ± 2265	bc	21097 ± 699	b	19747 ± 584	b	20048 ± 1056	b
ethyl hexanoate^{d}	1241	fruity, orange			590 ± 60	b	759 ± 38	a	972 ± 195	a	941 ± 162	a	852 ± 57	a	509 ± 51	b
total			35554 ± 2163		32054 ± 388		32865 ± 400		34498 ± 2265		30866 ± 699		26743 ± 584		20557 ± 1056	
aliphatic alcohols																
ethanol^{d}		solvent	128425 ± 2357	ns	92696 ± 59730	ns	116881 ± 1480	ns	127410 ± 3079	ns	72276 ± 50148	ns	113449 ± 1476	ns	105536 ± 2326	ns
1-octanol^{d}	1566	herbal	515 ± 69	bc	663 ± 55	ab	603 ± 36	ab	581 ± 136	ab	733 ± 15	a	401 ± 25	cd	284 ± 45	d
total			128940 ± 2357		93359 ± 59730		117484 ± 1480		127991 ± 3079		73009 ± 50148		113850 ± 1476		105536 ± 2326	
terpenic alcohols																
linalool^{d}	1554	floral, lemon	2502 ± 216	a	2984 ± 174	a	2833 ± 124	a	3133 ± 622	a	2546 ± 46	a	1571 ± 140	b	1508 ± 176	b
1,4-terpineol^{d}	1605	woody^{e}					849 ± 60	b	806 ± 157	b	941 ± 95	ab	1055 ± 97	a	626 ± 28	c
total			2502 ± 216		2984 ± 174		3682 ± 124		3939 ± 622		3487 ± 95		2626 ± 140		3656 ± 176	
monoterpens																
α-pinene^{d}	1020	citrus, woody			5378 ± 185	e	11884 ± 573	c	11168 ± 225	cd	16040 ± 772	a	15051 ± 349	b	10446 ± 759	c
δ-carene^{d}	1149	floral			2781 ± 267	e	6132 ± 218	b	4555 ± 555	c	9108 ± 443	a	5890 ± 78	b	3656 ± 351	d
β-myrcene^{d}	1168	peel, geranium	771 ± 98	f	19049 ± 2913	e	49749 ± 2420	c	50050 ± 6984	c	84154 ± 4263	a	72931 ± 2487	b	39605 ± 2391	d
limonene^{d}	1211	fruity, lemon	116756 ± 10383		1777639 ± 33909		3735564 ± 145083		3680845 ± 297084		5288008 ± 195441		4695133 ± 140646		2864035 ± 158395	
γ -terpinene^{f}	1242	floral			2815 ± 105	c	4424 ± 195	b	4296 ± 410	b	5257 ± 311	a	4962 ± 250	a	2850 ± 274	c
β-ocimene^{f}	1251	floral					839 ± 98	ab	911 ± 175	a	1107 ± 31	a	1058 ± 229	a	640 ± 21	b
p-cymene^{f}	1265	orange	1254 ± 313	c	6982 ± 632	b	9171 ± 407	a	8866 ± 1057	a	9233 ± 949	a	9372 ± 542	a	6633 ± 581	b
α-terpinolene^{f}	1279	citrus, pine^{e}			6072 ± 48	d	9094 ± 507	b	8325 ± 371	c	10490 ± 326	a	8974 ± 400	b	5883 ± 168	d
total			118781 ± 10383		1820716 ± 33909		3826857 ± 145083		3769016 ± 297084		5423397 ± 195441		4813371 ± 140646		2875200 ± 158395	
sesquiterpenes																
valencene^{d}	1713	woody, citrusy^{e}	8271 ± 848	e	15766 ± 683	d	22186 ± 922	c	27895 ± 4355	ab	29683 ± 1947	a	30399 ± 3376	a	24663 ± 1989	bc
α-selinene^{f}	1724	orange^{e}			658 ± 142	b	1552 ± 167	a	1792 ± 523	a	2194 ± 68	a	1876 ± 427	a	1773 ± 168	a
7-epi-α-selinene^{f}	1754				984 ± 92	b	1482 ± 335	ab	1664 ± 499	ab	2089 ± 129	a	1867 ± 464	a	1580 ± 183	ab
total			8271 ± 848				25220 ± 922		31351 ± 4355		33966 ± 1947		34142 ± 3376		28016 ± 1989	

[a] Along a single line, aroma amounts (expressed as total ion current) with the same letters are not statistically different among juice samples. [b] Retention index on DB-Wax column. [c] Descriptors resulting from the juice sample “J” through GC–olfactometry sessions using the same chromatographic and SPME conditions as flavor release analysis. [d] Identification assessed by mass spectrometry and retention index of pure reference compounds (INRAMASS). [e] From the literature. [f] Tentative identification using NIST mass spectra library.

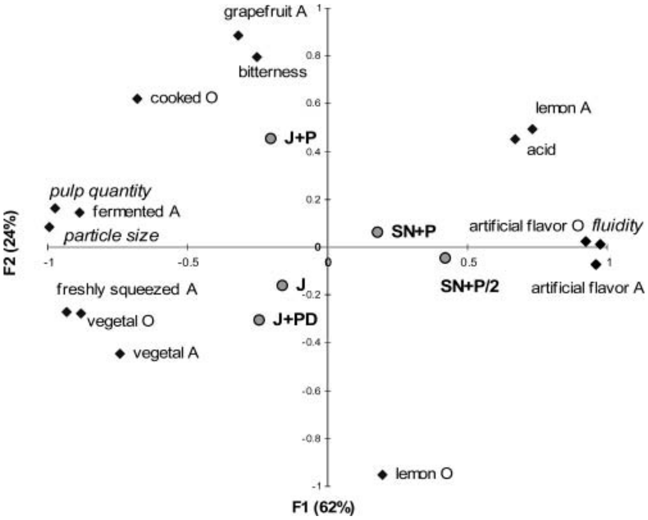


Figure 5. Biplot representation of PCA made on orange juice samples (in bold) and showing significant differences between products. Odor and aroma attributes have "O" and "A" letters, respectively. Texture attributes are in italics.

comparable. In general, the whole juice (J) accounted for the highest headspace aroma amount. In particular, terpenes such as limonene, β-myrcene, and δ-carene were significantly more abundant in J than in samples reconstituted from supernatant. This could be due to a matrix effect. Surprisingly, when whole juice was enriched with natural pulp (J+P), aroma release was not greater than in the J sample; however, hexanal release significantly increased with pulp amount. Conversely, when juice was enriched with a deodorized pulp (J+PD), a strong retention effect was detected for aldehydes and esters (except ethyl butanoate). However, no effect was observed for ethanol, 2-propanone, and other hydrophilic compounds dissolved in the aqueous phase of juices.

GENERAL DISCUSSION

We were the first to study the role of suspended solids in orange juice on the complex phenomenon of texture and flavor perception. The aim of this work was, first, to investigate whether these solids account for modifications in sensory perception and, second, to understand if these modifications were due to physicochemical changes in aroma release or to sensory processes as well.

Role of Cloud. Cloud naturally contains a non-negligible amount of aroma, in particular, monoterpenes and sesquiterpenes. This is the cause of the major difference in odor and overall flavor perception noted between W and SN (only the latter contains cloud) (**Table 3**).

Differences in odor were also confirmed by flavor release experiments (**Table 5**): in comparison with the serum sample (W), SN released additional aroma compounds (included in cloud). Moreover, this insoluble fraction also showed a strong retention effect on aroma compounds, which are prevalently present in the aqueous phase, such as ethyl butanoate or hexanal. This could be due to molecular interaction phenomena with the macromolecules forming the cloud fraction: pectins, lipids, or proteins as well. Further investigations are thus necessary to test this hypothesis on model systems and better understand the mechanisms involved.

Role of Pulp. Pulp strongly influenced not only texture but also flavor perception. In **Figure 5**, PCA shows juice samples according to significant texture, taste, aroma, and odor attributes; in this biplot representation, products (juice samples) and variables (sensory attributes) remain on the same principal plane. This representation has the advantage of showing sensory results from a global viewpoint. Texture properties discriminate juice samples along the first axis. Samples reconstituted from supernatant (SN+P/2 and SN+P) are characterized by the highest "fluidity" attributes but also by "acid" taste and "artificial" flavor. The juice enriched with a naturally flavored pulp (J+P) is discriminated from J and J+PD as having the lowest "lemon" odor and the highest "grapefruit" and "bitter" attributes. J+PD is characterized by a strong "vegetal" flavor.

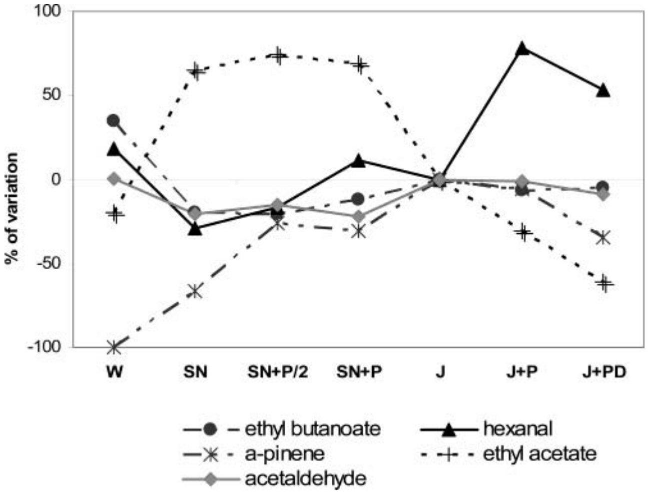


Figure 6. Percent of variation in released aroma compounds relative to the whole juice J.

Pulp accounted for two major physicochemical effects in orange juice samples: it retained a large amount of aroma compounds and modified the rheological properties of the juice matrix. Therefore, when we added a natural pulp to the whole juice, two contrary effects took place: (1) We increased aroma amount in the sample (an equilibrium was established between the absorbed and the free forms of aroma compounds). (2) We increased texture and viscosity and thus the difficulty for the free odor compounds to diffuse from the thickened juice to the vapor phase. This explains why flavor releases from J+P and from J are very similar (**Table 5**).

The different release-affecting effects due to cloud and pulp are plotted in **Figure 6** for some aroma compounds taken as examples. The graph shows the percent of variation in release relative to the whole juice J.

Ethyl butanoate (EB) is responsible for a fruity note in orange juice; it is the molecule with the highest odor impact in orange juice, as recently demonstrated by Buettner et al. (*17*). Like most short-chain esters, EB is present in high amounts in the headspace of W (+35% release relative to J). The cloud present in SN strongly affects EB release, whereas pulp (the natural and the deodorized type) did not modify EB release in any way. Linalool showed a similar trend.

Hexanal (H) is responsible for an herbal note in orange juice. As in the case of EB, its release from SN was strongly inhibited by cloud macromolecules. Nevertheless, H is present in large amounts in native pulp, so it increased with pulp amount (J+P showed a 78% increase compared to J). This increase persists even in the case of the addition of deodorized pulp to J (58% in J+PD). This could be due to the enzymatic formation of hexanal in the pulp fraction before juice pasteurization (*18*).

Ethyl acetate (EA) is another very important odor compound in OJ (orange note) and is one of the less hydrophobic orange juice esters. Unlike EB, this compound was present in very low amounts in W headspace.

Cloud strongly increased EA amount in SN (65%), whereas no effect was observed by adding pulp in SN+P/2 and SN+P. This is coherent with the results obtained by Brat et al. (*7*), who found a greater amount of EA in the cloud than in the pulp of a Naveline orange juice (3.4 and 1.6 $\mu g \cdot g^{-1}$, respectively, fresh weight). Interestingly, a strong EA retention effect is detected for the most highly textured juices: an increase in viscosity and texture could affect EA diffusion into the juice matrix, thus explaining the lower amount of released EA. In a previous study on pectin solutions, we showed that an increase in viscosity caused a decrease in the diffusion and, thus, in the release of very volatile esters (*19*).

Pulp strongly affects terpene release. α-Pinene (AP) was completely absent in W headspace (−100%) and gradually increased with the addition of pulp. This could be explained by high AP amounts recovered from naturally flavored pulp. Moreover, the simple retention effect of pulp is observable in AP release when we added deodorized pulp to the juice (−35% of AP released from J+PD). This retention effect could be due to absorption on the surface of pulp particles as well as to the diminished diffusivity caused by higher viscosity. The other terpenes followed the same trend, but sesquiterpene percent variations were less pronounced due to their lower volatility. Finally, ethyl hexanoate (EH), which demonstrates higher hydrophobicity than EA and EB, showed variations in release more similar to those observed for terpenes (data not shown).

Jordan et al. (*10*) found fairly similar trends for flavor release from orange juices with insoluble solid contents of 3 and 10−15% (they used polydimethylsiloxane and polyacrylate SPME fibers and simultaneous steam distillation−extraction), although their quantitative measures were affected by important standard deviations. However, they concluded that the reduction of insoluble solid content does not decrease the quality of the aromatic fraction present in orange juice, including aldehydes. These conclusions do not agree with previous literature or, clearly, with the physicochemical and sensory results demonstrated in the present study.

Sensory−Instrumental Correlations. Odor perception is directly related to the aroma compounds released in the vapor phase of juice. As previously stated, aroma amount is a function of the initial concentration in the juice as well as the physical parameters that determine molecular transfers in the headspace. Therefore, the odor differences detected by the sensory panel with increasing pulp amount (**Figure 4**) could be partly explained by the differences found in aroma release (**Table 5**). For example, the strong decrease in the “artificial flavor” intensity could be due to the strong decrease in aroma compounds characterized by strong “sweet orange” odor, such as ethyl acetate ($r = 0.90$), 2-propanone ($r = 0.95$), and octanal ($r = 0.81$). On the other hand, the strong increase in the “vegetal” odor in J+P and J+PD is correlated with the major increase in hexanal amount ($r = 0.86$), which has an herbal odor. Finally, the high perceived intensity of the “freshly squeezed” odor (in all samples except SN+P/2) is mostly due to the high amount of terpenic compounds. Moreover, the fact that this odor descriptor did not significantly change could be traced to acetaldehyde amount, responsible for a fruity/fresh note (**Table 5**), which was not affected by increasing pulp ($r = 0.96$).

Nevertheless, overall flavor perception is a very complex event in which many sensory modalities converge and influence each other. Therefore, when a food or beverage is taken into the mouth, aroma perception is influenced by interactions of taste and/or texture components. Some of these interactions are suggested in **Figure 5**: the “lemon” and “grapefruit” aroma descriptors are associated with acid and bitter tastes, respectively, and not with their corresponding odor descriptors. Further investigation is needed to clarify the role of taste in influencing aroma perception.

The perception of textural properties could affect overall flavor perception (*20*). Many authors, such as Baines and Morris (*21*) and Juteau et al. (*22*), found that the aroma perception of a model solution thickened with hydrocolloids generally decreased. In all of these cases, it has been hypothesized that aroma perception is changed by some sort of interaction among taste, aroma, and texture components of the system. The mechanisms by which interactions occur are not clear, and hypotheses based both on a change in flavor release and/or on some sort of cognitive interaction have been proposed. In a very early study conducted on a model solution, Ahmed attempted to understand the role of nonvolatile components such as sugar, pectin, and acid on the limonene retronasal threshold. He found only a significant increase in threshold due to organic acids, whereas pectin did not show any significant effect (*11*). In our case the problem is more complex because orange juice is a real and multiphase system, so cloud and pulp fractions (the mouthfeel agents) are already rich in aroma compounds. When we add pulp, therefore, we increase not only texture but also add additional aroma compounds.

The interactions between senses may, thus, occur at a central level where chemicosensory and somatosensory input converge, or even at a perceptual level where previous experiences could influence aroma judgment. In our case, as juice samples were discriminated for the “freshly squeezed” attribute only when they were assessed in-mouth, we could not exclude the occurrence of a cognitive bias. In fact, juices that were perceived in-mouth as being pulpous may have been associated with a “natural” impression and thus may have received a higher “freshly squeezed” and a lower “artificial flavor” notation during the evaluation in-mouth. This is why we added a pulpy juice (24%) made with a deodorized pulp (J+PD) to the experimental design. For this sample, the intensity of the “freshly squeezed” aroma is lower than for J+P and J, thus confirming that both cognitive and chemical effects occurred.

Conclusion. In this work we gain insights into the role of suspended solids on the sensory perception of aroma and texture in orange juice. We showed that in a hand-squeezed pasteurized orange juice, these solids modify the juice matrix, thus influencing rheological properties and flavor release in orange juice. As a consequence, the perception of texture and flavor properties is strongly modified. Cloud contributes to the flavor of a juice lacking in the coarsest particles. Moreover, in our juices cloud exhibits strong retention properties relative to some oxygenated compounds such as hexanal and ethyl butanoate. These key compounds are probably involved in molecular interactions with cloud macromolecules. Further investigations are necessary to better understand these mechanisms. As expected, pulp strongly influences the sensorial perception of texture properties, such as fluidity and pulp amount, but it also strongly influences odor, aroma, and taste perceptions: the addition of natural pulp to low-pulp juices increases the fresh orange juice character. This may be explained by both physicochemical (fresh pulp contains high amounts of key aroma compounds, including acetaldehyde and mono- and sesquiterpenes) and cognitive effects, mainly due to the tactile properties of the pulp. Nevertheless, it could be emphasized that the hand-squeezing process is quite different from the industrial processing, leading to different pulp content and composition (*23*). This suggests that other experiments suitable for industrially processed juices could be made to confirm our findings and eventually to provide further information for improving quality.

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